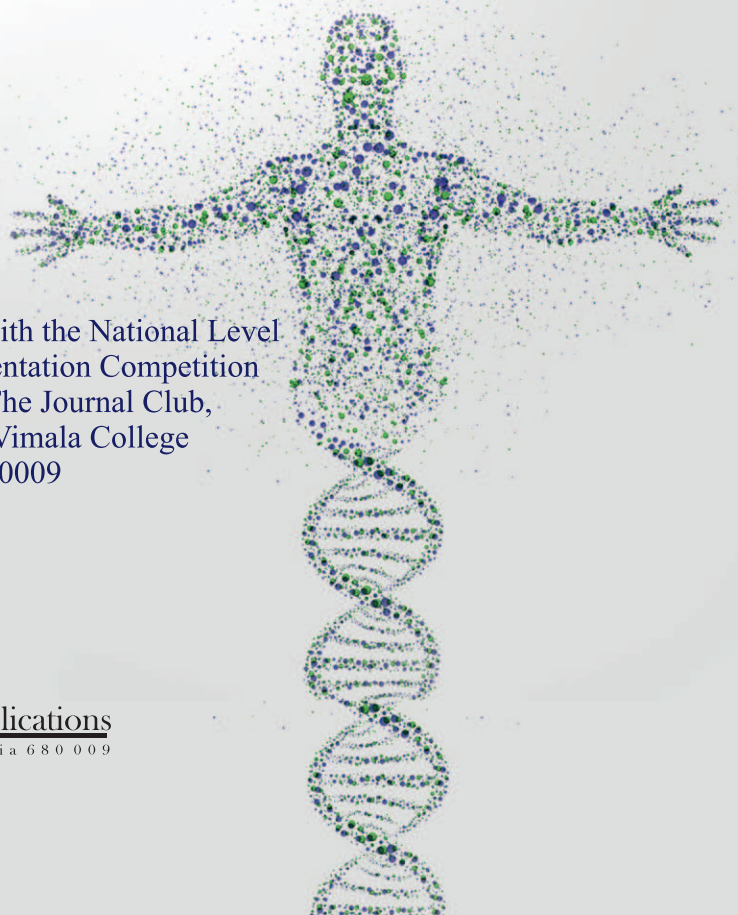
MODERN TRENDS IN BIOLOGICAL RESEARCH

Editors

Dr Indu M S | Dr Honey Sebastian | Dr Petrisia Joseph



A Handbook in connection with the National Level Virtual Scientific Paper Presentation Competition organized by ZOOTOPIA - The Journal Club, PG Department of Zoology, Vimala College (Autonomous), Thrissur – 680009

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Vimala College (Autonomous) , Thrissur – 680009
(NAAC Reaccredited (3rd cycle): A Grade-CGPA 3.5)
held on 9th January 2021

ABOUT THE INSTITUTION

Vimala College (Autonomous), Thrissur, a first grade women's College under the CMC Management established in 1967, is affiliated to the University of Calicut. The Institution is under the managementof the Nirmala Province, Thrissur, of the Congregation of Mother of Carmel (CMC). True to the ideals and heritage handed over to the Congregation by her founder, Blessed Chavara Kuriakose Elias, the Institution aims at the "pursuit of intellectual and professional excellence and encourages a holistic approach to education that not only ensures academic excellence but also equips young women to face the challenges in life by fostering values, imbibing emotional maturity, creating civic responsibility and building global competencies in a dynamic environment. Ever since its inception, the Institution has been in the forefront of higher education in the State. The Institution offers 18 undergraduate and 18 postgraduate courses and is a centre for research in English, Commerce, Physics, Economics, Social Work and Malayalam. Accredited at the national level with a Five Star status in 2001 by the NAAC, the institution has undergone two subsequent cycles of re-accreditation in 2008 and 2014 and presently holds the top grade A with a CGPA of 3.50 on a 4 point scale. The University Grants Commission (UGC) conferred autonomy in 2016 and identified her as a College with Potential for Excellence in 2016. Vimala College has a full-fledged DST – FIST funded laboratory and presently 6 science departments of college is supported under DBT-STAR College Scheme. The atmosphere of the Institution is charged with the noblest ideals of humanity and the spirit of secularism, justice and equality of opportunity enshrined in the Constitution.

ABOUT THE DEPARTMENT OF ZOOLOGY

The Department of Zoology became operational in 1967 and aims to provide quality education, to promote training in practical and conceptual skills in various sub-disciplines of biology and also inculcates the spirit of resource conservation and love for nature. The Department also never fails to motivate the students for self employment in various applied branches of Zoology and to orient the students in developing research skills. Both under graduate and post graduate courses are being offered by the Department, which is facilitated through excellent infrastructure and qualified faculty members. The Department of Zoology has one UG and two PG Programmes in Zoology. Self-financing M. Sc Zoology with specialization "Fishery Science" started in the academic year 2013-2014 and regular M. Sc Zoology with specialization in 'Structure, physiology, development and classification of animals' was introduced in the academic year 2020-2021. With the aim of educating students about the fish culture techniques, the department has initiated a start-up programme - "Aquaphilia" in June 2018. The department has tie up with various research organizations/institutions and has functional MoUs with them. The department is funded by DBT-STAR college scheme in 2020.

ZOOTOPIA - The Journal Club, Department of ZOOLOGY
Vimala College (Autonomous), Thrissur
organizes
National Level Virtual Scientific Paper Presentation Competition - 9th January 2021

9.00 AM to 09.30 AM	Inaugural Function Meeting Link: https://meet.google.com/pgx-ekko-vyu
9.30 AM to 12.30 PM (Category A & Category C) 3.00 PM to 6.00 PM (Category B)	Scientific Presentations

Category A: Health and Diseases
Link: https://meet.google.com/pgx-ekko-vyu
Chair Persons:
Dr Shaji E M, HOD, KKTM College, Pullut
Dr Seema Menon, Assistant Professor, KKTM College, Pullut

Category B: Biodiversity and Environment
Link: https://meet.google.com/nnc-nxvz-trj
Chair Person:
Mr Jain J Therattil, Assistant Professor, St. Aloysius College, Elthuruth, Thrissur

Category C: Biotechnology & Pharmacology
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MODERN TRENDS IN BIOLOGICAL RESEARCH

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Principal's Message

It is indeed a special privilege and pleasure to publish MODERN TRENDS IN BIOLOGICAL RESEARCH - A Handbook in connection with the National Level Virtual Scientific Paper Presentation Competition organized by ZOOTOPIA - The Journal Club, Department of Zoology, Vimala College (Autonomous), Thrissur held on 9th January 2021. The conference provided a fertile ground for the academic deliberations and dispersal of knowledge. I would like to congratulate the Department of Zoology for taking this initiative and publish the full papers for the benefit of society

Dr. Sr. Beena Jose
Principal
Vimala College (Autonomous)
Thrissur - 680 009

Message

I am very happy to be part of success of the National Level Virtual Scientific Paper Presentation Competition organized by ZOOTOPIA - The Journal Club, Department of Zoology, Vimala College (Autonomous), Thrissur. On behalf of Department of Zoology, I extend our sincere gratitude to the respected Principal, Dr Sr Beena Jose, who has always been a constant supporter and motivator to the planning and execution of the event. I would like to express my earnest thanks to Dr Sr Beena T L & Dr Minimol K Jose, Vice principals whose support were inevitable in successfully planning and conducting this programme. I extend my sincere thanks to all the resource persons for accepting our invitation to be part and parcel of this programme, listen to our participants, adjudicate them and be moderators of all paper presentations. The success of any conference is the participants who turn up. I sincerely thank all the delegates for your valuable response and participation. I would like to thank all faculty members and students of Vimala College for the success of the programme

Dr. Honey Sebastian
HoD, Department of Zoology
Vimala College (Autonomous)
Thrissur - 680009

Preface

The success of any research is the acceptance of the findings by the experts and peer researchers after sufficient debates and deliberations. The research will also be benefitted only if it is spread in the society and used beneficially for the betterment of the society. The world-wide crisis of COVID-19 pandemic has disrupted daily lives, global economies and educational systems. Schools and colleges in India and abroad are still under different phases of lock-down to maximize social distancing and minimize the spread of infection amongst students and teaching staff. The science and research field are forced not to have physical meetings and to adopt non-contact teaching and research. With the excellent capacity of human beings to adapt to any constraining conditions we saw an upsurge in the field of digital learning and virtual platforms. To overcome the stagnant phase of research and communications, we the Department of Zoology, Vimala College (Autonomous), Thrissur planned to gather experts and scholars around the country with the aim to continue the academic exchange and disseminating the latest advanced research in the field of Biological Science among researchers. A National Level Virtual Scientific Paper Presentation Competition was organized by ZOOTOPIA - The Journal Club, Department of Zoology, Vimala College (Autonomous), Thrissur on 9th January 2021where researchers and post graduate students all over the country were invited for exposing the budding scientists to scientific community. The presentations were done under 3 categories: Health and Diseases, Biodiversity and Environment and Biotechnology and Pharmacolgy. In connection with the event, I am very happy to publish the full papers of all the research findings presented during the conference. I take this opportunity to extend my earnest thanks to all who worked hard to make this event a grand success

Dr Indu M S
Coordinator
Assistant Professor on Contract
Department of Zoology
Vimala College (Autonomous)
Thrissur - 680009

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Category A
Health and Diseases

Analysis of chromosomal polymorphism in relation to reproductive failure

Anjana Menon*, Lekshmi Jayakrishnan*
* Department of Zoology, St. Aloysius College, Elthuruth

Abstract

A chromosome polymorphism is a variation in chromosome morphology which includes changes in their size and shape that affects heterochromatic segments, satellites and satellite stalks. Polymorphic variants occur in the heterochromatin on the long arm of chromosomes 1, 9 and 16, the short arm of D and G group chromosomes and the distal heterochromatin of Y chromosomes. The causes of infertility among women include tubal diseases, ovulatory dysfunction, cervical or uterine abnormalities, endometriosis related infertility and RSA. Male infertility can be classified as azoospermia, oligozoospermia, asthenozoospermia, teratozoospermia and aspermia. The objectives of this investigation included the evaluation of incidence and types of polymorphism and its effect on fertility related processes and clinical picture in patients.

Keywords: Chromosome Polymorphism, Karyotyping, Reproductive failure

Introduction

The recent progresses in the study of cell nucleus have increased the interest in euchromatin and heterochromatin, the two functionally different parts of the genome, which can be visualized on chromosomes. The most active and gene rich region of the genome is euchromatin. Heterochromatin, a gene poor region, is of two types: constitutive, a stable form present in polymorphic variants; and facultative, a reversible form as seen in Barr body. Constitutive heterochromatin contains tandemly organized highly repetitive sequences of satellite DNA which do not encode for any proteins and has no coding potential. The exact role of heterochromatin in the human genome remained unknown for a very long time as its frequent polymorphisms did not have any functional or phenotypic effects. It has now been acknowledged that heterochromatin is non-inert and plays a major role in cell and organismal viability in multicellular eukaryotes. Genes required for normal chromosomal inheritance, viability and fertility are located in heterochromatin. It also plays an essential role in spindle attachment, chromosomal movement, meiotic pairing and sister chromatid cohesion.

A chromosome polymorphism is defined as a variation in chromosome morphology which includes changes in their size and shape. The polymorphism includes heterochromatic segments, satellites and satellite stalks. Cytogenetic heteromorphisms are referred to as

variations at specific chromosomal regions with no impact on phenotype. Polymorphic variants on chromosomes usually occur in the paracentric heterochromatin on the long arms of chromosome 1, 9 and 16, the short arm regions of D and G group chromosomes and the distal heterochromatin of Y chromosome. Increases in length of the heterochromatic regions on the long arms of these chromosomes are designated as 1qh+, 9qh+, 16qh+ and Yqh+. Increase in length of the short arm satellite and stalks of acrocentric D and G group chromosomes (13, 14, 15, 21 and 22) are designated as, for example, 14ps+ and 13pstk+. The increases in the length of short arm are designated as p+. The short arms and satellites of acrocentric chromosomes contain heterochromatin while the stalks contain repeats for genes that encode 18S and 28S rRNA and ribosomal proteins that coalesce to form the nucleolus are known as nucleolar organizing regions (NOR). Human beings are polymorphic with respect to a number of such polycistronic genes.

Infertility is a major social and health problem affecting 10% to 15% of sexually active couples who are unable to conceive after one year without contraception.

Most polymorphic variants are familial and follow Mendelian inheritance from one generation to other with a slow mutation rate. De novo polymorphic chromosomal variants are rare and can occur possibly as a result of unequal crossover between heterochromaticregions of homologous chromosomes in meiosis or due to conjugation of repeated DNA sequences. These variants are large in size and are associated with clinical conditions. However, there is increased frequency of variants in association with different clinical conditions such as reproductive failure, recurrent spontaneous abortions and even psychiatric disorders.

For a long time, chromosomal polymorphism, most of which take no effect on phenotypes, have been considered harmless, receiving less attention. Evidences suggest that these variations may be associated with deleterious reproductive consequences, recurrent pregnancy loss and reproductive failure or infertility.

Materials and Methods

Peripheral Blood Lymphocyte Culture using a modified Moorhead and Hungerford (1960) protocol

Taken 5mL of culture medium (RPMI 1640; SIGMA) pH 7, in a centrifuge tube and added 0.6mL Foetal Bovine Serum (FBS,INVITROGEN) and 0.2mL Phytohemagglutinin (PHA, INVITROGEN) and added 0.5mL peripheral venous blood into it; mixed well and the culture was incubated in the water bath at 37 for 69 hours.For the mitotic cell arrest, at 67th hour of incubation, 50 µL colcemid (GIBCO, KARYOMAX) was added to arrest the cell division at metaphase stage and again incubated it for 2 hours.

For harvesting the arrested lymphocytes, at 69th hour of incubation the cell suspensions were centrifuged for 10 minutes at 1600 rpm, the supernatant was discarded, and pellet was treated with 5mL hypotonic solution (0.56% KCl) then incubated for 15 minutes. Following the incubation, the samples were centrifuged carefully at 1600 rpm for 8 minutes and fixed using fixative (methanol and acetic acid in the ratio 3:1). At the beginning, the fixative was wisely added and made up to a volume of 5mL and mixed well by using vortex mixture and made up to 9mL and then incubated it for 20 minutes at room temperature. After this, suspension was centrifuged at 1600 rpm for 10 minutes. Then tubes were kept in refrigerator overnight.

The next day, after centrifugation at 1600 rpm for 10 minutes, the supernatant was discarded and the pellet was used for making the slides. The slides were prepared by air drop method. The slides were placed in hot air oven for 1 hour for ageing for GTG banding.

GTG Banding (G – Bands by Trypsin using Giemsa)

After 1 hour of ageing the slides were treated with PBS and then trypsin (0.032g in 600mL PBS) and the slides were placed in Giemsa stain for 1.30 minutes. Then the slides were air dried.

Karyotyping

Karyotyping was performed from the stained slides by capturing 20 metaphase plates using spectral imaging software.

Results and Discussion

A total of 10 cases were taken up from patients referred to CIMAR for evaluation of chromosomal abnormalities related to reproductive failures within a time span of two months.

Case no# 3 (figure 1) showed an elongated q arm of sub-metacentric chromosome 9 of group C with an extra block of heterochromatin in the above mentioned arm. The patient showed symptoms like primary infertility and improper spermatogenesis. The karyotype of this patient was 46, XY, 9qh+. Recent studies suggest that classicalvariants of 9qh+ and heteromorphism on chromosome 6q may be responsible for recurrent abortions (Calgayanet al.2009).However, in this study we found a statistical association between the chromosomal polymorphism, namely, 9qh+, and infertility. The frequency of 9qh+ was statistically significantly increased in women with primary infertility and in men with azoospermia which was confirmed by other studies (Minocherhomji et al. 2009). The 9qh region possess gaps and splits more frequently than any other region in synaptonemal complexes which has been pointed out in several studies (Codina-Pascaul et al.2006; Sun et al.2007).

Case no# 8 (figure8) showed an elongated satellite on the acrocentric chromosome 15 of D group. The karyotype of this patient was 46, XY, 15ps+.Unexplained Recurrent Miscarriage (URM) and infertility were the symptoms associated with this patient. Heteromorphism of ps+ in all D and G groups was observed leading to multiple clinical effects in the female patient with a Bad Obstetrics History (BOH) (Vaghasia et al.2017). Madon et al.2005 documented that these variants were correlated with reproductive effects. Both these studies supported the data in this study as 15ps+ karyotyping was conducted for a female patient with BOH.

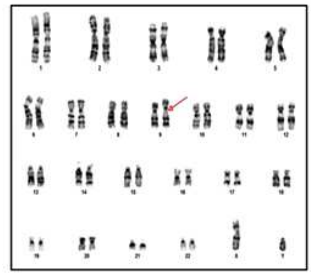

Figure 1: Case# 3 : 46, XY, 9qh+

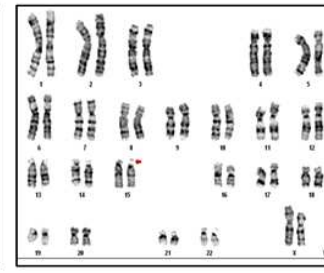

Figure 2: Case# 8 : 46, XY, 15ps+

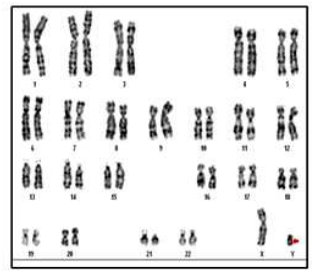

Figure 3: Case# 6 : 46, XYqh-

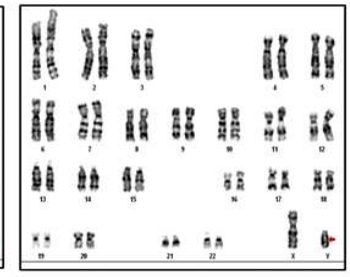

Figure 4: Case# 2 : 46, XYqh+

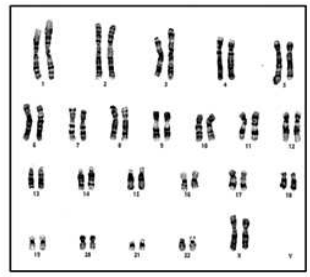

Figure 5: Case# 9 : 46, XX, 1qh+

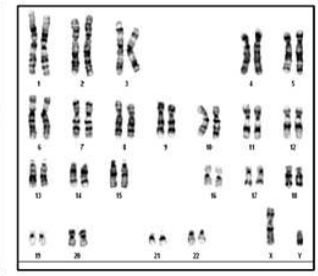

Figure 6: 46, XX, Normal female

Case# 6 (figure 3) showed a deletion of a heterochromatin region in the acrocentic Y chromosome. The patient was diagnosed with infertility. 46, XYqh- was the karyotype of this patient.

Case no# 2 (figure 4) karyotype is 46, XYqh+. Symptoms like improper spermatogenesis was shown by the patient.Y chromosome polymorphisms could also discourage homologous

chromosome pairing during the cell division phase, thus causing disorders such as cell division disorder, embryonic developmental disorder, teratogenic disorders, stillbirth and miscarriage (Akbas et al. 2012). The spouses of those carrying the Y chromosome polymorphisms exhibited a shorter gestational age, higher frequency of miscarriage and longer interval between pregnancies (Yan Wang et al.2017). Azoospermic Factor (AZF) microdeletions was observed in patients with XYqh-. Carriers of karyotypic Y chromosome abnormalities have the potential to be at risk from Y chromosome microdeletions, and these microdeletions can be transmitted from infertile fathers to their oûspring (Pan et al. 2018).

Case no# 9 (figure 5) is 46, XX, 1qh+ which is an extra heterochromatin region on the q arm of chromosome 1. The patient was diagnosed with infertility. The frequency of 1qh+ was significantly increased in women with primary infertility and in men with azoospermia (Minocherhomji et al. 2009).

Out of the 10 patients, 5 patients (figure 6) showed normal karyotype (46, XX/XY).

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Analyzing efficacy of Black Cumin Oil (*Nigella Sativa*), 95% Curcumin and their combination using biological assays

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Abstract

In the present study, preliminary cell-based assays were performed on human breast cancer cell line SKBr3 to evaluate the biological properties of *Nigella sativa* (Black Cumin oil) and 95 % pure Curcumin powder and their combination to be used as an anticancer nutraceutical agent. Biological assays such as DPPH assay cytotoxic assays and staining assays were carried out to study the compounds' activities. From the outcomes obtained from the present study, it was inferred that 95% pure curcumin and Black Cumin oil have the potential antiproliferative activity and induce cytotoxicity in breast cancer cells andfurther advanced studies and validation could provide a possibility to be used as a natural anti-cancerous agent. A significant finding observed in the study was that the combination of both Black Cumin oil and 95% Curcumin showed proliferation and cell survivability in cancer cell lines. In addition to this, the combination was found to have more application as a strong antioxidant property than an individual application.

Keywords : *Black Cumin oil*, *Curcumin*, Anticancer, Antioxidant activity, Antiproliferative activity, Cytotoxicity

Introduction

India is one of the 12 mega diversity countries globally, with its unmatched diversity accountable to the 11 different phytogeographical zones. The country is a treasure trough of traditional knowledge and holds a vast repository of medicinal plants used in traditional treatments. Medicinal plants are essential for pharmacological research and drug development. Extensive investigations are carried out to identify and exploit the medicinal plants because of the detailed understandings of the potential activities of natural drugs and notable adverse effects produced by synthetic drugs [1]. The plant constituents directly function as therapeutic agents and precursor materials for the synthesis of drugs or as models for pharmacologically active compounds [2]

The most prevalently diagnosed malignancy amidst women is breast cancer, which accounts for about 30% of the currently detected cancer in women on a global scale [3]. 20-25% of cases in invasive breast cancers exhibit HER2, which significantly gave rise to an aggressive

phenotype and low recovery rate. SKBr3 are human breast cancer cell lines that overexpress HER2 proteins (ER^-, PR^- and $HER\text{-}2^+$) [4, 5]. Many anticancer medications that are used recently have reported cytotoxicity in normal tissues, and hence plant-based medicines are gaining attention [6]. Various studies suggested that dietary phytochemicals can prevent cancer [7].

Materials and Methods

Out of many herbal compounds used in traditional medicine, two essential compounds *Nigella sativa* {Black cumin oil (BKO) and 95% pure curcumin (CCR) and their combination were selected for the present study. The compounds were obtained from Akay Flavours and Aromatics Pvt. Ltd, Ernakulam, Kerala and their combination. These were dissolved in 1% DMSO. The stock solution concentration of 1mg/ml was prepared. Different concentrations of the compound were made by serial dilution.

Cell culture and maintenance

The cell line used for the study was human breast cancer cell line SKBr3. It overexpresses the HER2/c-erb-2 gene product. Cell lines were cultured using Dulbecco's Modified Eagle Medium–High glucose (DMEM) in a CO_2 incubator (5% CO_2, 37^0c).

For antioxidant property estimation

The DPPH assay for antioxidant property was evaluated according to Maksimoviæ*et al.,* 2011 with some modifications.

For the cell viability and proliferation estimation

For the investigation of cell viability, MTT assay was performed with some modifications to Bahguna*et al.,* 2017.

For cell to cell interaction estimation

The cells were incubated for three days with regular treatment of the compound at the specified concentration after compound treatment. The cells were fixed with 200µl 4% paraformaldehyde and stained with 500µl crystal violet (0.5%) after removing the fixative. The cells were incubated for 15 minutes and observed under the microscope.

For cytotoxicity estimation

For cytotoxicity determination Acridine orange and ethidium bromide (AO/EB) dual staining assay was performed following Majeed *et al.,* 2019 with some modifications.

Statistical Analysis

To compare statistical significance, one way ANOVA test was done using SPSS software. A P-value of less than 0.05 was used as a criterion for estimating the significance. The statistical analysis of the IC50 value was obtained using Microsoft Excel.

Results

Estimation of antioxidant property

The results show that the IC50 value of ascorbic acid, CCR, BKO, and its combination were obtained as 9.39µg/ml, 38.28µg/ml, 136.46µg/ml, and 15.42µg/ml, respectively. The combination of BKO and CCR showed an IC50 value near to the value obtained for ascorbic acid (standard).

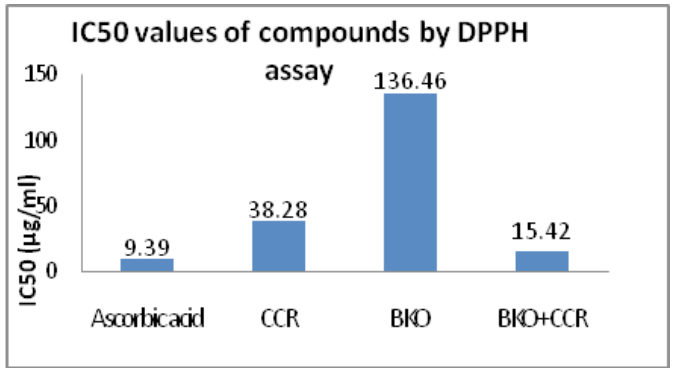

Figure 1: Graph showing results of antioxidant property

Estimation of cell viability and proliferation

The MTT results of the test compounds showed that CCR and BKO have significant anti-proliferative properties. This was significantly different from control ($p \leq 0.05$). In CCR, a dose-dependent decrease in the cell viability was observed from 96.6% to 71% as the concentration increased from 5µ/ml to 25µ/ml, and in BKO a moderate rate from 92.12% to 77.72% respectively. On the other hand, the anti-proliferation in cells treated with the combination was not significantly different from the control ($p \geq 0.05$). Instead, at their higher concentration, a significant proliferative property near the value obtained for control was observed (96.69%).

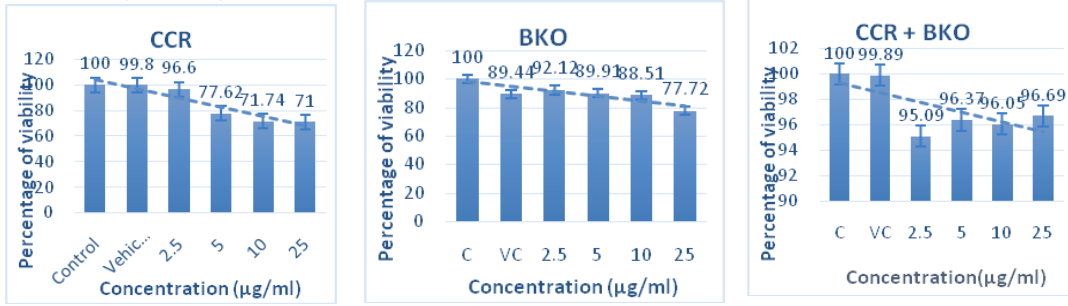

Figure 2: Graphs showing results of MTT assay

Estimation of the cell to cell interaction

The results from crystal violet staining showed a change in cell morphology. Reduction in density and size and elevated contact inhibition was observed in cells when treated with BKO and CCR separately in a dose-dependent manner. This provided evidence for less survivability of cells. Mesenchymal and epithelial cells were also observed. In the case of the combination, the administration of compounds at higher doses increased cell to cell contact attributing to cell survival and proliferation.

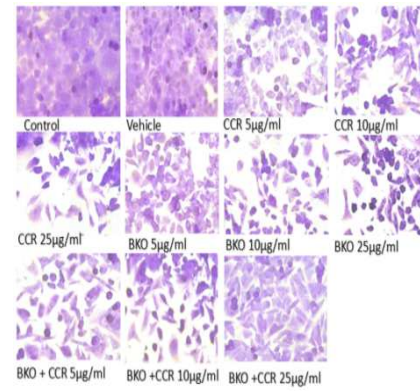

Figure 3: Microscopic image (10X) showing crystal violet stained SKBR3 cells

Estimation of cytotoxicity

During AO/EB staining, a dose-dependent increase in the number of late apoptotic or necrotic cells can be observed in cells treated with CCR and BKO when compared to control, which has a uniform green fluorescence with a negligible amount of dead cells emitting red fluorescence. The combination has a remarkable difference in their cytotoxicity where the ratio of dead to living cells was decreasing in a dose-dependent manner, which was comparable to the control.

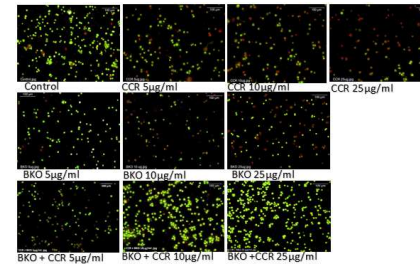

Figure 4: Microscopic image (10X) showing AO/EB stained SKBR3 cells

Discussion

By analyzing the DPPH assay results, the combination of CCR and BKO exhibited a significant antioxidant property than compounds when treated individually. The results depicted that the combination had elevated the free radical scavenging potential, reflecting a synergic effect on the two compounds' antioxidant nature. Previous works validate this finding [11, 12]. When comparing the antioxidant property of BKO with CCR and their combination, BKO showed the least antioxidant potential. This supports the report of El-Beshbishy *et al.*, 2009 that the ethanolic solution of curcumin showed more antioxidant potential than Black cumin seed extract. In the previous studies, Black cumin was reported to be an excellent antioxidant [14]. The composition of the oil and difference in the origin of the cell line can contribute to less anti-oxidant activity.The compounds' composition and quality dependon the source, method of extraction, geographical, and storage conditions [15].

Findings of MTT assay and cytotoxicity estimation provided a light towards the anticancerous property of BKO and curcumin. In contrast, the combination increases the proliferative property at higher concentrations. The anti-proliferative property of curcumin is reported in several previous studies. Curcumin provides protection against lipid and protein peroxidation and enhancing the performance of antioxidant enzymes such as SOD and glutathione peroxidase [16]. Curcumin reset the imbalance between ROS production and antioxidant activity and lowers the TNF-α and IL-1 expression [17].

Curcumin could induce cytotoxicity through apoptosis and modulate cell cycle regulating proteins thereby, lowering cell viability. Studies reported the ROS-mediated mitochondrial pathway of apoptosis [18] caused by curcumin, enhancing the Bax expression, and reducing Bcl-2 and Bcl-xL in small cell lung cancer cells NCI-H446 [19]. Curcumin regulating cell cycle with its antiproliferative property was demonstrated on breast cancer cell lines T47D, MCF7 [20].

Crystal violet staining and AO/EB dual staining showed cellular morphology changes, cell to cell contact, and detection and quantification of apoptotic cells, respectively. Thymoquinone is a monoterpene that is the predominant biologically active BK extract component in the essential and fixed oil [21]. The majority of the studies show that the TQ and its derivatives can cause cytotoxicity in cancerous cells and induce apoptosis resulting in a reduction in cell viability, angiogenesisinhibition, and antimetastasis [22]. Reduction in cell volume, chromatin condensation, and cell shrinkage are associated with apoptosis [23].TQ could hinder the incorporation of Thymidine in the DNA [24], induce telomere shortening [25], and increases the production of pro-apoptotic molecules, and decreases anti-apoptotic molecules in cancer cells thereby [26, 27]. The DNA damage caused by curcumin is demonstrated by Cao *et al.*, 2016.It is well-known that cell to cell contact is essential for cell survival. But in the present study, individual administration of the compound induced cell to cell contact inhibition.

The dose-dependent decrease in antiproliferation observed in co-treatment could be due to the enhanced cell arrest in tumor cells. This has provided ample time to modulate the DNA damage level and DNA repair to resume the cell cycle progression and protect the cells from death [29]. It is evident from the earlier studies that both curcumin and TQ can affect the cell cycle and alter the regulatory proteins, and induces a cell arrest at S and G2/M phase, preventing the damaged cell from entering into the M phase. Another suggestion for the observation is the remarkable anti-oxidant property exhibited by the combination. This may have altered the redox environment making them susceptible to ROS concentration changes and redox manipulation [30].The co-treatment has enhanced the contact resulting in proliferation. A similar result was observed in the study of combined formulation of TQ and curcumin on cisplatin-induced toxicity in HEK 293 cells [31].The co-treatment helped to provide the cells' conditions to proliferate and remain viable by exhibiting higher antioxidant

potential and providing enough time for DNA repair during the cell arrest as the applied concentration raised.Further studies are needed for strong validation of these findings.

Conclusion

In the present study of a preliminary evaluation of the properties of CCR, BKO, and their combination for its therapeutic application in cancer therapy, it is inferred that CCR and BKO have the potential anti-proliferation activity in breast cancer cells. This can be utilized as a chemotherapeutic by serving as a raw material for pharmaceutical industries. In the case of the combination, proliferative property suggests limited chemotherapy application. Nevertheless, the combination's high antioxidant activity can be exploited in regular cell lines. The investigation must be extended in normal cells to check for its carcinogenicity of co-treatment. But at this stage, there is not much scientific evidence for its effect on normal cells since it has not been assessed in the present study. Furthermore, research is required to have a deeper understanding of the anti-cancerous potential of individual compounds and proliferation potential of combination, bringing these fantastic compounds to the forefront of novel therapeutics.

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Cytogenetic profile of children with suspected genetic disorders

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Abstract

A chromosomal disorder, anomaly, aberration, or mutation is a missing, extra or irregular portion of chromosomal DNA. It can be from an atypical number of chromosomes or a structural abnormality in one or more chromosomes. The blood samples were collected from the genetic lab and undergo the PBLC using 5 mL RPMI, 0.6 mL FBS and 0.2 mL PHA and 0.5 mL of peripheral venous blood then incubated at 37! for 69 hours. At 67th hour arrest the reaction for getting metaphase stage then harvesting at 69th hour and the suspension slide and undergo GTG banding. Then the slides were air dried Karyotyping was performed from the stained slides by capturing 20 well spread metaphases using spectral imaging software.Chromosome analysis was performed at CIMAR. Metaphase plates analysed and karyotyped. Out of 10 children five of them shows normal karyotype. Thus, most of the commonly observed chromosome abnormalities was seen; 2 children tested positive for DS & rest 3 each tested as ES, TS and 1 Cri du chat syndrome.

Keywords: Chromosomal disorder, Genetic diseases, Karyotyping

Introduction

Chromosomal abnormalities, alterations and aberrations are at the root of many inherited diseases and traits. Chromosomal abnormalities often give rise to birth defects and congenital conditions that may develop during an individual's lifetime. Examining the karyotype of chromosomes in a sample of cells can allow detection of a chromosomal abnormality. Chromosome abnormalities usually happens as a result of an error in cell division (mitosis & meiosis). If a woman is 35 years old, the eggs in the ovaries are also 35 years old. Errors in meiosis may be more prone to happen as a result of the aging process. Men, on the other hand, produce new sperm continually. There is, therefore, no increased risk for chromosome abnormalities to occur based on the age of the father. Numerical abnormalities are whole chromosomes either missing or extra to the normal pair. Structural abnormalities are when the part of an individual chromosome is missing, extra, switched to another chromosome or turned upside down. Most people with aneuploidy have trisomy instead of monosomy.DS is probably the most well-known example of a chromosomal aneuploidy.

Down's syndrome: First discovered by John Langdon Down in 1866. 95% of Down's syndrome is caused by non-disjunction with the error being predominantly in meiosis I. The syndrome is characterised by mild or moderate intellectual disability. Some individuals are

also reported to have a congenital heart diseases, high risk of acute leukaemia and susceptibility to respiratory infection.

Edward's syndrome: 90% of infants with this syndrome have complete trisomy and a small percentage this is an autosomal lethal mutation, where children usually die shortly of the birth. A new born child will exhibit developmental abnormalities.

Patau's syndrome: It's an autosomal abnormality caused by the trisomy of chromosome 13. Patau's syndrome is a lethal condition because multiple birth defects of brain, heart and other vital organs so the babies survive only for few months. Mortality rate is very high during the first three month of life.

Klinefelter's syndrome: This sex chromosome aneuploidy was first described by Harry Klinefelter in 1942 and is caused because of an extra X chromosome in a male. The affected individual is sterile with tall but narrow shoulders, broad hips, small or immature testes and no production of sperm & having gynaecomastia.

Turner's syndrome: It's a sex chromosomal monosomy found in females and is first discovered by Henry Turner and his team in 1938.The genetic reason for causing this syndrome is the non-disjunction of X chromosome during gametogenesis. Individuals are characterised by absence or reduced secondary sexual characters.

Cri du chat syndrome: It's an autosomal abnormality caused by a rarely occurring deletion mutation; first reported in 1963. Mainly it characterised by severe physical and mental retardation, congenital heart diseases, a condition of microcephaly etc. The more curious factor that the affected child cry exactly similar to the crying sound of a cat and very weak. Hence the syndrome is also known as the "Cat cry syndrome".

Materials and methods

Study population based on chromosome analysis was performed at the Centre. A detailed history was taken on a pre-designed proforma to include pedigree and clinical history.

Blood Collection: About 5ml of heparinized peripheral venous blood sample was collected from each patient aseptically and each sample was given a unique laboratory number.

Peripheral Blood Lymphocyte Culture using a modified Moorhead and Hungerford (1960) protocol by this method

Take 5ml of culture medium (RPMI 1640) pH 7, in a centrifuge tube and added 0.6ml Foetal Bovine Serum (FBS) and 0.2ml phytohemagglutinin (PHA) and add 0.5ml peripheral venous blood into it; mixed well and the culture was incubated in the water bath at 37 for 69 hours. For the mitotic cell arrest, at 67th hour of incubation, 50 µl colcemid was added to arrest the cell division at metaphase stage; and again incubated it for 2 hours. For harvesting the arrested lymphocytes, at 69th hour of incubation the cell suspensions were centrifuged for 10 minutes at 1600rpm, the supernatant was discarded, and pellet was treated with 5ml hypotonic solution (0.56% KCl) then incubated for 15 minutes. Following the incubation, the samples were

centrifuged carefully at 1600 rpm for 8 minutes and fixed by using fixative (methanol and acetic acid in the ratio 3:1). At the beginning the fixative was wisely added and made up to a volume of 5ml and mixed well by using vortex mixture and make up to 9ml and then incubated it for 20 minutes at room temperature. After this, suspension was centrifuged at 1600 rpm for 10 minutes. Then tubes were kept in refrigerator overnight. The next day, after centrifugation at 1600 rpm for 10 minutes, the supernatant was discarded and the pellet was used for making the slides. The slides were prepared by air drop method. The slides were placed in hot air oven for 1 hour for ageing for GTG banding.

GTG Banding (G – Bands by Trypsin using Giemsa) After 1 hour of ageing the slides were treated with PBS and then trypsin (0.032g in 600ml PBS) and the slides were placed in Giemsa stain for 1.30 minutes. Then the slides were air dried Karyotyping was performed from the stained slides by capturing 20 metaphases using spectral imaging software.

Result

A total of 10 children who were suspected of a chromosome abnormality were included in this study. Out of 10 children five of them shows normal karyotype. The results have been tabulated in Table 1.Thus, most of the commonly observed chromosome abnormalities was seen; 2 children tested positive for Down's syndrome, 1 for Edward's syndrome, 1 for Turner's syndrome and 1 for Cri du chat syndrome. Representative images of the cases have been presented in Figures. Klinefelter's syndrome is rarely detected during the childhood because its symptoms are visible during puberty. Other syndromes such as Edward's syndrome and Patau's syndrome is also rare because most of cases during the gestational period, termination takes place.

Case number	Age (years)	Karyotype	Diagnosis
1	8	46,XX	Normal
2	6	46,XY	Normal
3	7	46,XX	Normal
4	5	46,XX	Normal
5	2	46,XY	Normal
6	3	47,XY+21	Down's syndrome
7	2	47,XY+21	Down's syndrome
8	1	47,XY+18	Edward's syndrome
9	2	46,XX,5p-	Cri-du-chat syndrome
10	4	45,X	Turner's syndrome

Table 1 : Tabulation of the cytogenetic results of children suspected of a chromosome abnormality.

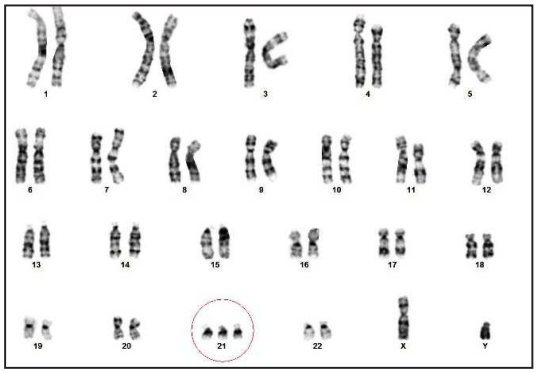

Figure 1: Karyotype of case 6: 47, XY,+21

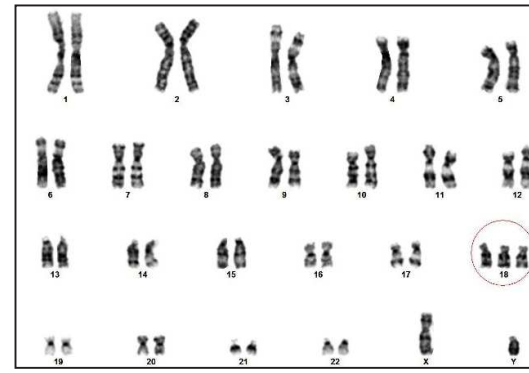

Figure 2: Karyotype of case 8:47,XY,+18

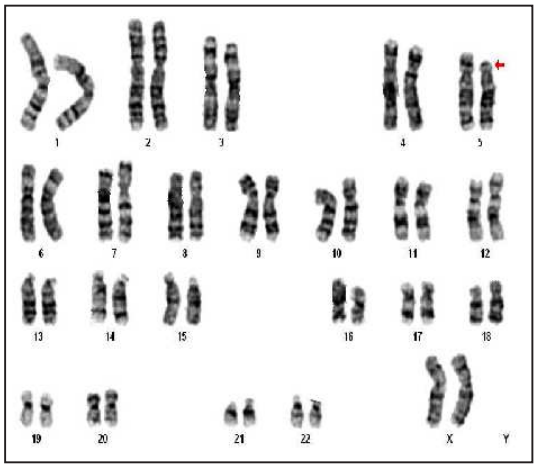

Figure 3 : Karyotype of case 9:46,XX,5p-

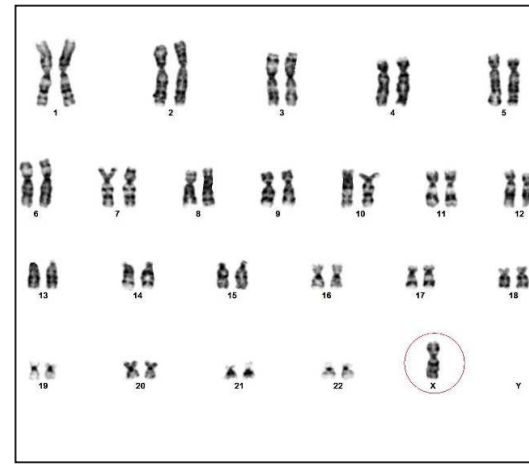

Figure 4: Karyotype of case 10: 45,X

Discussion

Cytogenetic analysis is a valuable investigation in the diagnostic work up of children with suspected chromosomal disorders. Chromosomal disorders may arise from either numerical and or structural changes in the autosomes or sex chromosomes. So far, approximately 1000 chromosome syndromes have been reported. Cytogenetic analysis is an essential component in the diagnosis and evaluation of children with various congenital abnormalities, dysmorphic features, developmental delay and intellectual disability. The frequency of major chromosomal abnormalities is estimated to be between 1 in 150 to 1 in 200 live births. According to Worton et al. (1977) surveys conducted among healthy adult populations have found lower frequencies of chromosomal abnormalities (probably as a result of the high mortality among affected neonates and infants who fail to survive into adulthood). Studies found a wide range of chromosomal aberrations in children with suspected chromosomal disorders who were referred for cytogenetic analysis.

In this study, DS was the commonest autosomal aneuploidy and the most frequent reason for cytogenetic analysis, and the majority of such patients were karyotypically confirmed as having DS. The findings in this study are in agreement with a previous study conducted by Jayasekara in 1988 on the spectrum of chromosome anomalies seen at the Human Genetics Unit, Faculty of Medicine, and Colombo. He reported a high proportion (76.3%) of Down syndrome cases among the 76 patients with chromosome anomalies. Among the DS children, there was a male predominance (58.6% males versus 41.4% females). In a meta-analysis of data from 55 independent studies, Kovaleva et al. (2002) reported similar male preponderance among DS patients.DS due to mosaicism (10.8%) was found to be higher than due to Robertsonian translocations between chromosome 21 and the acrocentric chromosomes (5 %). In the study, the frequency of pure trisomy, mosaicism and translocation was 87.9%, 7.7% and 4.4% respectively. Translocation carriers have a high risk of aneuploid offspring with every pregnancy, the recurrence risk depends on the sex of the carrier parents and the chromosomes that are fused. If one of the parents is the carrier of a balanced translocation involving the two chromosome 21s, the recurrence risk for DS is 100%.

TS was the commonest sex chromosomal aneuploidy accounting for 9.6% of cases and 6.4% of them were karyotypically confirmed as having TS. TS usually results from total or partial absence of one of the two X chromosomes normally present in females. It may also result from a structurally abnormal X chromosome in which deletion or duplication of genetic material has occurred. TS is commonly diagnosed at puberty because of failure of sexual maturation resulting from ovarian dysgenesis. However, an increasing number of patients are now being recognized during infancy and childhood because of clinicians' increased awareness of other stigmata such as peripheral lymphedema, growth failure etc. TS variants (54.0%) were found to be commoner than the classic 45, X karyotype (46.0%). Various mosaic forms in association with a 45, X cell line were the commonest TS variants seen in 50% of cases.

A recent study found chromosomal abnormalities in 13.6% of 568 children with intellectual disability, dysmorphic features, congenital anomalies and developmental delay. In this study, 4 children were diagnosed with a terminal deletion of 5p characteristic of Cri-du-chat syndrome [del(5)(p15.2pter)], 1 child with Wolf-Hirschhorn syndrome [del(4)(p15.3pter)], 1 with Jacobsen syndrome [del(11)(q23.2qter)] and another child suspected with Angelman syndrome [del(15)(q11.2q12)] based on their clinical features. Yashwanth et al. (2010) found that evaluation of chromosomal abnormalities is important in understanding the underlying etiology of congenital malformations and intellectual disability. However, clinical diagnosis with molecular cytogenetic techniques (fluorescence in situ hybridization (FISH) and micro-array) in such patients could be improved.

Conclusion

A variety of chromosomal abnormalities were identified in children undergoing cytogenetic analysis. This demonstrates the importance of cytogenetic evaluation in children with various congenital abnormalities, dysmorphic features, developmental delay and/or intellectual disability. The types of chromosomal abnormalities identified in this study were similar to those found in other studies. Down syndrome is the most prevalent aneuploidy compared to other syndromes. From this studies Down syndrome is most prominent in male compare to the females. Turner syndrome is common sex chromosomal abnormality because it can be identified through other stigmata's in childhood. Other syndromes are rare compare to the Down syndrome.

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Antihyperlipidemic effects of Lemon Leaves (*Citrus limon* L.) in Alloxan induced diabetic mice

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Abstract

Diabetes mellitus is one of the most chronic progressive disease which caused by the impairment of insulin production by pancreatic â cells or by peripheral insulin resistance. Insulin resistance can alter systemic lipid metabolism which then leads to dyslipidemia development. Hyperlipidemia is the most hazardous factor in coronary heart disease. Since the beginning of man on earth, Nature is seen as a source of medicinal agents for many years. Citrus is the main medicinal plant of the Rutaceae family. *Citrus limon* has well known nutritional and medicinal properties. The present study was carried out to determine the presence of antihyperlipidemic activity of methanolic extract of *C. limon* leaves in alloxan (alloxan 70 mg/kg b.w.) induced diabetic mice. After 72 hrs Alloxan induction, diabetic mice received a methanolic extract of *C. limon* leaves orally at a dose of 100 and 200 mg/kg body weight daily for 28 days. A significant reduction in total cholesterol, triglycerides (TG), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL) and increase in High-density lipoprotein (HDL) were observed after 28 days of treatment of methanolic extract of *C. limon* leaves. It was observed that the methanolic extract of *C. limon* leaves possess good antihyperlipidemic effect.

Keywords: Diabetes mellitus, Hyperlipidemia, Cholesterol, *Citrus limon.*

Introduction

Diabetes mellitus is a comprehensive and multifactorial metabolic syndrome, characterized by a disturbance in insulin secretion and insulin receptor or post-receptor events with abnormalities in the metabolism of carbohydrate, protein, and lipid and results in chronic hyperglycemia (American Diabetes Association, 2010). Hyperglycemia and hyperlipidemia is the most common features of diabetes mellitus, contribute to the microvascular and microvascular developments in diabetic complications, which account for the mortality and morbidity of diabetes (Taskinen, 2002).WHO reported, it is estimated that 3% of the world's population suffer from diabetes and the prevalence of the disease is expected to double (6.3%) by the year 2025. The drugs used are restricted by the pharmacokinetic properties, secondary failure rates, and accompanying side effects. Thus a new class of compounds is necessary to get rid of diabetes problems. (Sabjan KB, 2012). Raised cholesterol level in the blood is due to abnormalities in lipoprotein level, the particles that transfer cholesterol into the bloodstream. This may be associated with diet, genetic factors (such as LDL receptor mutations in familial hypercholesterolemia), and the presence of other types of diseases such as diabetes and an underactive thyroid (Durrington et al., 2003). The important characters

of diabetes mellitus are hyperglycemia and hyperlipidemia (Sheetz, 2002).Hyperlipidemia is a state when abnormally high levels of lipids (fatty substances) are found in the blood. Many plants derivative and home remedies have been screened for the action of hypolipidemia. (Kumar et al., 2013).*Citrus limon* have well-known nutritional and medicinal properties. The whole part of *citrus limon* is used as traditional medicine. Citrus lemon belongs to the family rutaceae (Chaturvedi, et al., 2016). In this study, we evaluated the antihyperlipidemic effects of methanolic extract of lemon leaves at 2 doses (100, 200 mg/kg) in the diabetic mice.

Material and Methods

Chemicals

Alloxan used in the study was procured from SRL (Sisco Research Laboratories Pvt. Ltd. Chennai, India). The standard drug used in this experiment was Glibenclamide. The other drug and chemicals used were of analytical grade and Distilled water and normal saline also were used in this study.

Preparation of coarse powder and methanolic extract of *Citrus limon* leaves

Fresh *C. limon* leaves were collected locally and authenticated from the (No. RUBL211732) Department of Botany, Rajasthan University, Jaipur. These leaves were dried completely shed and ground with an electric grinder into coarse powder and stored in an airtight container. The extraction was carried out by the soxhlet apparatus (hot percolation method). The used solvent was methanol. About 50 gm of lemon leaves powder was extracted with 300 ml of methanol. The extract was concentrated to dryness under controlled temperature 40-50°C. The extract was stored in an airtight container and preserved in the refrigerator till further use.

Animals

Normal healthy weight of 20-50g of both (male and female) sex mice were obtained from CPCSEA registered animal house of Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan. The mice were housed in the cages at a constant temperature of 23^0C ± 1 and 12 hrs light/ dark cycle with free access to water and food.

Alloxan induced diabetic rats

For inducing diabetes, a single intraperitoneal (i.p) injection of 70 mg/kg body weight of alloxan monohydrate dissolved in saline to overnight-fasted mice (Aruna et al., 1999). After three days of injection, blood glucose level was monitored by a glucometer and mice with blood glucose level more than 250 mg/dl were considered as diabetic.

Treatment groups

Mice were randomly divided into 5 groups (n=6). Group 1 or normal control (NC) group received only normal saline via gavage. Group 2 or diabetic control (DC) group received

only one i.p injection of alloxan monohydrate. Group 3 (GB5) firstly received i.p injection of alloxan monohydrate and then was daily treated with drug Glibenclamide orally via gavage, Group 4 (CLM100) and group 5 (CLM200) firstly received i.p injection of alloxan monohydrate and then were daily treated with 100 and 200 mg/kg of extract respectively orally via gavage for 28 days.

Biochemical Analysis

Blood samples were collected from the heart and blood glucose levels were estimated using a one-touch glucometer (Accu-chek® Active Glucometer, Roche Diagnostic Corporation, Germany). Serum samples collected at the end of dosing were utilized for assessment of lipid profile using standard reagent kits. All the values were expressed as the mean and SD. Analysis of variance was performed and p values were observed. Values of $p<0.05$ were considered significant.

Results and Discussion

S.N.	Groups	Cholesterol Level (mg/dl)	Triglycerides Level (mg/dl)	HDL Level (mg/dl)	LDL Level (mg/dl)	VLDL Level (mg/dl)
1.	**NC**	68.46±1.04	67.54±1.38	39.25±1.2429	15.71±1.39	13.51±0.27
2.	**DC**	122.07±9.56	132.25±6.83	25.26±2.01	70.25±7.280	26.45±1.36
3.	**GB5**	89.92±3.17	76.26±2.31	43.22±1.66	31.61±4.003	15.308±0.45
4.	**CLM100**	96.75±1.41	66.495±2.07	37.99±1.23	45.46±1.67	13.29±0.42
5.	**CLM200**	93.16±1.77	86.65±1.817	40.51±2.37	35.23±2.35	17.33±0.36

*Results are presented as mean ± SD; No. of mice =6 in each group, Significance level considered as $p<0.05$

The alternation of the serum lipids level is one of the diabetes symptoms. There was a significant (PÂ0.05) increase in cholesterol, triglyceride, LDL, VLDL and decrease of HDL (figure1) in the alloxan-induced diabetic group compared with the normal control group.

Table1:Lipid profile level (mg/dl) after treated with methanolic leaf extract of *C limon*
Effect of methanolic *C. limon* leaves extract on cholesterol and triglyceride in diabetic mice

The results (figure 1 and table 1) showed a significant increase in cholesterol and triglyceride levels in the alloxan-induced diabetic group compared with the normal control group, treated the mice with methanolic lemon leaves extracts at concentration 100 and 200 mg/kg of body

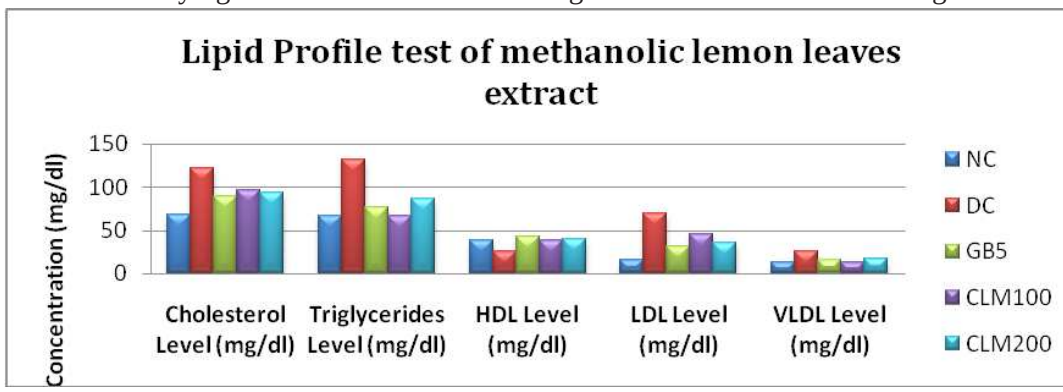
weight for 28 days, the results showed a significant reduction in Cholesterol, Triglyceride levels compared with diabetic control (DC) group.

Figure1. Effect of methanolic *C. limon* leaves extract on cholesterol, triglyceride, HDL, LDL and VLDL in diabetic mice

Effect of methanolic *C. limon* leaves extract on HDL, LDL, and VLDL levels in diabetic mice

The results in figure 1 showed a significant increase in levels of LDL and VLDL in alloxan treated group compared with the Normal control group, Treated the diabeticmice groups with methanolic lemon leaves extract at concentration 100 and 200 mg/kg of body weight for 28 days showed a significant decrease in levels of LDL and VLDL compared with DC group, and a significant reduction in level of (HDL) in alloxan treated groups compared with the control, Diabetic mice treated with the methanolic lemon leaves extract at concentration 100 and 200 mg/kg of body weight for 28 days showed a significant enhancement in HDL level compared with DC group.

Alloxan destroying beta cells in the islets of Langerhans cause a massive shortage of insulin



secretion. The absolute shortage of insulin in animals results in metabolic changes including increasing the level of glucose and cholesterol in the blood (Sreelatha and Inbavalli, 2012 and Rathnakar et al., 2011).ECl seed showed the changes that occur in levels due to the presence of hesperidin, it is the most important flavanone of Citrus sp., significantly increased HDL and significantly reduced cholesterol, LDL, and triglyceride plasma levels (Monforte et al.,1995).The juice of the citrus lemon declared a significant reduction in serum cholesterol, triglycerides, and LDL levels that resulted in an increase in HDL levels. These findings suggested the hypocholesterolemic effects of citrus lemon juice may be due to antioxidant effects (Khan et al., 2010).*Citrus limon* juice significantly reduced cholesterol and LDL levels, so it can be concluded that citrus lemon can prevent atherosclerosis and significantly increased serum HDL levels (Stein, 1999).

Conclusion

The results of this study indicated that 2 different doses of methanolic lemon leaves extract were effective on reducing blood cholesterol, triglycerides, LDL and VLDL and effectively increase the HDL level in diabetic mice.

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Population dynamics of mosquitoes in various breeding habitats at Vilvattom area, Thrissur district

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Abstract

Mosquitoes are one of the deadliest animals in the world with their ability to carry and spread disease to humans causes millions of death every year. Many species of mosquitoes inject or ingest disease causing organisms with a bite and thus becomes a vector for the transmission of diseases such as Malaria, Yellow fever, Chikunguniya, Dengue fever, Zika virus and other Arbo viruses. Most commonly there are 3 species of mosquitoes in Kerala and they are *Aedes, Anopheles, Culex*.The study area selected was Vilvattom Village in Thrissur District.As this area was reported as the dengue hit area during the rainy season in 2014 the main focus was on the *Aedes* species (*Aedes aegypti* and *Aedes albopictus*) causative agent of Dengue fever. Main methods adopted for getting the density of mosquitoes was by conducting surveys in the selected area and calculating the House Index and Container Index during the summer and rainy seasons of 2018, finally taking a comparison between both indices. Mosquito larvas were collected from their natural habitat from the surveyed area and larval analyses were conducted in the presence of District Malaria Officer. This study helps in the identification of mosquito larva, to calculate their relative density during various seasons and also to spread awareness in people about the significance of keeping their house premises free of mosquito breeding finally eradicate mosquitoes and bring a healthy society.

Keywords: Aedes mosquito, Anopheles Mosquito, Culex Mosquito, Vectors, Proboscis, Mosquito Larva, House Index, Container Index

Introduction

Mosquitoes are one of the deadliest animals in the world [1]. Their ability to carry and spread disease to humans causes millions of death every year. In 2015, Malaria alone caused 4, 38,000 deaths. The worldwide incidence of dengue has risen 30-fold in the past 30 years, and more countries are reporting their first outbreaks of the disease. Sustained mosquito control efforts are important to prevent outbreaks from these diseases. The world has been witnessing theemergence and re-emergence of an array of 'mosquito-borne diseases' in the recent decades. [2] Mosquitoes are a group of about 3500 species of small insects that belongs to the order *Diptera*. Within that order they constitute the family *Culicidae*.[3] The life cycle consists of the egg, larvae, pupae and adult. Eggs are laid on the water surface; they hatch into motile larvae which feed on aquatic algae and organic material; pupae are breathing non-flying primitive adults. Mosquitoes are very distinct from other members of Nematocera in having their long proboscis [4]. Females of most species have tube-like mouthparts (called

a proboscis) which can pierce the skin of the host (colloquially but incorrectly referred to as a "bite") in order to extract blood which contains protein and iron needed to produce egg. The saliva of the mosquito transmitted to the host with the bite can cause itching and a rash. In addition, many species of mosquitoes inject or ingest (or both) disease-causing organisms with the bite and are thus a vector for the transmission of diseases such as malaria, yellow fever, Chikungunya , West Nile virus, dengue fever, filariasis, Zika virus and other arboviruses. Different mosquito species belonging to genera *Culex, Aedes* and *Anopheles* serves as vectors for many diseases. They are considered as most significant vectors for various diseases because of their abundance, vector capability, recurrent infection and diversity [5].Kerala state is highly vulnerable to vector born diseases because of conductive temperature throughout the year significant annual rainfall and presence of many sources are responsible for breeding of mosquitoes.

Scope and Significance

To find the relative density of different species at different seasons and to formulate, control strategies. To spreadawareness in people about the significance of keeping their house premises free of mosquito breeding finally eradicate mosquitoes and bring a healthy society.

Materials and Methods

Mosquito larval survey was organized in association with the Primary Health Center, Vilvattom and District Medical Office, Thrissur. The group members conducted a survey in the 7_{th} division of Thrissur Corporation which was reported as the dengue hit area during the rainy season in 2014. The survey was done during the summer and rainy seasons of 2018. The program was officially inaugurated at Public Health Centre, Vilvattom followed by a discussion regarding challenging issue of spreading Dengue Fever in connection with the following monsoon season in the selected area. The survey covered 136 houses of the selected area. The houses were surveyed for the detection of vectors, their breeding places and existing conditions having the potential to breed mosquitoes. Larvae were collected from their natural breeding habitat using glass beakers and pipette from open sources and tree holes. Small containers were emptied in to plastic bottle. The number and type of positive containers as well as the number of water filled containers are inspected and recorded. Collections from each site were maintained separately in suitable containers. In the lab, larvae were kept in beakers covered with a mosquito net and after few days, some of them pupated and emerged as adults. Details of survey were recorded and analyzed.

Awareness Programme

Group members conducted an awareness program in connection with the survey. The house members were made aware of the mode of transmission of Dengue Fever and the significance of keeping their house premises free of mosquito breeding places.

Larval and adult identification

Larva and adults were identified according to the apparent morphological features with the help of a hand lens and using standard keys and catalogues [6.] Identification was done under

the guidance of Dr.Sasi Kumar, Malaria officer, District Medical Office (H), Thrissur. In the larval survey, various indices were used to record the density of mosquito species. The indices were House index (HI) and Container index (CI).

House index (HI)

= Percentage of houses positive for Mosquito larvae

HI= Number of house positive for Mosquito larvae ×100
No. of houses inspected

Container index (CI)

= Percentage of water holding containers positive for Mosquito larvae.

CI = No. of positive containers for mosquito larvae×100
No of containers inspected

Result

Table 1: Mosquitoes recovered from various temporary breeding habitats during Rainy season.

Rainy season	Plastic	Tin	Discarded container	Mud pot	Tire	Tank	TOTAL
June	4	Nil	3	2	2	Nil	11
July	3	Nil	5	4	2	1	15
August	2	Nil	1	Nil	Nil	Nil	3

Table 2: Container index of mosquitoes during rainy season.

Month	Total container checked	Positive container	Container index
June	248	11	4.43%
July	83	15	18.07%
August	40	3	7.5%

Table 3: House index of mosquitoes during rainy season.

Month	Total houses checked	Positive houses	House index
June	11	2	18.18%
July	10	1	10%
August	12	1	8.33%

Table 4: Mosquitoes recovered from various breeding habitats during summer season.

Summer season	Plastic	Tin	Discarded containers	Mud pot	Tire	Tank	Total
January	Nil	Nil	1	Nil	Nil	Nil	1
February	1	1	Nil	Nil	Nil	Nil	2

Table 5: Container index of mosquitoes during summer season.

Month	Total containerschecked	PositiveContainer	Containerindex
January	63	1	1.58%
February	60	2	3.33%

Table 6 : House index of mosquitoes during summer season.

Month	Total houses checked	PositivesHouses	House index
January	52	1	1.92%
February	51	2	3.92%

Table 7: Comparison of container index and House index during rainy season and summer season.

	Rainy season			**Summer season**	
	June	**July**	**August**	**January**	**February**
Container Index	4.43%	18.07%	7.5%	1.58%	3.33%
House Index	18.18%	10%	8.33%	1.92%	3.92%

Discussion

Mosquito control manages the population of mosquitoes to reduce their damage to human health and economy. This survey collected mosquitoes during rainy season and summer season along with the identification of different mosquito species. During rainy season, plastic containers turned out to be the most preferred breeding ground followed by discarded containers whereas, in summer season, plastic containers along with the metal tins and discarded containers were the perfect breeding grounds. But, the figures were lesser than the rainy season.

Apart from the study of breeding grounds, different species of mosquito larvae were also collected to identify the premature larval stages. Three species belonging to three different genera *Aedes*,*Anopheles* and *Culex* were observed during study. The species were identified

on the basis of an awareness class conducted by the zoology department, Vimala College Thrissur. A survey was conducted before the commencement of the project. During the course of survey, mosquito larvae from various containers were collected in our zoology lab. The mosquito egg hatches and becomes a larva. The larva has a well-developed head with mouth brushes for feeding, a large thorax with no legs and a segmented abdomen. Larvae usually breathe through spiracles located on their eight abdominal segments or through a siphon. They usually feed on algae, bacteria and other microbes [7]. *Culex* larvae are bottom feeders. During air intake, their head hangs downward at an angle with water surface. Palmate hairs are absent in abdomen [8]. Their respiratory siphon is long and placed on the eighth abdominal segment [9]. *Anopheles* larvae are surface feeders. During air intake, their head lies horizontally parallel to the water surface [10]. Palmate hairs are very small or absent in abdomen [8].Their respiratory siphon is very short. They either swim through propulsion with their mouth brushes or by jerky movements of their entire body. *Aedes* larvae are bottom feeders with no palmate hairs in abdomen. Their head hangs vertically downwards from the water surface. Their respiratory siphon is thick and short [8].The larvae then, grow into pupa. They are comma shaped. The head and thorax together merge into a cephalothorax, with the abdomen curving around underneath. After few days, depending on the temperature and other factors, the pupa rises to the water surface, the dorsal surface of its cephalothorax splits, and the adult mosquito emerges out.

After the larval identification, these larvae were reared in our zoologylab. Within one week, the larva grows into mosquito based on their external morphology. *Anopheles* can be easily identified by the typical resting position i.e., abdomen sticking up in the air making an angle of 45Ú with the surface. Their wings carry dark spots. *Aedes* has a black and white striped body with a white lyre on her thorax with two yellow chords in the centre. It rests with abdomen parallel to the surface making a slight hump. *Culex* are mostly brown or grey with stout body, unspotted wings and sitting posture parallel to the surface. *Culex* lays around 200 to 400 eggs in a cluster, in stagnant water such as polluted ponds, ditches and tanks. She arranges the eggs by her hind legs, which are cemented together to form a small floating raft. The eggs of Anopheles are cigar shaped with floats down their sides. *Aedes* generally drop their eggs singly on damp mud or other surfaces near the water edges. Such an oviposition site is commonly hollow stumps or discarded containers [10].

After identification, we calculated the container indices of both the seasons. The container index is higher in rainy season (30%) rather than summer season (4.91%). Similarly, house index was also calculated. It shows that rainy season has higher house index (36.51%) compared to summer season (5.84%), [Table 7].

The larval stages are largely aquatic. These stages last from 4 to 14 days depending on the type of species and temperature. During summer mosquitoes undergo a diapauses stage due

to which there is a delay in their development. This contributes to their population decline in summer as compared to the rainy season.

Conclusion

Mosquitoes are not just a menace; they can also cause health hazards to human beings. At the same time it is a carrier of many diseases. Mosquitoes breed mostly in plastic containers, also found in tins, can, buckets, discarded tires that holds stagnant water. *Culex, Anopheles* and *Aedes* were also collected from various containers. The *Aedes* have higher density, than other groups of mosquitoes. House and containers indices were noted. Rainy season have higher container and house indices compared to summer season. This survey has given more information about the occurrence and frequency of mosquito affected in that area so that required control measure can be adopted to safeguard that particular area.

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Protective effect of non-fermented coconut inflorescence sap on cyclophosphamide induced hepatotoxicity in mice

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Abstract

Neera, a non-fermented sap (NFCS) from unopened inflorescence of *Cocos nucifera* L is a well-known traditional natural beverage. This sweet sap havehigh nutritional value and its composition contains sugars, phytochemicals, electrolytes, proteins and vitamins is promising. But, its pharmacological properties are not well studied.Hence, present study assesses the effect of NFCSoncyclophosphamide (CTX) induced hepatotoxicity in mice. Swiss albino mice were treated with different doses of NFCS (250 and 500 mg/kg b.wt.) for 10 days. Cyclophosphamide (CTX, 25 mg/kg b.wt.) given orally for 10 days to induce hepatotoxicity and animals were maintained for 20 days. The hepatic function parameters, oxido-reduction status and histopathological analysis of liver tissue was done. The CTX-induced hike in thiobarbituric acid reactive substances (TBARS) and liver marker enzymes in mice was marginally decreased by administration of NFCS. Additionally, the activities of antioxidant enzymes including catalase, superoxide dismutase and glutathione peroxidase, together with cellular glutathione level, were found to be improved in these animals. Histopathological analysis of liver suggests that NFCS could reduce the CTX-induced damage. This study revealed the efficacy of NFCS to ameliorate the cyclophosphamide induced hepatotoxicity attributed through its free radical scavenging activities. The use of NFCS may prevent the oxidative stress mediated degenerative ailments.

Keywords: Hepatotoxicity; antioxidants; natural drink; polyphenols; oxidative stress

Introduction

Nowadays, a number of chemotherapeutic agents are used for the treatment of various types of cancers. Unfortunately after the complete reduction of tumours, chemotherapy cannot achieve an adequate therapeutic outcome due to its numerous side effects at therapeutically effective doses. Cyclophosphamide (CTX) is a well-known antineoplastic agent belong to nitrogen mustard subclass mainly used for the treatment of various human malignancies (Shathish et al., 2012). Recently, side effects including immunosuppression, myelosuppression, anemia, cardiotoxicity, hepatotoxicity and renal toxicity are reported to be associated with this alkylating agent which restricted its use (Nair et al., 2016). The secondary metabolites (phosphoramide mustard and acrolein) of CTX generates free radicals which interfere with the antioxidant defense system of body. In recent days, supplementations

of nutrient rich dietary agents with free radical scavenging properties have reported to be beneficial in ameliorating the toxic effects of chemotherapeutic drugs (Jose et al., 2017).

Non-fermented coconut inflorescence sap (NFCS) or *Neera* is one of natural soft drinks, being traditionally tapped from unopened spadix of *Cocos nucifera*. This sap contains sugars, protein, fat, vitamins, electrolytes and phytonutrients. In traditional medicine, this sap is used in the ailment of various diseases due to its high rejunation and detoxification effect (Asha et al., 2019). Additionally, the richness of polyphenols and vitamin C provides antioxidant efficacy to this natural beverage (Silpa et al., 2020). Even though, it is considered as a nutritional drink, a few scientific studies are carried out to reveal its health beneficial efficacy. From these aspects, the present study aimed to evaluate the efficacy of NFCS on alleviating hepatotoxicity induced by CTX in mice.

Materials and methods

Chemicals

Nitroblue tetreazolium (NBT), 5,5' dithiobis (2-nitrobenzoic acid) (DTNB), reduced glutathione (GSH), thiobarbituric acid (TBA) and riboflavin were purchased from Sisco Research Laboratories Pvt Ltd, Mumbai, India. The kits for estimating various biochemical parameters were obtained from Agappe Diagnostics, India. All other chemicals and reagents used were of analytical grade and acquired from reputed Indian manufactures.

Sample collection

The fresh non-fermented coconut inflorescence sap (NFCS) was collected from coconut spadix by traditional tapping method, filtered and immediately transferred to an autoclaved container. In order to avoid microbial contamination kept the sap in deep freezer (-80^{o}C).

Animals

Male Swiss albino mice (25 – 30g) were purchased from Small Animal Breeding Station, Kerala Veterinary and Animal Science University, Thrissur, Kerala. The animals were maintained under standardized environmental conditions of light and humidity and provided with standard diet (Sai Durga Feeds and Foods, Bangalore, India) and water *ad libitum*. In this study, animal experiments were carried out with the prior consent from Institutional Animal Ethics Committee (IAEC/ACRC/16-12/17 dated 19/12/2016) and were strictly following the guidelines of IAEC.

Experimental plan

Forty four male Swiss albino mice (25-30 g) were divided into six groups (n=6). Group I was kept as normal without any treatment and group II as control (CTX 25 mg/kg b.wt.). Animals from group III and IV were administered NFCS orally at the dose of 250 and 500 mg/kg b.wt., respectively for ten days. To induce hepatotoxicity, cyclophosphamide (CTX, 25 mg/kg b.wt.) was given to all animals except group I for ten days after six hrs of drug administration (Pratheeshkumar and Kuttan, 2010). All the animals were maintained for 20 days and euthanized on 21st day. Blood was collected by cardiac puncture, serum was separated

and activities of liver function marker enzymes like serum glutamate oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphate (ALP) and total protein were determined using kits of Agappe Diagnostics. Liver was dissected out, washed with ice cold saline (0.9%) to remove blood contaminants if any. Then homogenate (10%) was prepared with 0.1 M tris-HCl (pH 7.4) for the estimation of antioxidant enzymes such as superoxide dismutase (SOD) (McCord and Fridovich, 1969), catalase (CAT) (Beers and Sizer, 1952) and glutathione peroxidase (GPx) (Hafeman et al., 1974). Additionally, the level of GSH was measured at 412 nm using DTNB (Moron et al., 1979). The level of TBARs, an oxidative stress marker was also measured at 532 nm (Ohkawa et al., 1979). A small portion of liver from each group was taken and fixed in 10% neutral buffered formalin solution. Then the sections with a thickness of 4 μm were taken, deparaffinized, hydrated and stained using haematoxylin–eosin. The sections were examined for the extent of tissue damages.

Statistical analysis

The values are expressed as mean ± SD for six animals per group and statistically analyzed with one way ANOVA using graph pad Instat 3 software (Graph Pad Software, Inc. La Jolla, USA) traced by Dunnett multiple comparison test.Significant levels of treated animals were determined by comparison with control group.$^{*}p<0.05$ and $^{**}p<0.01$ was considered to be statistically significant.

Results

The effect of NFCS administration on ameliorating the toxicity of CTX was summarized in table 1. Compared to normal animals, the serum markers including total bilirubin (0.690 ± 0.04 mg/dL), SGOT (187.0 ± 9.8 U/L), SGPT (59.9 ± 2.6 U/L) and ALP (83.1 ± 2.3 U/L) were elevated significantly ($p<0.01$), indicates hepatic injury caused by CTX. While the activities of SGOT, SGPT and ALP in animals treated with NFCS at the doses of 250 and 500 mg/kg b.wt didn't show such an increase. The effect of NFCS on alleviating redox imbalance in liver caused by CTX was depicted in table 2. As a result of CTX intoxication, a hike in the level of lipid peroxidation (nmol of MDA/mg protein) with concomitant decline in the activities of antioxidant enzymes including SOD, CAT, GPx and GSH was noticed in control animals. While the administration of NFCS at the doses of 250 and 500 mg/kg b.wt. markedly reduced the level of lipid peroxidation. Additionally, animals treated with NFCS (250 and 500 mg/kg b.wt.) have shown significantly ($p<0.01$) higher activities of SOD, CAT, GPx and GSH than control. The liver tissue of CTX treated animals has shown severe damages including haemorrhage, infiltration of inflammatory cells, fat deposition and necrosis (Figure 1). However, the liver tissue architecture of NFCS treated groups were found normal indicates their ability to restore the damages caused by cyclophosphamide.

Table 1: Protective effect of NFCS administration on liver function parameters in CTX intoxicated mice.

Group	Total bilirubin) (mg/dL	SGOT(U/L) (g/dL)	SGPT(U/L)	ALP(U/L)	Total protein
Normal	0.321 ± 0.02	146.5 ± 13.1	31.9 ± 8.1	45.8 ± 1.8	6.70 ± 0.08
Control	0.690 ± 0.04	187.0 ± 9.8	59.9 ± 2.6	83.1 ± 2.3	5.10 ± 0.09
NFLD	0.353 ± 0.05**	119.3 ± 21.6**	39.9 ± 3.7**	65.7 ± 1.5**	6.70 ± 0.11**
NFHD	0.314 ± 0.06**	93.8 ± 11.5**	34.4 ± 6.6**	60.5 ± 5.4**	6.20 ± 0.13**

Values are represented as mean ± SD per group (n=6); **$p<0.01$ compared to control.

Table 2: Effect of NFCS on hepatic antioxidant status in CTX intoxicated mice

Group	SOD (U/mg protein)	CAT (U/mg protein)	GPx (U/mg protein)	GSH (nmol mg protein)	LPO (nmol mg of MDA/mg protein)
Normal	1.11 ± 0.24	14.25 ± 1.91	26.87 ± 3.03	13.64 ± 2.34	1.24 ± 0.21
Control	0.36 ± 0.08	7.65 ± 0.87	12.64 ± 2.55	7.97 ± 1.31	7.55 ± 0.97
NFLD	0.90 ± 0.30**	11.61 ± 0.99*	24.00 ± 1.18**	12.94 ± 3.49*	2.92 ± 0.62**
NFHD	0.95 ± 0.09**	13.32 ± 2.84**	22.23 ± 2.98**	13.48 ± 1.64**	1.55 ± 0.36**

Values are represented as mean ± SD per group (n=6); **$p<0.01$ and *$p<0.05$ compared to control.

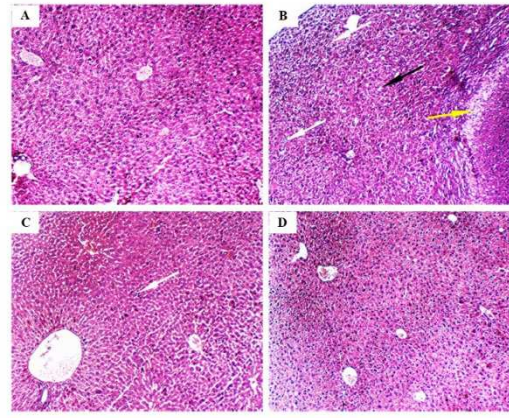

Figure 1: Hematoxylin/Eosin stained sections of liver tissues of CTX intoxicated mice - (A) Normal, (B) control (C) NF 250 mg/kg b.wt. and (D) NF500 mg/kg b.wt. The yellow arrow represents fat deposition, black arrow represents necrosis and white arrows represents infiltration of inflammatory cells (200 X magnification).

Discussion

Although CTX is a drug widely applied in the treatment of malignant and nonmalignant tumors, the clinical outcomes of treatments with these agents are severely limited, mostly due to its toxicity to normal tissues. Therefore, it is necessary to develop adjuvant therapy which may be used in combination with CTX to improve the efficacy of the treatment or reduce the associated undesirable side-effects. Herbal products can be used as alternate agents which have reported advantages over synthetic drugs. The active ingredients included in such products interact easily and help to lower the side effects of chemotherapeutic drugs (Jose et al., 2017). Considering this, current study aimed to exploring the efficacy of coconut inflorescence sap on alleviating cyclophosphamide induced hepatotoxicity in mice.

Hepatocytes are the primary site of activation of drugs, hence hepatic dysfunction is the important consequence related to CTX toxicity. A hike in the activity of ALP, SGPT and SGOT indicates the hepatotoxicity by CTX (Sun and Peng, 2008). NFCS administration restored the drop of these serum markers indicates the protective efficacy of coconut saps towards liver. Furthermore, hike in level of lipid peroxidation with concomitant reduction in the activity of various antioxidant enzymes including SOD, CAT and GPx were noticed in the liver of CTX intoxicated mice in this study. NFCS markedly alleviate these argumentative conditions by elevating antioxidant status to a considerable extent, indicating their ability to neutralize oxidative stress in liver. These results are confirmed by the histopathological observations and these findings also supported protective efficacy of NFCS on alleviating toxicity of various chemotherapeutic drugs.

The pharmacological properties exerted by natural products are mainly contributed to the synergistic action of various compounds present in them. NFCS contain various phenolic compounds including chlorogenic acid, apigenin, caffeic acid, gallic acid and protocatechuric acid with excellent antioxidant and chemoprotective efficacy (Oyagbemi et al., 2016). Most of the anticancer agents induce oxidative stress and create electrophilic aldehydes that targets and kill cancer cells and intake of antioxidants along with such drugs found to be effective in decrease the oxidative stress occurred during chemotherapy (Conklin, 2004). Hence, the chemoprotective efficacy exerted by NFCS may be due to the combined action of these antioxidant compounds.This healthy beverage can be used as an effective dietary supplement for long term without any adverse effects especially in peoples who suffers the side effects of chemothepeutics for long term consumption.

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Category B
Biodiversity and Environment

Current status of molecular taxonomy of spiders in India

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Abstract

Morphological taxonomy is a very well-established field and never loses its importance in the field of biodiversity. Still the reliability solely on the morphology can led to the misidentification and synonymy. Aid of molecular techniques can somewhat overcome the problem and help the classical taxonomy to reach a newer height of increased credibility with less discrepancies. In India the use of molecular taxonomy is still coping up the pace. Total 247 published records of 139 species of spider DNA barcodes from India are there in Barcode of Life Data System. In India Araneidae is the most represented spider family in BOLD with 59 entries followed by Oxyopidae with 32, Salticidae and Theraphosidae with 30 entries. In 2019 Thyagi et al. conducted the first large scale DNA barcoding on Indian spiders which includes 101 morphospecies belongs to 72 genera. Caleb et al. in 2017 utilized the scope of molecular taxonomy in the discovery of two new species of jumping spiders (Salticidae) *Epocilla* and *Mogrus* from India. Kulkarni et al. in 2017 done the phylogenetic analysis of Theridiid genus *Meotipa* to understand placement of the genus. In 2017 Chatterjee et al. prepared the molecular barcode of *Menemerusnigli*during the first report of the species from India. Chatterjee et al. in 2018 utilized the sequence of mitochondrial cytochrome c oxidase subunit 1 in the first report of *Psechrus inflatus* and *Hyptiotesaffinis* from India. Collaboration of molecular techniques with classical taxonomy is need of the hour in this era of anthropological mass extinction.

Key Words: Spiders, Molecular taxonomy, Biodiversity, DNA barcode

Introduction

Spiders belong to the most abundant group of predators in most of the ecosystems, but have received much less attention than other insect predators (Whitehouse & Lawrence, 2001). Spiders are among the most abundant insectivorous predators of terrestrial ecosystems (Nyffeler & Benz, 1987; Wise, 1993). Not only abundant, but they are one of the most diverse arthropod orders, with diverse species and exhibit a great variety of foraging strategies (Coddington & Levi, 1991). The various foraging strategies can be attributed to better predatory control of insects and ecological importance. The diet of spider is made up primarily of insects from various taxa, and also of other spiders (Nyffeler, 1999). Eggs, larvae, and adults of many different insect pests are major diet of spiders (e.g. Whitcomb, 1974; Nyffeler et al., 1990; Young & Edwards, 1990). Another important aspect about spiders is spider silk and spider venom therapeutics.(Malik, 2018) (Saez et al., 2010). In order to make all these possible applications initially taxonomy of the taxa should be done. Developments

and lack of confusions in taxonomic field will aid in the betterment of applied research.

Taxonomy, the classification of living organisms gains immense importance in current scenario where there is species loss in alarming scale due to anthropogenic activities. The database of every organism is necessary to estimate the loss as well as design various proactive and reactive strategies to prevent and cope up the species loss. The extent of undescribed species will be much higher for invertebrates than vertebrates. Using novel technologies of molecular biology in combination with classical taxonomy, the aim of quantification of biodiversity of our nature is very likely. Usage of molecular technologies in taxonomy will improve the credibility of description of particular species. The errors caused by pure morphological taxonomy can be overcome by the aid of molecular technologies. In India the use of molecular taxonomy is still coping up the pace. Use of molecular techniques with traditional taxonomy will make a paradigm shift in the taxonomic field in India. India is one of the mega diverse countries in the world. In order to quantify biological wealth of such a land mass molecular taxonomy should be considered as the optimal practice. Molecular taxonomy utilizes various molecular markers for the creation of molecular barcodes. Cytochrome oxidase Subunit 1 (COI) is considered to be the best and hence widely used molecular marker. Current status of molecular taxonomy of spiders in India is not appreciable. Compared to many other countries the entries from India is much lesser. The data about molecular taxonomy of spiders from India is mined from the website of Barcode of Life Database (BOLD Systems). Recently most of the taxonomists are showing interests in addition of molecular techniques along with classical approach. So it is evident that in the near future the contribution from India to BOLD systems will increase in alarming rate.

Results and Discussion

In India 287 published record of spider DNA barcode are there (Barcode of Life Data System). Barcode of 247 species of spiders, of which 139 are given full species name are found in BOLD. 40 unspecified species are also there. Araneidae is the most represented spider family in BOLD with 59 entries followed by Oxyopidae with 32 entries Salticidae and Theraphosidae with 30 entries each.(Fig – 1). Poecilotheria and Oxyopes are the most represented Genera with 25 entries each followed by 21 Unspecified and Neoscona with 16 entries.

Caleb et al. in 2017 utilized the scope of molecular taxonomy in the discovery of two new species of Jumping spider (Salticidae) *Epocilla* and *Mogrus* from India. After morphological assessment DNA extraction was performed by means of QIAamp® DNA Investigator Kit, following the instructions of manufacturer. Amplification of partial fragment (~650bp) of mitochondrial cytochrome C oxidase subunit I (mtCOI) gene were performed and sequencing was done by the means of the BigDye® Terminator Cycle Sequencing Kit (v3.1) on 3730 DNA Analyzer. The species were named *Epocilla sirohi* sp.n. and *Mogrus rajasthanensis* sp.n. The resulted sequence was submitted in GenBank, NCBI and BOLD.

Kulkarni et al. in 2017 done the phylogenetic analysis of Theridiid genus *Meotipa* to understand the placement of the genus. A previously published set of 242 morphological characters for Theridiidae phylogeny (Agnarsson 2004) with simple modifications were scored for these species. Parsimony and Bayesian analysis were done. The analysis revealed the monophyletic nature of the taxa.

In 2017 Chatterjee et al.prepared the molecular barcode of *Menemerus nigli* during the first report of the species from India. The species was described by Wesolowska & Freudenschuss, 2012 and the type locality was Pakistan. DNA barcode was utilized to confirm the identity of the specimen collected from West Bengal, India. Partial amplification of cytochrome C oxidase subunit I (mtCOI) was carried out for the purpose of barcoding. The sequencing was done at ZSI and sequence was uploaded to Barcode of life Data System (BOLD). The sequence developed in the study showed 100% similarity with sequences of *M. nigli* from Pakistan.Chatterjee et al. in 2018 utilized the sequence of mitochondrial Cytochrome c oxidase subunit 1 in the first report of *Psechrus inflatus* Bayer (Araneae: Psechridae) from India. The species previously known from China is recorded for the first time in India. Partial sequence data of mitochondrial cytochrome C oxidase (mtCOI) was generated and is submitted in BOLD. The barcode which is generated from 650 bp of mtCOI was used for a similarity search in NCBI and found to be 98% similar to the specimen from China.Chatterjee et al. in 2018 confirmed the identity of *Hyptiotes affinis* Bösenberg & Strand, 1906 by sequencing mt COI and results in the first report of the species from India. This species was previously known from China, Japan and Taiwan. During this study they found the species from Assam and utilized molecular techniques for the conformation.

Thyagi et al. in 2019 done the first large scale attempt on DNA barcoding of spiders from India with 101 morphospecies of 72 genera under 21 families. Cryptic species and species complex are also analyzed during the study. Data from these study showed that DNA barcoding is a valuable tool for species identification and species discovery.

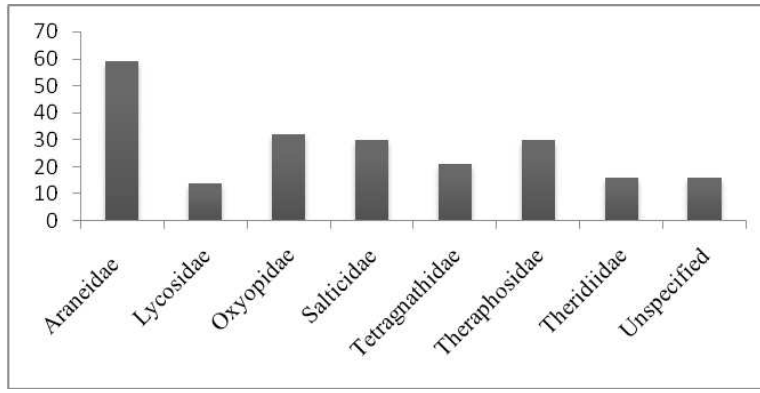

Figure1: Bar diagram showing different spider families entries from India deposited in BOLD Systems

Conclusion

Compared to other countries contribution from India to molecular taxonomy of spiders is only at initial stage. In India 287 published record of spider DNA barcode are there ((*Record List | Public Data Portal | BOLDSYSTEMS*, n.d.) . Barcode of 247 species of spiders, of which 139 are given full species name are found in BOLD. 40 unspecified species are also there. Araneidae is the most represented spider family in BOLD with 59 entries followed by Oxyopidae with 32 entries Salticidae and Theraphosidae with 30 entries each. In the case of genera Poecilotheria and Oxyopes are the most represented genera from India. The interesting part is that more than 75 percentages of entries of Indian spiders are done in foreign universities. This data showing that still molecular taxonomy is not common among Indian researchers. But as hope of light, many large scale molecular taxonomy works are being initiated in India which may jeopardize the anomalies in existing spider taxonomy along with better explanation of evolution.

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Application of molecular taxonomy in lynx (Oxyopidae) spiders

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Abstract

The family Oxyopidae comes under order Araneae,which includes lynx spiders. Their cryptic behaviour, sexual dimorphism and unavailability of taxonomic keys for juveniles make the taxonomic studies in this group strenuous. Recently DNA barcoding contemplate as a state-of-the-art in taxonomic appraisals,especially in spider taxonomy.Species specific studies in Oxyopidae gain importance by accounting their hunting behaviour and its significance in integrated pest management. Members under Oxyopidae enjoy a wide distribution across the tropics and subtropics.Even so the molecular taxonomic studies among the lynx spiders focused at most in southern Asia.As of November 2020, the World Spider Catalog accepts 438 species in 9 genera of lynx spiders. Among this not more than 30 species are identified by using molecular taxonomic tool. Most of the molecular taxonomic studies in Oxyopidae employed Cytochrome Oxidase 1 (COI) as a molecular marker.DNA barcoding paved the way for a comparative study among the members of the same family and others by obtaining the DNA sequences from BOLD or GenBank using BLAST algorithms, which further helps to generate phylogenetic relations using computer software.A close phylogenetic relationship of *Oxyopeshupingensis*with *O. sertatus* confirmed by DNA barcoding. Barcoding of complete mitochondrial genome of striped lynx spider *O. sertatus*, using the circular genome is 15,078 bp in length, revealed 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, 2 ribosomal RNA genes, and a control region. Barcoding of diversity and structure of bacterial communities in the gut of Oxyopidae impart the ecology and feeding behaviour of its members.

Keywords: *Araneae, CO1, DNA Barcoding, Identification, Oxyopidae*

Introduction

The family Oxyopidae comes under largest arachnid order Araneae distributed predominantly in the equatorial region and some part of the temperate zone as well. These spiny legged hunting spiders are proficient in fast running and jump on the prey as a wild cat. Many lynx spiders are important predators in agricultural systems as biological control agents (Meng et al., 2014).A total of 438 species in 9 genera of lynx spiders so far reported all over the world (WSC, 2020). Hexagonally arranged eight eyes accompanying prominent spins on the legs discern themfrom the remaining spider families. Spiders of the family Oxyopidae have

received very little taxonomic attention in India(Gajbe, 1999).The traditional classical taxonomic studies mislaid many of its members into other families on account of its cryptic behaviour.

Molecular taxonomy using DNAbarcoding shows a mushrooming trend in the field of species identification, phylogenectic studies and other related taxonomic appraisals. Its widely accepted that molecular taxonomy is the most reliable and accurate taxonomic tool available today. Molecular genetic information is rapidly gaining support as an ample source of easily quantifiable, discrete taxonomic characters that can often be homologized over a wide range of taxa and allow rapid standardized analysis even by nonspecialists (Astrin et al., 2006). Establishing molecular characters as a standard taxonomic tool equivalent to, or even superior to, morphological data has been met with harsh criticism (Will &Rubinoff, 2004).Forgoing works in spider DNA barcoding pay attention to the mitochondrial genome by considering its more conserved nature collate to the nuclear genome. Neoteric works in DNA Barcoding changes its track into nuclear genomic sequencing by using specific molecular markers. COI sequences are most frequently used molecular marker in worldwide molecular taxonomic studies. Besides 12srDNA, 16srDNA, elongation factor alpha, histone (H1,H2,H2B,H3) genes of nuclear genome used as molecular markers.

Molecular taxonomy in species specific studies

Gaikwad et al (2017) have generated DNA barcodes for 60 species of spiders for the first time from India including Oxyopidae. First large scale attempt on DNA barcoding of spiders from India with 101 morphospecies of 72 genera under 21 families including Oxyopidae done by Tyagi et al. (2019) show that DNA barcoding is a valuable tool for specimen identification and species discovery of Indian spiders. Pan et al. (2014) traced the complete mitochondrial genome of striped lynx spider *Oxyopessertatus* containing a circular molecule of 14,442 bp in length, containing 13 protein-coding genes, 2 ribosomal RNAs, 22 transfer RNAs, and a control region. Using the circular genome is 15,078 bp in length, containing 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, 2 ribosomal RNA genes, and a control region complete mitochondrial genome of the lynx spider *Oxyopeshupingensis* was characterized by Yang et al. (2019). Kumari et al. (2018) conducted a study of identification of spiders prevalent in Rajasthan, India through DNA barcoding.In their study barcoding of COI gene confirmed the morphological identification of four species i.e., *Neosconavigilans*, *N.theisi*, *Pardosa birnamica* and *Oxyopes* sp. Standard barcode region of CO1gene of 64 samples were amplified,the sequences of 658 base pairs were recovered from 62 samples, representing 7 families, 20 genera and 27 species including oxyopids done by Tahir et al (2020) concluded that CO1 has potentially enough information for fast and accurate identification of spiders.

Molecular taxonomy to trace phylogeny among Oxyopids

Afzal et al. (2020) distinguished five species of spiders includingOxyopidaeusing their CO1 gene sequences and compared them with 40 earlier published sequences, retrieved from GenBank on the bases of maximum similarity index. The sequences of gene encoding CO1 of all five spider species were deposited in GenBank. Zhang et al. (2013) investigated phylogenetic relationships of the jumping spider by molecular phylogeny obtained from Bayesian, likelihood and parsimony methods.They also derived the divergence time and biogeography of the same.Yang et al. (2019) found close phylogenetic relationship of *O.hupingensis* with *O.sertatus* by comparing COI sequence of the two. The phylogenetic analysis show *Oxyopes sp.* done by kumari et al. (2018) is discovered a close relation between Indian population with their Chinese counter parts. According to Yuan et al. (2020) donemolecular species delimitation based on Automatic Barcode Gap Discovery(ABGD), P_{ID} (Liberal), and generalized mixed Yule coalescent (GMYC) were incongruent in species assignment and he showed that the interspecific genetic divergence between *O. sertatus* and *O.taiwanensis* was relatively low (1.28 ± 0.43%), and the intraspecific genetic divergence of *O. striagatus* was relatively high (1.69 ± 0.35%).Jalajalakshmi and Usha (2019) conducted a BLASTanalysis(maximum likelihood) of Mito COX sequence of six species including *O.lineapties* revealed that these six species were diverged from a common ancestor.The barcode gap analysis by Ashfaq et al. (2019)showed that maximum intraspecific distance for all but one of the 90 species in his samples is less than its Nearest Neighbor distance where *O.azhari* has an exception it overlaps with *O.oryzae*.

Aid to investigate the ecology and feeding behaviour of the individuals

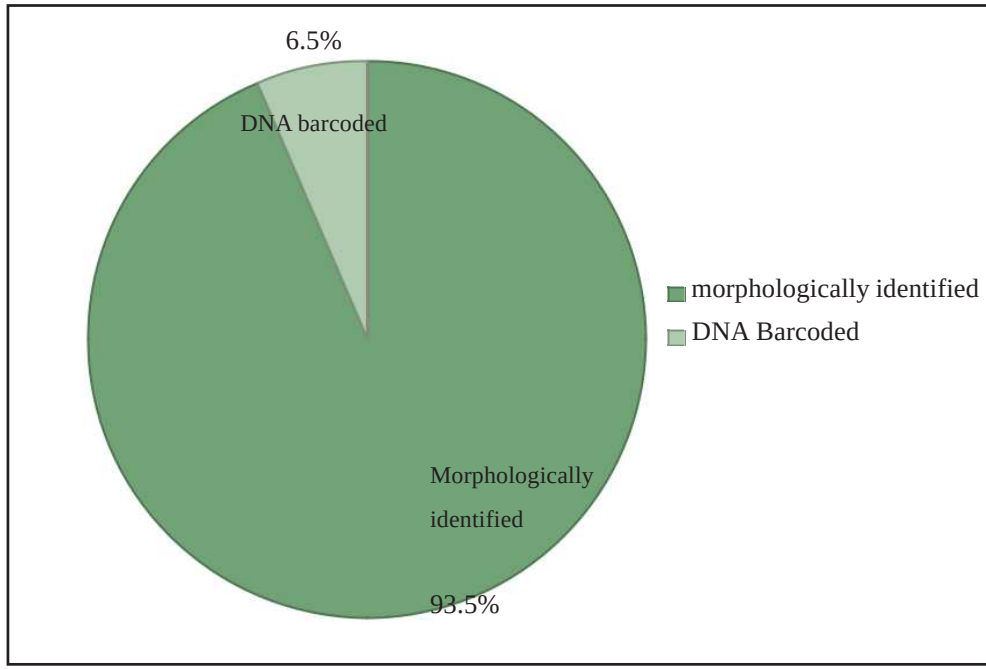
A preliminary study on isolation and patterns of population genetic structure in Taiwanese *Oxyopes*done by Yuan et al. (2020) using DNA barcode data.Kumari et al (2020) studied the diversity and structure of gut bacterial communities among the seven species of twofamilies belonging Oxyopidae and Thomisidae, from different states of India by using barcoding tools. Their data revealed a total of 16 bacterial phyla with Proteobacteria as the predominant group in Thomisidae and Firmicutes in Oxyopidae. The core bacterial communities in the spider guts include the generaof *Acinetobacter, Staphylococcus*, *Corynebacterium*, *Cutibacterium* and *Pseudomonas*. The data also indicated a higher gut bacterial community similarity between spider species belonging to Thomisidae as compared to those belonging to Oxyopidae bacteria.

Result and Discussion

Cryptic behaviour, sexual dimorphism and unavailability of taxonomic keys for juveniles turn down the credibility of classical taxonomic studies Oxyopidae. Molecular taxonomy using DNA barcoding currently contemplate as an advanced tool in species identification and derive the phylogenectic relationships among the spider families. In accordance with

world spider catalog Novmber 2020 there are 438 species of 9 Genera are identified using classical taxonomic appraisals, however only less than 30 species of oxyopids are DNA barcoded (Figure 1). These groups distributed both in equatorial and temperate zones. Even so the lion's share of molecular taxonomic work conducted in south and south East Asia. The major studies have been happening in Pakistan and china. In India these groups are mostly ignored. Apart from some preliminary studies few notable molecular studies happened in Rajastan and WesternGhats.

Figure 1: Number of Oxyopids identified using morphology and molecule



It is undisputed that DNA barcoding is competent in species identification. Species specific studies focused in mitochondrial genome, which is helpful in the species identification as in the case of *O.seratus*its mitochondrial genome is 14,442 bp length where as circular genome is15,078 bp length in *O. hupingensis*. Barcoding of COI gene confirmed the morphological identification of four speciesi.e.,*Neoscona vigilans, N.theisi, Pardosa birmanica* and *Oxyopes* sp.These shows that the COI sequences considered as most preferable molecular marker in barcoding. The access of COI sequences from the international databases like NCBI GENBANK and BOLD using BLAST tool enable to examine the interrelationships between the members of a family all over the world. As an extension phylogenetic relationships among them can be obtained from Bayesian, likelihood and parsimony methods. The divergence time and biogeography of the of the oxyopidae can also derived from barcoded data.This application further lead to the pick out the close phylogenetic relationship of

O.hupingensis with *O. sertatus.*Apart from its wide geographical variations, comparison of barcodedsequences are revealed a close relationships *Oxyopes* sp. in India and China.Low interspecific genetic divergence between *O. sertatus* and *O. taiwanensis* pointed a common ancestry among the two and high the intraspecific genetic divergence of *O. striagatus* and *O. sertatus* shows lack of nearest common ancestor.

Molecular taxonomy now advances its applications beyond the taxonomic and phylogenetic studies to ecological works. The study of diversity and structure of gut bacterial communities ofoxyopidae using DNA barcoding tool is novel to the ecological studies. Higher gut bacterial community similarity between spider species belonging to Thomisidae as compared to those belonging to Oxyopidae. There is higher gut bacterial community similarity between spider species belonging to Thomisidae as compared to those belonging to Oxyopidae. This type of studies will help to identify the ecology and feeding habit of members of Oxyopidae family.

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Study of arthropod-induced leaf gall diversity in Nemmara, Palakkad

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Abstract

Galls are swollen growths on the external tissues of plants, induced by various parasites (fungal and bacterial) or by arthropods. These are organized structures and the cause of the gall can often be determined without the actual agent being identified. Most galls are caused by irritation or stimulation of plant cells due to feeding or egg-laying by insects. Each type of gall-producer is specific to a particular kind of plant. The present study focused on the leaf gall diversity in NSS College Campus, Nemmara, Palakkad District. The visual analysis of galls was done using stereo-zoom microscope, to locate larval or adult stages of the inducers, facilitating identification. The enumeration of galls per collected leaves was also carried out. Out of 15 galls collected from 15 different host plants, majority (10) were found to be insect-induced, whereas 4 were mite-induced, the fifteenth sample, remaining unidentified. Out of the fifteen types of galls, most were epiphyllous, excepting those obtained from *Mangifera indica*, *Chionanthus* sps. and *Tectona grandis*. Microscopic analysis aided in identifying three inducers (*Pseudophacopteron tuberculatum* on*Alstonia scholaris, Trioza jambolana* on *Syzigium cumini and Pauropsylla depressa* on *Ficus racemosa*) upto their species level, all belonging to order Hemiptera. The genera *Trioza* on *Terminalia paniculata, Apsylla* on *Mangifera indica, Asphondyla* on *Tectona grandis* and *Contarina* on *Calycopteris floribunda* were also identified. No spotting of any inducer or its life stage was obtained from *Diospyros cordifolia*, which revealed only the exit holes under microscopic examination. Enumeration data revealed that galls induced by Gall mite on *Thespesia populnea* (122.7 ± 28.4) *and Mallotus philippiensis* (53.3 ± 11.8) were maximum in number, whereas those of *Pauropsylla depressa* on *Ficus racemosa* (1.6 ± 0.003), *Trioza jambolana on Syzigium cumini* (4.2 ± 0.05) and *Contarina* sps (Gall midge) on *Calycopteris floribunda* (4.4 ± 1.7) were minimum.

Keywords: cecidia, arthropod, leaf galls

Introduction

Cecidia or galls are kind of swollen growths on the external tissues of plants, which are induced by various parasites (fungal and bacterial) or by arthropods. Galls are formed by feeding or egg-laying activity of insects, mites, wasps or midges. Induction of gall formation occurs either by mechanical damage or salivary secretions (introduced by arthropods), which

initiate increased production of normal plant growth hormones. These plant hormones cause localized plant growth that can result in increases in cell size (hypertrophy) and/or cell number (hyperplasia, resulting in an abnormal plant structure called a gall. Once gall formation is initiated, many galls will continue to form even if the insect dies. In addition, most galls are usually not noticed until they are fully formed and remain on plants for extended periods of time (more than a season) (Wawrzynski et al., 2018).Most galls are caused by irritation and/or stimulation of plant cells due to feeding or egg-laying by insects such as aphids, midges, wasps, or mites.More than 13,000 insect species are capable of inducing neoplastic formation or galls in plants(Stone and Scho¨nrogge, 2003). Gall-making habit is found in six orders of insects: Coleoptera (beetles), Lepidoptera (moths and butterflies), Hemiptera (aphids), Thysanoptera (thrips), Diptera (flies), and Hymenoptera (sawflies and wasps). Factors such as weather, plant susceptibility, and pest populations affect the occurrence of galls on plants from year to year. Increased species richness with decreasing altitude/latitude appears to be a global phenomenon in insects. The same phenomenon has also been reported for galling insects along altitudinal and latitudinal gradients. Temperature and moisture are the actual factors responsible for this. The altitudinal gradient negatively influenced galling richness in xeric habitats, but there was no significant correlation between galling richness and altitude in mesic habitats. The mean number of galling insects was higher on woody than on herbaceous plants, but did not differ between shrubs and trees, refuting the plant architecture hypothesis (Lara et al., 2002).

The present study focuses on the leaf gall diversity in NSS College Campus, Nemmara, Palakkad District. Leaf galls are the commonest of all gall types, and hence, they were selected for the study. The visual analysis of galls was carried out using stereo-zoom microscope, so as to locate larval or adult stages of the inducers, facilitating the identification process. The enumeration of galls per collected leaves was also carried out.

Materials and Methods

Galled leaves were collected from various plant species of NSS College Campus, Nemmara, Palakkad, during the period from December 2019 to February 2020. From each plant, 20 leaves with galls were collected and were enumerated by counting. The number of each category (number of galls per leaf) was expressed as Mean ± SD.The collected leaves with galls were photographed using Redmi Note 8 9PKQ1.190616.001 Android version phone camera. The galls were then photographed (in both unopened and opened condition) using Leica MC170 HD Camera of stereozoom microscope, maintained in the research lab of Zoology Department, St. Thomas College, Thrissur, under higher magnifications to locate life cycle stages, if any, of the cecidogenic arthropod.

Results and Discussion

Leaf galls were obtained from fifteen types of host plants in the campus of NSS College,

Nemmara, of which ten of the causative organisms were insects [Families Phacopteronidae, Triozidae (of Order Hemiptera) and Family Cecidomyiidae (of order Diptera)], and four were arachnids (Family Eriophyiidae; Order Trombidiformes) as enlisted in table 1. The gall obtained from the host plant *Diospyros cordifolia* did not reveal the presence of the inducer in microscopic examination, and hence, remains unidentified. In contrast to other types of plant/insect interactions, galls have been widely studied only by insect ecologists. Therefore, within recent decades, galls were analyzed mostly for their favorable effect on insect fitness (Alford, 2012). Gall tissues guarantee nutrition, protection and a favorable microenvironment to the gall inducer. Sites of hyperplasia and hypertrophy are commonly reported for arthropod galls, and are commonly related to the feeding habits of each taxon (Raman et al., 2005, Isaias et al., 2014).Our study aimed at studying leaf gall diversity in NSS College, Nemmara campus. Out of 15 galls collected from 15 different host plants, majority (10) were found to be insect-induced, whereas 4 were mite-induced, the fifteenth sample, remaining unidentified. The identification was carried out by referring to previous works conducted as well as with the expertise of researchers (Narendran, 2012, Saleem and Nasser, 2015).

Sl No:	Type of inducer (classification)	Inducer name	Host plant	Gall no:/leaf (Mean ± SD)*	Description of gall
1	Insecta Hemiptera Phacopteronidae	*Pseudophacopteron tuberculatum*	*Alstonia scholaris*	14.4 ± 1.05	Epiphyllous and perfoliate balls
2	Insecta Hemiptera Triozidae	*Trioza jambolana*	*Syzigium cumini*	4.2 ± 0.05	Epiphyllous lumps
		Trioza sps.	*Terminalia paniculata*	8.3 ± 0.85	Epiphyllous warts
		Pauropsylla depressa	*Ficus racemosa*	1.6 ± 0.003**	Epiphyllous, balls
		Apsylla sps.	*Mangifera indica*	11 ± 0.88	Hypophyllous wart
3	Insecta Diptera Cecidomyiidae	*Asphondyla* sps. (Gall midge)	*Tectona grandis*	12.5 ± 1.8	Hypophyllous warts
		Contarina sps (Gall midge)	*Calycopteris floribunda*	4.4 ± 1.7	Epiphyllous
		Gall midge (Genus name unknown)	*Chionanthus* sps	.16.5 ± 0.03	Hypophyllous knobs
			Hydnocarpus pentandrus	8.2 ± 0.13	Epiphyllous lumps
			Terminalia elliptica	21.6 ± 2.3	Epiphyllous warts

4	Arachnida Trombidiformes Eriophiyidae	Gall mite (Genus name unknown)	*Cinnamomum malabatrum*	28.6 ± 5.03	Epiphyllous and perfoliate lumps
			Pongamia pinnata	17.4 ± 0.9	Epiphyllous and perfoliate erineums
			Thespesia populnea	122.7 ± 28.4	Epiphyllous warts
			Mallotus philippiensis	53.3 ± 11.8	Epiphyllous warts
	Unidentified gall inducer		*Diospyros cordifolia*	42.4 ± 12.4	Epiphyllous warts

Table 1: showing the list of galls collected from NSS College, Nemmara Campus, their description, number and the arthropod inducers identified

Out of the fifteen types of galls, most were epiphyllous, excepting those obtained from *Mangifera indica, Chionanthus* sps. and *Tectona grandis*, an observation which is consistent with previous literature (Mani, 1964, Raman, 2007). Moreover, all mite induced galls obtained were epiphyllous. Gall structures were found to be diversified as the collection included galls in the forms of balls, lumps, warts, knobs and erineums. Microscopic analysis aided in identifying three inducers (*Pseudophacopteron tuberculatum* on*Alstonia scholaris, Trioza jambolana* on *Syzigium cumini and Pauropsylla depressa* on *Ficus racemosa*) upto their species level, all belonging to order Hemiptera. The genera *Trioza* on *Terminalia paniculata, Apsylla* on *Mangifera indica, Asphondyla* on *Tectona grandis* and *Contarina* on *Calycopteris floribunda* were also identified. No spotting of any inducer or its life stage was obtained from *Diospyros cordifolia*, which revealed only the exit holes under microscopic examination. Enumeration data revealed that galls induced by Gall mite on *Thespesia populnea* (122.7 ± 28.4) *and Mallotus philippiensis* (53.3 ± 11.8) were maximum in number, whereas those of *Pauropsylla depressa* on *Ficus racemosa* (1.6 ± 0.003), *Trioza jambolana on Syzigium cumini* (4.2 ± 0.05) and *Contarina* sps (Gall midge) on *Calycopteris floribunda*(4.4 ± 1.7) were minimum. Parasites and natural enemies have recognized as major selective agents in the evolution and maintenance of gall diversity (Stone and Schönrogge, 2003). Hence, the study holds good in its taxonomic relevance as well as its evolutionary significance. The study also throws light on its further scope of identifying more of twig, petiole, flower and bud galls, apart from leaf galls, extending our understanding of cecidogenic arthropod diversity.

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Analysis of well water potability in selected areas of Palakkad district post Kerala floods 2018

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Abstract

From the 8th of August 2018, severe floods affected the south Indian state of Kerala, due to unusually high rainfall during the monsoon season and was the worst flood in Kerala in nearly a century. Apart from the damage inflicted to the whole state's infrastructure, a drinking water crisis also arose due to the contamination of wells. The aim of the present study conducted in December 2018 was to analyze and compare selected physico-chemical and biological indicators of water quality of well water samples collected from ten places in Palakkad- (Nemmara, Karimkulam, Nenmeni, Karippode, Alathur, Thrippalur, Vandazhy, Pallassana, Mudappallur, Vadakkencherry). The potability of these samples was tested based on colour, turbidity, taste, odour, pH, total hardness, calcium hardness, magnesium hardness and presence of coliforms (based on MPN index). In the study conducted, it was found that all water samples met acceptable physical and chemical characteristics recommending their use as drinking water, except the sample from Karippode, which showed increased water hardness, suggesting the need of remedial measures. Ground water undergoes faecal contamination, if there are septic tanks in the vicinity or a contamination pathway exists between a source of bacteria (surface water, septic system, animal waste, etc.) and the water supply. Disease-causing bacteria may use this pathway to enter the water supply. On analyzing the probable number of coliform levels, we could find that the levels were alarmingly high in samples from Karimkulam, Karippode, Alathur, Pallassana, Mudappallur and Vadakkencherry, which indicated that they were unsuitable for drinking, and may be in proximity to sources of faecal contamination. This suggested the need for immediate decontamination steps, unless which they cannot be used for drinking purposes.

Keywords: well water potability, calcium hardness, magnesium hardness, total coliforms

Introduction

The ground water potential of Kerala is very low as compared to that of many other states in the country with an estimated ground water balance of 5590 mm^3. Dug wells are the major ground water extraction structure in Kerala. The open well density in Kerala is perhaps the highest in the country – 200 wells per sq.km in the coastal region, 150 wells per sq.km in the

midland and 70 wells per sq. km in the high land (Rajeevan, 2014). Groundwater has been the mainstay for meeting the domestic needs of more than 80% of rural and 50% of urban population besides, fulfilling the irrigation needs of around 50% of irrigated agriculture. The ease and simplicity of its extraction has played an important role in its development. Since well water is a source of human water consumption, it is a necessity that periodic monitoring of its quality has to be carried out (Thomas KS, 2000, e-Article, 2001, e-Article, 2009, Brian Oram, 2014, Perlman, 2014).

From 8 August 2018, severe floods affected the south Indian state of Kerala, due to unusually high rainfall during the monsoon season. It was the worst flood in Kerala in nearly a century. Apart from the damage inflicted to the whole state's infrastructure, a drinking water crisis also arose due to the contamination of wells. The aim of the present study is to analyse and compare selected physico-chemical and biological indicators of water quality of house-hold water samples used by the project fellows for domestic purposes in their homes.

Materials and Methods

Collection of water samples

Water samples were collected from different areas of Palakkad district viz., Nemmara, Karimkulam, Nenmeni, Karippode, Alathur, Thrippalur, Vandazhy, Pallassana, Mudappallur and Vadakkencherry.

Assessment of chemical parameters

The determination of pH, total hardness and calcium hardness was done using reagents, chemicals and apparatus provided by the laboratory of Zoology Department, NSS College, Nemmara. All reagents and chemicals used were of analytical quality. pH of the samples was determined using pH meter.

Determination of faecal contamination

The laboratory tests for faecal contamination begin with a broad estimation of the probable number of coliform bacteria in the sample. Multiple tube fermentation method was used for faecal contamination detection. This is a test for determining the most probable number (MPN) of bacteria in water, first described by Mc Grady. The resultant MPN was presently determined from published table.

Results and Discussion

Water quality is a requisite as a measure of the physical, chemical, biological, and microbiological characteristics of water. Chemical attributes of water can affect aesthetic qualities such as how water looks, smells, and tastes. They also affect its toxicity and safety. Since the chemical quality of water is important to the health of humans as well as the plants and animals that live in and around streams, it is necessary to assess the chemical attributes of water. Moreover, in the scenario of post-flood effects on aquatic systems, it is very necessary that all household and domestic purpose wells need to be analysed for ensuring their water quality. Deterioration of water quality can result in major health hazards.

The chemical parameters measured for the ten well water samples are tabulated in table 1. Extremes of pH can affect the palatability of water but the corrosive effect on distribution systems is of more concern. Here, in the study, all samples showed value within pH range of 6.5 to 8.5, as per IS, 10500:2012. Hardness was initially considered as the capacity of water to destroy the lather of soap, but now as the determination of calcium and magnesium which are the main constituents of hardness. Although barium, strontium and iron can also contribute to hardness, their concentrations are normally so low in this context that they can be ignored. Thus, total hardness is taken to comprise the calcium and magnesium concentrations expressed as mg/l CaCO3. The widespread abundance of these metals in rock formations leads often to very considerable hardness levels in surface and ground waters. Hardness is a natural characteristic of water which can enhance its palatability and consumer acceptability for drinking purposes. Health studies in several countries in recent years indicate that mortality rates from heart diseases are lower in areas with hard water. Among the samples tested, sample from Karipode showed a total hardness of 372.8 mg/L, which was found to be higher than the acceptable limit (200 mg/L). According to Indian standards as well as EPA (Environment Protection Agency) standards, Ireland, water with hardness of above 200 mg/L is not palatable (e-Article, 2010). All the other samples showed values within normal range. However, water with increased hardness is not recommended for bathing purposes.

Similar to the results of total hardness, calcium hardness was highest in sample from Karipode (79.2 mg/L). The acceptable limit as per IS: 10500-2012bis 75 mg/L. All the other samples showed normal value. High levels of calcium may be beneficial and waters which are rich in calcium (and hence are very hard) are very palatable. There is some evidence to show that the incidence of heart disease is reduced in areas served by a public water supply with a high degree of hardness, the primary constituent of which is calcium, so that the presence of the element in a water supply is beneficial to health. But increased amounts are not considered as desirable (e-Article, 2001). Magnesium hardness was also higher than acceptable limit (125 mg/L) in sample Karipode (293.6 mg/L).Increased intake of magnesium salts may cause a change in bowel habits (diarrhea). Drinking water in which both magnesium and sulfate are present in high concentrations (~250 mg/l each) can have a laxative effect (Sengupta, 2013). The other samples showed values within acceptable limits.

Table 2 shows the MPN index of the water samples based on gas production. It was conclusive by comparing the standard MPN chart that the samples from Karimkulam, Karipode, Alathur, Pallassana, Mudappallur and Vadakkencherry were non-potable and need immediate decontamination treatment. Sample collected from Nemmara was potable as per the MPN index; however, it was the only sample of water that is subjected to chlorination. Ground water undergoes faecal contamination, if there are septic tanks in the vicinity or a contamination pathway exists between a source of bacteria (surface water, septic system, animal waste, etc.) and the water supply. Disease-causing bacteria may use this pathway to enter the water supply (e-Article, 2017).

Our study was focused on determining the quality of well water samples collected. Due to timely constraints and insufficient funds, we did not extend our work to take up the remedial measures. But we used the opportunity to inform the well users about the problems detected by us and suggest protective steps such as immediate disinfection using shock chlorination and thorough boiling of water before cooking and drinking. UV sterilization, iodination and ozonation are also effective, depending upon the affordability of treatment.

Table 1 : Comparison of chemical parameters of well water samples

Sample ID	pH	Total Hardness (mg/L)	Calcium Hardness (mg/L)	Magnesium Hardness (mg/L)
Nemmara	6.6	24.2	6.3	17.9
Karimkulam	7.7	115.2	27.8	87.4
Nenmeni	7.2	72.0	16.4	55.6
Karippode	8.5	372.8	79.2	293.6
Alathur7.1	86.4	23.2	63.2	
Thrippalur	7.2	86.4	24.3	62.1
Vandazhy	7.1	43.2	17.2	26.0
Pallassana	7.1	48.0	11.6	36.4
Mudappallur	7.0	30.4	7.3	23.1
Vadakkencherry	6.9	73.6	17.3	56.3

Table 2 : showing the MPN index of the water samples based on gas production

Sample ID	10 ml tubes			1 ml tubes			0.1 ml tubes			Reading	MPN	Potability
	1	2	3	1	2	3	1	2	3			
Nemmara	–	–	–	–	–	–	–	–	–	0,0,0	<2	Yes
Karimkulam	+	+	+	+	+	+	+	+	+	3,3,3	>2400	No
Nenmeni	+	+	+	–	–	–	+	–	–	3,0,1	11	Yes
Karippode	+	+	+	+	+	+	+	+	+	3,3,3	>2400	No
Alathur	+	+	+	+	+	+	+	+	+	3,3,3	>2400	No
Thrippalur	+	+	+	+	+	+	+	_	_	3,3,1	17	Yes
Vandazhy	–	–	–	–	–	–	–	–	–	0,0,0	<2	Yes
Pallassana	+	+	+	+	+	+	+	+	+	3,3,3	>2400	No
Mudappallur	+	+	+	+	+	+	+	–	+	3,3,2	1100	No
Vadakkencherry	+	+	+	+	+	+	+	+	+	3,3,3	>2400	No

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First record of two Theridula Emerton, 1882 species (Aranaea: Theridiidae) from India

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Abstract

Theridiidae is one of the largest families of spiders in the world with about 2472 species in 124 genera (World spider catalog, 2020). These incorporate 58 Indian species belonging to 19 genera (Sebastian&Peter, 2009). Despite rich diversity, studies about Indian Theridiids are exceptionally ignored, probably likely because of their little size and absence of relevant literature (Siliwal, 2009). Around 17 types of Theridula have been accounted from various parts of the world however there is no report of genus Theridula Emerton, 1882 from India so far. In this paper we depict two species of the genus Theridula, *Theridula opulenta* (Walckenaer, 1841) and *Theridula gonygaster* (Simon, 1873) collected from Kerala. Photographs, distribution, and morphology of the specimens will be discussed.

Keywords: First record, India, Theridiidae, Theridula

Introduction

The spider genus Theridula was established by Emerton in 1882. Around 17 types of Theridula have been accounted from various parts of the world; however, there is no report of genus Theridula from India so far. Our study on the diversity of theridiid spiders of Kerala led to the collection of two species of Theridula the Idukki district of Kerala. Detailed descriptions of specimens collected are provided, alongwith illustrations.

Materials and Methods

Specimens were collected by hand Idukki district of Kerala. The material was preserved in 75% alcohol. Live images of the specimens are taken with a Digital Camera, fitted with macro lens. Microphotographs were taken by Digital Camera attached to a Labomed CZM6 Stereozoom Microscope using Canon EOS Utility Software. The measurement and illustration of the specimen was done using drawing tube in the laboratory. Legs and pedipalp measurements were taken from their dorsal side of the body and are given in the order: Femur, Patella, Tibia, Metatarsus (except palp), Tarsus and Total. The eye measurements were taken with calibrated ocular micrometer. Female epigyne was cleared in situ with clove oil. The description was done by immersing the spider in a petri dish containing 70% alcohol. All measurements are in millimeters.

Abbreviations: AL-Abdomen length; AW-Abdomen width; CL-Cephalothorax length; CW-Cephalothorax width; TLTotal length; AME- anterior median eyes; ALE- anterior lateral eyes, PME-posterior median eyes, PLE- posterior lateral eyes; Fig-Figure.

Results and Discussion

Theridiidae Sundevall, 1833

Theridula Emerton, 1882

Theridula opulenta (Walckenaer, 1841) (Figure 1)

Description: Colour blackish with yellow colored spots towards posterior end of abdomen.Venter similarto dorsal side. Colour faded in preservative. Total length mm; 2.99 Carapace L 1.05; W 0.95; Abdomen L 1.94, W 2.42. Cephalothorax longer than wide, no patterns. Eyes heterogenous, eye region slightly elevated**,** eight in number arranged in two rows. PME white, larger than others. Ocular quadrangle wider anteriorly. Eye rims present. Distance between eyes: PME-PME 0.12; PME-PLE, 0.08; AME-AME, 0.23; PME-AME 0.09, PLE–ALE contiguous. Eye diameter: AME 0.14, PME 0.17, PLE 0.07, ALE 0.05. Maxillae and Labium colouration similar to ventral side of abdomen, chelicerae small, fags brown.Sternum heart shaped, wider near first coxa. Posterior end patterened with some light yellowish spots. Clothed with very fine hairs.Long slender legs. Light yellowish in colour. Clothed with fine black hairs. Measurements of palp and legs (Femur, patella, tibia, metarsus (except palp) and tarsus): Palp 0.46

[0.16, 0.12, 0.10, 0.08]; Leg I 1.84 [0.70, 0.25, 0.47, 0.25, 0.17], II 1.17[0.55, 0.19, 0.22, 0.14, 0.07], III 1.23 [0.36, 0.17, 0.29, 0.28, 0.13], IV 2.12 [0.69, 0.23, 0.44, 0.55, 0.21]. Leg formula; 4132

Abdomen black, with two yellow spots. Wider than long, clothed with very fine hairs. Venter similar to dorsal with two yellow spots towards the posterior end. Epigyne small, spermatheca round sclerotized facing each other**.** Copulatory duct open in a dark pit. **Habitat:** Collected from the bottom of a leaf, **Distribution:** North America, Southern Europe, India (New Record)

Theridula gonygaster (Simon, 1873) (Figure 2)

Description: Colour blackish with two white spots towards posterior end of abdomen.Ventral regionblack with white patch at the posterior end. Colour faded in preservative. Total length mm; 2.99 Carapace L 0.99; W 1.05; Abdomen L 2.00, W 2.95. Cephalothorax longer than wide ,no patterns. Eye region slightly elevated**.** Eyes Heterogenous, eight in number arranged in two rows. PME white, larger than others. Occular quadrangle wider anteriorly. Eye rims present. Distance between eyes: PME-PME 0. 21; AME-AME 0.49; ALE-AME 0.19, AME–PME 0.24, ALE-PLE contiguous. Eye diameter: AME 0.30, PME 0.32, PLE 0.23, ALE 0.24. Maxillae and Labium colouration similar to ventral side of abdomen, chelicerae small, fags brown.Sternum heart shaped, wider than long, wider near first coxa. Clothed with fine black

hairs. Legs long slender . Light yellowish in colour. Clothed with fine black hairs. Measurements of palp and legs (Femur, patella, tibia, metarsus (except palp) and tarsus): Palp 0.50 [0.17, 0.13, 0.11, 0.09]; Leg I2.01 [0.66, 0.25, 0.39, 0.50, 0.21], II 1.13[0.52, 0.18, 0.23, 0.13, 0.07], III

1.20[0.34, 0.15, 0.30, 0.27, 0.14], IV1.79 [0.72, 0.22, 0.44, 0.25, 0.16], Leg formula; 1432. Abdomen wider than long, diamond shaped with two white spots posteriorly, laterls and posterior ends pointed, dorsal portion convex, ventral region lighter in shade than dorsal region, sternum not equal in size. Epigyne small, spermatheca round separated by half of their diameter, their posterior borders joins (Levi, 1967c). **Habitat:** Collected from the bottom of a leaf

Distribution: Central and South America, Caribbean. Introduced to Europe, Congo, Madagascar,Seychelles, Georgia, China, Japan, India (New Record)

A. Dorsal view (Top view)
B. Dorasl view (Back view)
C. Ventral view
D. Sternum, chelicerae and Epigyne
E. Eyes
F. Epigyne

A. Habitat view
B. Dorasl view
C. Ventral view
D. Eyes
E. Sternum & Epigyne
F. Epigyne

1.***Theridula opulenta*** **(Walckenaer, 1841), 2.*Theridula gonygaster* (Simon, 1873)**

Conclusion

Even though the genus Theridula was established long back ago, no species of the genera was reported from India so far. The present paper reports two new Theridula species from Kerala. The present record seems to be significant because the report shows the new distributional ranges. The topography and climatic conditions of Kerala support a rich growth of theridiids. However, lack of relevant literature and small size of these creatures made them out of conservation network. Due to this many species remain unknown.

Acknowledgements

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Comparison of Odonata (Insecta) diversity in Kole wetlands and selected man-made ponds of central Kerala

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Abstract

Diversity of Odonata (dragonflies and damselflies) in Kole wetlands, a Ramsar site and twenty man-made ponds in central Kerala were studied and compared. A total of 44 species (30 dragonflies and 14 damselflies) belonging to 33 genera and eight families were recorded in the study, out of which 14 species were exclusively found in the wetlands. All the species recorded in the man-made ponds were also recorded from the wetlands.

Keywords: Insect diversity, ecosystem, Ramsar site, wetlands, biodiversity, conservation

Introduction

Dragonflies and damselflies (Odonata) are good indicators of the freshwater ecosystem health because of their amphibious life history, relatively short generation time, high trophic position, and diversity (Corbet 1993).They are beneficial insects because they prey upon agricultural pests and mosquitoes. They also act as prey for bigger animals in the food-web such as birds. A global decline of insect diversity is underway because of habitat loss, pollution, biological factors including pathogens, introduced species, and climate change (Sánchez-Bayo & Wyckhuys 2019). Because insects constitute the world's most abundant and speciose animal group and provide critical services within ecosystems, such an event cannot be ignored and should prompt decisive action to avert a catastrophic collapse of nature's ecosystems (May 2010). The situation urgently demands carrying out insect diversity studies in tropical countries like India, from where such information is lacking (Poorani & Abraham 2015). Wetlands of Kerala host rich biodiversity but are under pressure due to pollution, eutrophication, encroachment, reclamation and mining (Kokkal et al., 2008).Ponds are home to a diverse community of specialized plants and animals and are hence of great conservation concern. Through land-use changes, ponds have been disappearing rapidly and the remaining ponds are often threatened by contamination and eutrophication, with negative consequences for pond-dependent taxa like Odonata (Janssen et al., 2018).

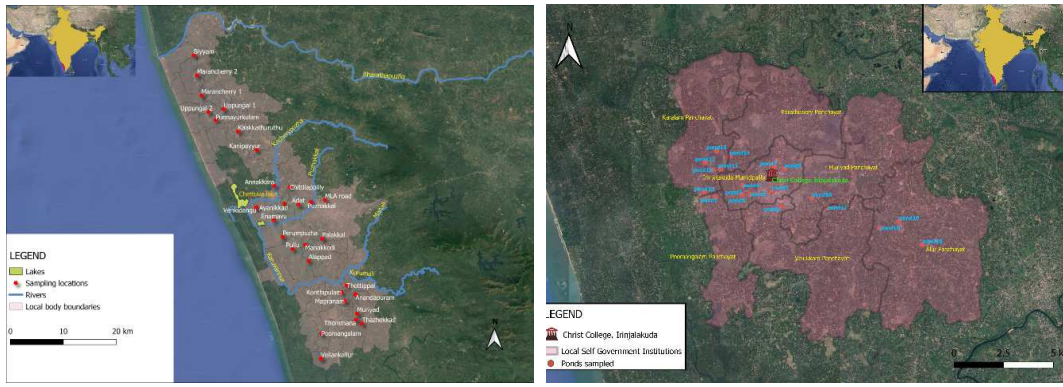
Material and Methods

Study period: September 2019 to February 2020 (6 months)

Study areas: The Kole wetlands are spread over Thrissur and Malappuram districts in Kerala, covering an area of 13,632 ha. They remain submerged under floodwater for about six months

in a year during the southwest monsoon. Paddy is cultivated in the post monsoon season after draining the excess water (Johnkutty & Venugopal 1993). Thirty sampling locations were chosen randomly intending maximum spatial coverage of the Kole wetlands (Figure 1).

Irinjalakuda is a municipal town in Thrissur District, Kerala, India. Irinjalakuda has a number of public and private ponds like most parts of the state. Twenty man-made ponds with public access were selected randomly in and around Irinjalakuda for sampling odonates (Figure 2).



Figures 1 & 2: Maps showing sampling locations in Kole wetlands and the ponds sampled

Visual Encounter Surveys (VES) were done in the sampling locations in fine weather between 9 AM and 3 PM. The observer walked along the edge of the water body at each location for 30 minutes and noted down the species richness and abundance of Odonata. The species were photographed with a Nikon Coolpix P-900 camera and identified using field guides (Subramanian 2005; Kiran & Raju 2013) and taxonomic monographs (Fraser 1933, 1934 & 1936). Species difficult to identify were caught using a sweeping net, examined closely using a hand lens, photographed and released.

For each sampling location, Shannon-Wiener diversity index was calculated using the

$$H' = -\sum_{i=1}^{S} p_i \ln p_i$$

where p_i is the proportion of individuals belonging to the *i*th species and S is the total number of species (Species richness).

Simpson's diversity index was calculated using the formula:

$$D = 1 - \sum_{i=1}^{N} (p_i)^2$$

where p_i is the proportion of individuals belonging to the *i*th species and N is the total number of individuals.

Results

A total of 44 species (30 dragonflies and 14 damselflies) belonging to 33 genera and eight

families were recorded in the study, out of which 14 species were exclusively found in the wetlands. All the species recorded in the man-made ponds were also recorded from the wetlands (Table 1).*Orthetrum sabina* was the most common species in the wetlands, while it was *Brachythemis contaminata* in the ponds. Species richness was higher for wetland locations (18.4 ± 4.53) compared to ponds (9.7 ± 3.45). However, both Shannon-Wiener and Simpson's diversity indices were significantly higher for the ponds (t = 4.45, p = 0.05 and t = 7.23, p = 0.05 respectively).

Sl No.	Name of the Species	Common English Name	Pond	Wetland
	Class: Insecta			
	Order: Odonata			
	Suborder: Zygoptera			
	Family: Lestidae			
1	*Platylestes platystylus* (Rambur, 1842)	Green-eyed Spreadwing	×	✓
	Family: Chlorocyphidae			
2	*Libellago indica* (Fraser, 1928)	Southern Heliodor	×	✓
	Family: Platycnemididae			
3	*Copera marginipes* (Rambur, 1842)	Yellow Bush Dart	✓	✓
	Family: Coenagrionidae			
4	*Aciagrion occidentale* Laidlaw, 1919	Green-striped Slender Dartlet	✓	✓
5	*Agriocnemis keralensis* Peters, 1981	Kerala Dartlet	✓	✓
6	*Agriocnemis pygmaea* (Rambur, 1842)	Pygmy Dartlet	✓	✓
7	*Ceriagrion cerinorubellum* (Brauer, 1865)	Orange-tailed Marsh Dart	✓	✓
8	*Ceriagrion coromandelianum* (Fabricius, 1798)	Coromandel Marsh Dart	✓	✓
9	*Ischnura rubilio* Selys, 1876	Western Golden Dartlet	✓	✓
10	*Ischnura senegalensis* (Rambur, 1842)	Senegal Golden Dartlet	✓	✓
11	*Paracercion calamorum* (Ris, 1916)	Dusky Lilly-squatter	✓	✓
12	*Pseudagrion australaisae* Selys, 1876	Look-alike Sprite	✓	✓
13	*Pseudagrion decorum* (Rambur, 1842)	Three-lined Dart	×	✓
14	*Pseudagrion microcephalum* (Rambur, 1842)	Blue Grass Dart	✓	✓
	Suborder: Anisoptera			
	Family: Aeshnidae			
15	*Anax guttatus* (Burmeister, 1839)	Pale-spotted Emperor	×	✓
16	*Anax indicus* Lieftinck, 1942	Lesser Green Emperor	×	✓
	Family: Gomphidae			
17	*Ictinogomphus rapax* (Rambur, 1842)	Indian Common Clubtail	✓	✓
18	*Paragomphus lineatus* Selys, 1850	Common Hooktail	×	✓
	Family: Macromiidae			
19	*Epophthalmia vittata* Burmeister, 1839	Common Torrent Hawk	✓	✓

	Family: Libellulidae			
20	*Acisoma panorpoides* Rambur, 1842	Trumpet Tail	✓	✓
21	*Aethriamanta brevipennis* (Rambur, 1842)	Scarlet Marsh Hawk	✓	✓
22	*Brachydiplax chalybea* Brauer, 1868	Rufous-backed Marsh Hawk	✓	✓
23	*Brachydiplax sobrina* (Rambur, 1842)	Little Blue Marsh Hawk	×	✓
24	*Brachythemis contaminata* (Fabricius, 1793)	Ditch Jewel	✓	✓
25	*Bradinopyga geminata* (Rambur, 1842)	Granite Ghost	✓	✓
26	*Crocothemis servilia* (Drury, 1770)	Ruddy Marsh Skimmer	✓	✓
27	*Diplacodes nebulosa* (Fabricius, 1793)	Black-tipped Ground Skimmer	×	✓
28	*Diplacodes trivialis* (Rambur, 1842)	Ground Skimmer	✓	✓
29	*Hydrobasileus croceus* (Brauer, 1867)	Amber-winged Marsh Glider	✓	✓
30	*Lathrecista asiatica* (Fabricius, 1798)	Asiatic Bloodtail	×	✓
31	*Neurothemis tullia* (Drury, 1773)	Pied Paddy Skimmer	✓	✓
32	*Orthetrum chrysis* (Selys, 1891)	Brown-backed Red Marsh Hawk	✓	✓
33	*Orthetrum pruinosum* (Burmeister, 1839)	Crimson-tailed Marsh Hawk	×	✓
34	*Orthetrum sabina* (Drury, 1770)	Green Marsh Hawk	✓	✓
35	*Pantala flavescens* (Fabricius, 1798)	Wandering Glider	✓	✓
36	*Potamarcha congener* (Rambur, 1842)	Yellow-tailed Ashy Skimmer	×	✓
37	*Rhodothemis rufa* (Rambur, 1842)	Rufous Marsh Glider	✓	✓
38	*Rhyothemis variegata* (Linnaeus, 1763)	Common Picturewing	✓	✓
39	*Tholymis tillarga* (Fabricius, 1798)	Coral-tailed Cloudwing	×	✓
40	*Tramea limbata* (Desjardins, 1832)	Black Marsh Trotter	✓	✓
41	*Trithemis aurora* (Burmeister, 1839)	Crimson Marsh Glider	×	✓
42	*Trithemis pallidinervis* (Kirby, 1889)	Long-legged Marsh Glider	✓	✓
43	*Urothemis signata* (Rambur, 1842)	Greater Crimson Glider	✓	✓
44	*Zyxomma petiolatum* Rambur, 1842	Brown Dusk Hawk	×	✓

Table 1 : Check list of Odonata recorded from kole wetlands and the selected ponds

Discussion

Even though they are human-made/altered ecosystems, both the ponds and the Kole wetlands host rich odonate fauna. 25% and 17% of the odonate species recorded from Kerala were seen in Kole wetlands and ponds respectively (Society for Odonate Studies 2020). The endemic *Agriocnemis keralensis* was found from both habitat types, while the rare *Platylestes platystylus* was found to be an exclusive wetland-dweller. The wetlands have greater species richness probably because they offer more microhabitats in the form of grass beds, canals, marshlands etc. Also, while the ponds are entirely lentic, wetlands have both lentic and lotic regions. Ponds score higher in the diversity indices because the species show greater evenness in ponds, while in the Kole wetlands a few species such as the migratory *Pantala flavescens*

were seen in disproportionately large numbers. Pollution caused by waste dumping was evident in both ecosystem types, but the wetlands had the additional burden of agrochemical pollution. Despite this, dominance of the pollution indicator dragonfly, *Brachythemis contaminata* (Subramanian 2005) was seen in the ponds probably because they are closed systems having higher concentrations of pollutants. Further studies on the ecology of Odonata in both Kole wetlands and the ponds are necessary to understand how they can be used as indicators of pollution and ecological change.

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Taxonomic studies on shrimps of Kannur district, Kerala

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Abstract

Decapods including the most commercially important crustaceans such as prawns, crabs and crayfish have the most diverse range of body forms. The present study analyzed the taxonomy of shrimp species in estuarine and marine waters of Kannur district. Collections were made from Kannur, Valapattnam, Thalassery, Darmadam and Muzhappilangadufrom March 2018 to July 2018. The specimen identified belong to three families, Penaeidae, Palaemonidae and *Alpheidae*. Identified penaeidan shrimps include the genera, *Fenneropenaeus, Metapenaeus, Penaeus*, *Parapenaeopsis* and T*rachypenaeus*. One identified specimen belonging to the family Palaemonidae, include the genus *Palaemon*, which could not be identified at species level. The identified specimen belonging to the family Alpheidae also could not be identified at genus/species level. Regularly updated checklists on regional biodiversity would aid in the efficient conservation plans in shrimp diversity.

Keywords : Taxonomic study, decapods, diversity

Introduction

Decapods are one of the most important groups of benthic macrofauna. They range from inconspicuous to large forms, including shrimps, crayfishes, lobsters and crabs and are distinguished by their high diversity and importance in fisheries and trophic dynamics. Decapod crustaceans are found richly in the marine, estuarine, and freshwater bodies. Among decapods, shrimp and prawn fauna constitute a diverse group of crustaceans (Thorp and Covich, 2009).

The body of shrimp is divided into cephalothorax and abdomen (Figure 1). Head includes a pair of compound eyes, mandibles, antennae, a pair of maxillipeds and 5 pairs of pereopods. Head is protected by a sheet called carapace. The front of the carapace tapered to form rostrum. Cephalothorax consisted of 13 segments. The abdomen possessed 6 segments. Each segment encompasses a pair of swimming feet called pleopods, 6^{th} segment changing its form into a tail fan called uropod. Above the uropod, there is a tail that tapered at the edge called telson. Abdominal segments are connected one another by a thin membrane (Marin, 2012).

Sexual dimorphism is a dominant condition between species of shrimps (Figure 2). The female shrimp is usually larger than male. Male shrimps are identified by the large copulatory organ, known as the petasma that found between the foremost pair of swimming legs. Corresponding structures are not present in the females and the

foremost pairs of pleopods are identical with the other four pairs. Female shrimp possesses a structure known as thelycum that found in between the last pair of pereopods which provides an anchor site for the male sperm packet received during copulation (Marin, 2012).

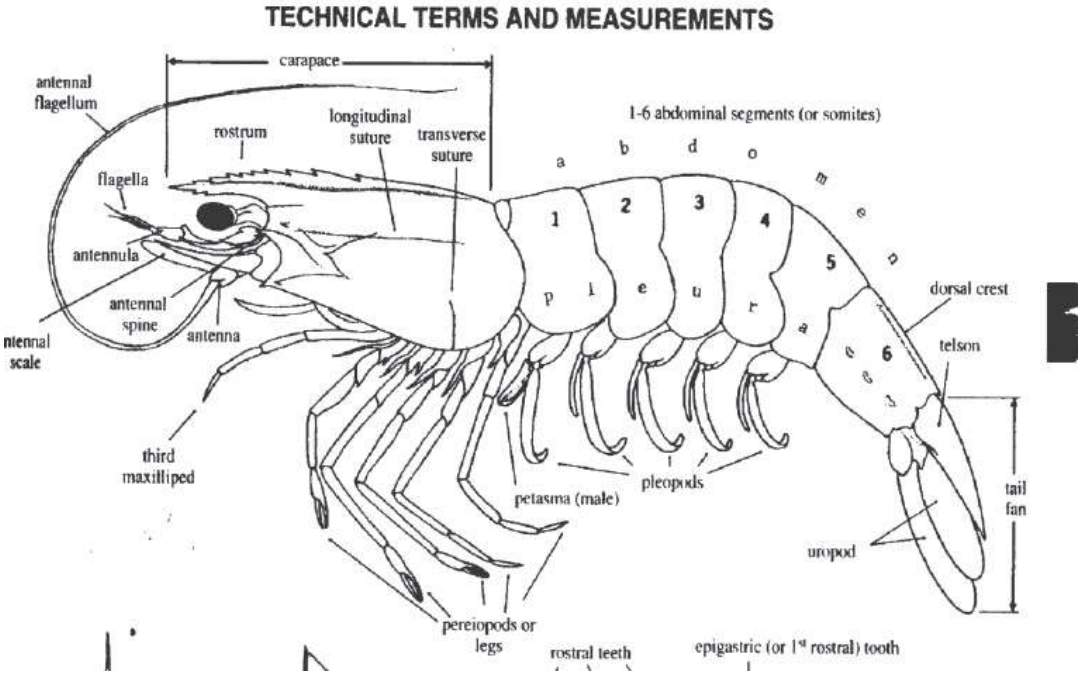

Figure - 1: Diagrammatic sketch of a typical penaeid shrimp

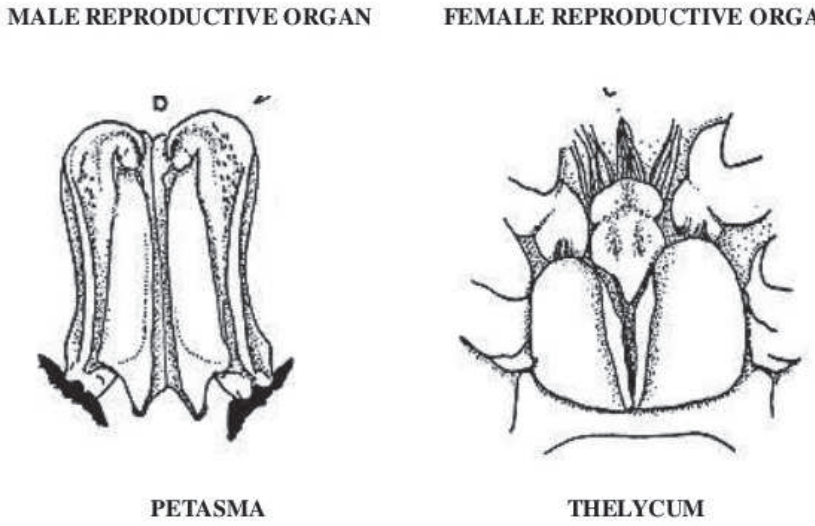

Figure – 2 : Diagrammatic sketch of male and female reproductive organ of shrimps

The caridean fauna inhabiting the marine, estuarine and freshwater ecosystems of India are diverse and fairly well known. Significant contributions to systematics of prawns of Indian region were made by Lester and Sewell (1934), Doughtie and Rao (1984) and Rajool Shanis et al., (1996). As reviewing through literature, it was noted that there are many studies on the prawn resources of Kerala waters. However, very few studies addressed the taxonomy and diversity of shrimp species in estuarine and marine ecosystems of Kerala. The present study analyzed the taxonomy and diversity of shrimp species inhabiting the estuarine and marine waters of Kannur district which would be a baseline data for further studies in this area. Regularly updated checklists on regional biodiversity would aid in the efficient conservation plans in shrimp diversity

Materials and Methods

Collections were made from five research stations for a period from March 2018 to July 2018. Samples were collected from five different sites which include Kannur, Valapattnam, Thalassery, Darmadam and Muzhappilangadu. Collections were made with the help of local fishermen of these areas and by using cast nets and also by using dragging a rectangular piece of mosquito nets (2m x 1m) along the banks. Specimens preserved in 5% formalin and identified upto species level (Farfante, 1988; Flach and Bruin, 1994)

Results

A total of 85 specimens were collected from the study areas of Kannur district. And the specimen identified belong to three families, Penaeidae, Palaemonidae and Alpheidae. Identified *penaeidan shrimps include the genera, Fenneropenaeus, Metapenaeus, Penaeus*, *Parapenaeopsis* and T*rachypenaeus*. The genus *Palaemon*, belonging to the family Palaemonidae and one specimen belonging to the family Alpheidae, could not be identified at species level.

Family Penaeidae : - Penaeid shrimps are widely distributed in the tropical and sub tropical areas of the world. They are particularly abundant in South East Asia. Body of the shrimps are almost always laterally compressed, the rostrum usually compressed and toothed and the abdomen long, longer than the carapace. Antennules and antennae are generally large and plate-like. The pereiopods or legs are usually slender, but in some, a single leg or pair of legs may be stout and some pereiopods end in pincers or chelae. The pleopods or abdominal appendages used for swimming are well developed and except in a few species, are present on all five anterior abdominal segments.

Fenneropenaeus indicus (Common name: Indian white prawn and Local name: Naaran chemeen/ vellaran chemeen):- Uniformly glabrous carapace and abdomen. Hepatic and antennal spines on carapace but no orbital spine. Sigmoidal, curved rostrum armed with seven or eight dorsal and five or six ventral teeth. Telson without lateral spine. Shallow convexed rostral crest; adrostral carina reaches epigastric teeth; well defined gastro-orbital

carina, which occupies 2/3 distance between hepatic spine and margin of carapace. Telson without lateral spine. Antennal flagellum yellow or greenish yellow. Posterior half of uropod yellow.

Metapenaeus affinis(Common name: Jinga Prawn and Local name: Kazhanthan chemmeen):- Rostrum bears 9 teeth on dorsal (epigastric tooth included) margin. No tooth on its ventral margin .Adrostral carina and sulci absent. Only basial spine present on second and third pereiopods. Merus of fifth pereiopod bears a protrusion on poste-rior margin . No cicatrix on fifth or sixth abdominal segment. Posterodorsal spine present and no dorsolateral sulcus on six abdominal segment. Telson with dorsal groove throughout its length and without fixed or movable spine. Body pale greenish to pale pinkish or pink brownish with green or red brown specks.

Metapenaeus dobsoni (Common name : Flower tail prawn/ Pink shrimp and local name: PoovalenChemeen / Thelly chemmeen):-Body pubscent with small patches. Rostrum long, extended beyond antennular peduncle and armed with 7-9 dorsal teeth, almost half of its distal half toothless. Adrostral crest reaching as far as epigastric tooth. Telson armed with spinules. Petasma with each distomedian projections form short filament or tubular structure culminating in a pair of simple distomedian spouts. Distolateral projections directed forward. Thelycum having long tongue shaped anterior plate bearing a groove, lateral plates horse shoe-shaped.

Metapenaeus monoceros :- (Common name : Brown Chemmeen / Speckled chemmeen and Local name : Choodan chemmeen/ Kuzhiyan chemmeen):- Body pubescent, often small patches /stripes present in larger specimens. Dorsal part of rostrum armed with 9-12 evenly placed teeth. Adrostral crest extended beyond second rostral tooth, adrostral groove reaching behind epigastric tooth. Telson without spinules. Ischial spine of first walking leg distinct. Distomedian projections of petasma convoluted, swollen and bulbiform hiding distolateral projection. Anterior plate of thelycum long and deeply grooved having small ball like structure at both ends; lateral plates very small, egg shaped surrounded by large raised lateral margins.

Metapenaeus sp.:- Integument variably pubescent, sometimes almost entirely glabrous, rostrum armed with dorsal teeth only; epigastric tooth often conspicuously separated from first rostral tooth; orbital and pterygostomian spines lacking; antennal and hepatic spine pronounced; gastro-orbital carina absent; hepatic sulcus anterior to hepatic spine well defined and accompanied by ventral carina often descending almost vertically from spine then turning towards pterygostomial angle; sulcus posterior to hepatic spine ill-defined or absent; telson lacking fixed subapical spines but bearing movable, sometimes minute and very numerous posterolateral once. Antennular flagella shorter than carapace; fifth pereiopod modified in male; Petasma symmetrical, semiclosed, depressed with median lobes usually produced into simply cureved, hoodlike or convoluted distal projections; Thelycum closed with paired

lateral plates of sternite XIV often continous across sternite, usually more or less enveloping posterior end of elongate median protuberance of sternite, XIII.

Parapenaeopsis stylifera (Common name : Kiddi Prawn and Local name: Karikkadi chemmeen):- Body smooth. Rostrum sigmoidal, sharply bent upward, distal half toothless rest part armed with 6-8 dorsal teeth. Epigastric tooth present. Telson armed with 1-2 pair of fixed spines. Distolateral projections of petasma slender, horn like and straight. Thelycum square-cut and concave with a slender stem like posterior process.

Penaeus mondon (Commom name : Tiger Prawn and Local name: Kaara chemmeen):- Rostrum well developed and toothed dorsally and ventrally. Carapace without longitudinal or transverse sutures. Cervical and orbito – antennal sulci and antennal carinae always present. Hepatic and antennal spines pronounced. Pterygostomain angle round. Stylocerite at first antennular segment. Basial spine on first and second pereiopods and exopods on the first to fouth pereiopods ususlly present. No fixed subapical spines on telson. Adrostral sulcus and carina short, not reaching posteriorly beyond mid length of carapace. Gastrofrontal carina absent. Female have closed-type thelycum. Petasma in male symmetrical with thin mediums lobes. Most distinct feature of identification of this species; fifth pereopods without exopod; hepatic carina horizontally straight; and gastro orbital carina occupying posterior half of distance between hepatic spine and postorbital margin of carapace. Body colour vary from green, brown, red, grey, blue and transverse band colours on abdomen and carapace alternated between blue or black and yellow.

Penaeus semisulcatus (Common name : Green Tiger Prawn and Local name: Kaara chemmeen):- Rostrum with 7 or 8 dorsal teeth and 3 ventral teeth.Atrodrostral crest and groove; carina, extends beyond epigastral tooth with post-rostral carina almost reaching to rear of carapace. Body color pale brown body which sometimes shows a greenish tint on the carapace with two yellow or cream transverse bands across the back of the carapace. The abdomen is banded with brownish grey and pale yellow transverse bands, while the antennae are banded brown and yellow. Uniformly smooth carapace and abdomen

Trachypenaeus curvirostris (Common name : Southern Rough Shrimp):- Rostrum armed with 7 to 11dorsal teeth, reaching distal half of second antennular article or little beyond, usually up tilted, straight or up curved; cervical groove very feeble; hepatic groove discernible; longitudinal suture short; pterygostomian angle blunt or sharp; abdomen with a small median tubercle on second segment and a mid-dorsal crest on last 4 segments; telson armed with 3 or 4 pairs of small movable lateral spines subequal in size; epipod present on first 3 pereopods; fifth pereopod usually well exceeding antennular peduncle, but in some populations reaching only second article of it. Petasma with broad,wing-like distolateral projections, directed laterally and curved dorsoventrally; distomedian projections small, curved ventrally. In females, anterior plate of thelycum concave anteriorly, with a middle groove posteriorly and

a bluntly pointed anterior margin; notched anteromedially; in fertilized specimens groove can be hidden and notch obliterated; coxae of 4th pereopods often with a small projection, always densely fringed with setae. Body pink to reddish brown, sometimes whitish on sides; abdominal crest whitish; pereopods white with some pink; pleopods white with red patches or reddish brown; uropods bright red to reddish brown, sometimes dark brown with distinct white margins

Family Palaemonidae:- Palaemonid shrimps live in a wide range of environments, from freshwater to the deep ocean. They have a long rostrum with teeth above and below. Their first two legs are chelate (clawed), second legs being larger and longer than the first. Carpus (segment next to claw) is undivided, and second abdominal segment overlaps first and third segment. Most are small, rarely reaching longer than 5cm , but some get large enough to be of some commercial value. This family includes many transparent or nearly transparent species. Many are commensal (living on or in another organism without causing harm to the host organism), attracted to hosts such as sponges, corals and anemones. More colourful ones able to alter their color to match their background or hosts.

Palaemon sp.:- Head and thorax protected by a relatively thin carapace which, as in many species of prawns and shrimps, drawn out into a projection between eyes known as rostrum. Distinctive rostrum can be used to distinguish common shrimps from other species. In this species rostrum curves upwards, divided in two at tip with six or seven teeth along its upper surface and four or five teeth on under side. First five segments of abdomen bear fringed appendages known as pleopods or swimmerets that used to propel this shrimp through water.First three appendages on thorax modified for use in feeding, and remaining five pairs known as pereopods. First and second pair of pereopods tipped with pincers.

Family Alpheidae:- They lives from intertidal zone to great depths occurring especially in coastal tropicaland sub tropical ecosystems such as estuaries, mangroves and coral reefs. First pair of pereiopods very asymmetric, and such asymmetry originates in the juvenile phase. Major chela of this pair of appendages extremely very well developed, with fixed and mobile fingers of a rather peculiar morphology (tooth-cavity system). Chela produces a snapping sound which one of the most audible sounds in the environments where these animals live. This cheliped is used for prey capture and in agonistic interactions.

Discussion and Conclusion

The diversity range of descapod crustaceans varies by spatial differences in environmental and oceanographic conditions, particularly by depth, bottom type and characteristics of the water masses (Carbonell and Abello,1988). In India, shrimp species have been recorded from both shallow and deep waters.

The present study analyzed the taxonomy and diversity of shrimps distributed in the coastal regions of Kannur district. In the present study, a total of 13 species of shrimps belonging

to six genera under three families such as Penaeidae, Palaemonidae, and Alpheidae were recorded. The highest contribution to species diversity was from the family Penaeidae (76.9%) containing five families and 10 species. Similar reports were made by Mogalekar (2015), and he reported about 7 species of Penaeid shrimps belonging to 2 genera, from Vembanad Lake, Kerala. Immanual et al. (2012) published an annotated checklist of the penaeoid, sergestoid, stenopodid and caridean shrimps of India and they recorded a total of 437 species out of which, 343 species were marine forms and 94 were freshwater. This is the latest checklist available for the shrimps along the Indian coasts. Since the present study was limited for a period of 6 months, extensive exploration could not be made. Even through the diversity of marine shrimps is very rich, a very small portion of the same is documented in the present study. There is ample scope for the diversity study of crustaceans. So the baseline data derived from the present study can be used for further research

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Studies on the bio control potential of *Amblyseius dioscoreae* to manage the two spotted spider mite - *Tetranychus urticae* (C. L. Koch) - infestation in Lac insect host plant - *Flemingia macrophylla* (Willd.) Merr.

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Abstract

Though India is the leader producer of lac in the world, selection of lac insect host plant according to the topographic and climatic condition of an area is very important in lac cultivation. *Flemingia macrophylla* is an Angiosperm plant in the family Fabaceae which is one of the most suitable plants for lac cultivation in Kerala. *Amblyseius dioscoreae* – a member of the Phytoseiidae family which comes under genus Amblyseius noted to be an effective bio control agent against *Tetranychus urticae* (two spotted spider mite) which causes serious threat to *F.macrophylla* by sucking the plant fluid from the foliage. This study attempted to elucidate the bio control potential of *A.dioscoreae* in controlling the pest *T.urticae* so as to avoid the use of chemical pesticide to manage the pest and would ensure the quality of host plant which in turn would help the production of qualitative and quantitative increase in the lac production.

Key words: Angiosperm, Bio control agent, Chemical pesticide.

Introduction:

India is the largest producer of lac in the world. Lac is produced by the phytophagus insect, *Laccifera lacca* which comes under Kerriidae family. It secretes true lac, the only natural resin of animal origin with immense industrial applications. Nearly 70% of the lac produced in India is exported. According to the difference in the climatic and topographic conditions of an area, selection of host tree is very important in lac cultivation. The quality of lac mainly depends on the variety of the host tree on which they feeds. *Flemingia macrophylla* commonly known as Kamatteri is an Angiosperm plant in the family Fabaceae which is one of the most suitable host plants for lac cultivation in Kerala. *Tetranychus urticae* is known as the pest of *F.macrophylla* which causes serious threat to *F.macrophylla* by sucking the plant fluid from the foliage. Heavy infestation causes discolouration, reduce the photosynthetic ability and premature drop of foliage. Under this circumstances *Amblyseius dioscoreae* – a member of the Phytoseiidae family which comes under genus Amblyseius noted to be an effective bio control agent against *Tetranychus urticae* (two spotted spider mite). This study

attempted to elucidate the bio control potential of *A.dioscoreae* in controlling the pest *Tetranychus urticae* so as to avoid the use of chemical pesticides in managing the pest.

Materials and Methods

Samples of predatory mites (*Amblyseius dioscoreae*) and their target pest (*Tetranychusurticae*) were collected from the Lac Insect Germplasm Centre of Kerala Forest Research Institute (KFRI) located at Peechi, in Thrissur District. Samples of infested leaves having mites were put in self sealing plastic bags for subsequent screening of predatory and pest mites in the laboratory. At the laboratory individual leaves was thoroughly examined under a Leica stereo zoom microscope (magnification ranging from 10x to 40x) for the recovery of the predatory as well as the pest mites. Successful rearing and maintenance of sufficient stock cultures of both mites in the laboratory were carried out by following the leaf flotation technique. Prey mites were provided to the predatory mites during the rearing period. Renewal of leaf disc was carried out at an interval of 2 – 3 days and regular supply of prey mites was ensured. Dehydrated speciemen of mites were slide mounted in Hoyer's medium for identification. Studies were conducted by placing the cultures of *A.dioscoreae* at room temperature (33^0c) and experimental temperature (20^0c) for analysing the influence of temperature in feeding rate of the predatory mite.

Result and Discussion:

Amblyseius dioscoreae belongs to Phytoseiidae family characterised by four pairs of long legs. Adults are pear shaped, transparent to slightly yellowish orange with an unsegmented body, completes its life cycle in five stages, which were identified based on their metabolic and physical activities, body size and frequency of their resting period. Different behavioural characters of *A.dioscoreae* were observed during the study. High intensity of searching behaviour (zig – zag motion) was shown by adults compare to immature stages. Presence of trichomes and tiny water drops on the leaf blade act as barrier which deflects them from their searching direction of interest. The activity and locomotion of *A.dioscoreae* was found to be reduced under lower and higher temperatures. *A.dioscoreae* feeds on all stages of the two spotted spider mite including eggs, larva, nymphal and adult stages. Majority of them feeds on the juvenile stages of *T.urticae* which were easy to cut the cuticle and also to locate them due to poor web production of pest mites as compared to adult mites. Hence the level of pest mites can be controlled to an extent by application of *A.dioscoreae* in early stages of pest infestation. Adult males of *A.dioscoreae* are slightly smaller than females. As they attain maturity the male predatory mites compete among themselves to find healthy females for mating. Duration of copulation ranges from 30 to 70 minutes during which the sperms were transferred to spermatheca of the female through insemination. Within 2 -3 days after internal fertilization, eggs are laid on trichomes (adaptation to avoid egg predators) by severe contraction of their body parts. Mature eggs were seen in the body of adult females by using stereo zoom microscope.

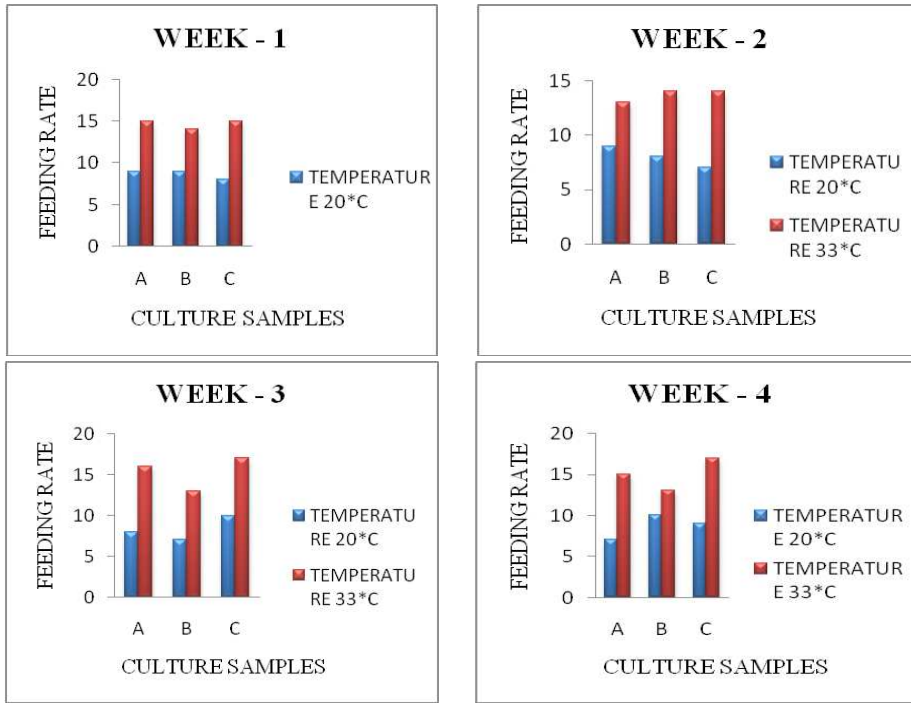
Temperature plays an important role in metabolic activities of predatory mites. Decrease in temperature results in decrease of hunger level and the reduction of searching behaviour in

A.dioscoreae. This result had been observed from the less feeding rate of *Amblyseius dioscoreae* on *Tetranychus urticae* under controlled condition (20^0c).

Conclusion:

The study shows the *Amblyseius dioscoreae* is an effective control against the *Tetranychus urticae* on the plant *Flemingia macrophylla*. But temperature plays an important role in the effectiveness of predatory mites against the prey. The feeding rate of *A.dioscoreae* is directly influenced by ambient temperature. Thus in high altitudes where temperature is low, results the effectiveness of this bio control agent may decline. Level of pest mites can be controlled by the application of *A.dioscoreae* in the early stages of pest infestation since majority of them prefers to feeds on juvenile stages of *T.urticae*. This study has shown that the use of *A.dioscoreae* during the early stages of the two spotted spider mites infestation can prevent the loss of foliage of the lac insect host plant - *F.macrophylla*. The result would help in avoiding the use of chemical pesticides to manage the pest and would ensure quality of the host plant which in turn would help the production of qualitative and quantitative increase in lac production.

Influence of temperature on feeding rate of *Amblyseius dioscoreae*



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Deep scattering layer of South Eastern Arabian Sea

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Abstract

Midwater sound-scattering layers containing aggregations of zooplankton andmicronekton prey form in response to a trade-off between predator avoidance atdepth and optimal foraging near the surface. Although the volume backscatterstrength of zooplankton aggregations have been extensively studied in the past, fewer studies have specifically examined other descriptive characteristics of these layers such as depth of layers, timing of migrations, and the presence of secondary scattering layers below the main scattering layer. In the present study, patterns of deep scattering layers (DSLs) were characterized using Acoustic surveys using SIMRAD EK 60 with either 38 KHZ or 120 KHZ depending up on the depth of DSL in South Eastern Arabian Sea.

Keywords: Deep scattering layer, Nekton, biomass, diurnal vertical migration, South Eastern Arabian Sea

Introduction

Oceans are the site for quite few unusual phenomena on whichour awareness is limited. One such Occurrence is the migratory Deep scattering layer (DSL) of mesopelagic and epipelagic depths in all world Oceans (Duvall and Christensen 1946; Eyring et al., 1948; Raitt 1948).DSL in the ocean was first recognized in 1942 and has since been found to be widespread in most of the major oceans except the Arctic and Antarctic (Dietz, 1948; Tucker, 1951). Marine organisms aggregate at specific depths in the ocean and these organisms can be documented as a scattering layer on the echogram of an echosounder. Thislayer has been observed in all the oceans (Sameoto et al., 1985). The DSLorganisms, which ascend around dusk and descend around dawn, apparently reflect the predator-prey tracking (Hays 2003). Often discrete layers are evident at different depths, each layer composed of different species or developmental stages. The DSL is a layer of living organisms, ranging from macrozooplankters like amphipods, chaetognaths, copepods, doliolids, euphasiids, isopods, lucifers, medusa, ostracods, pteropods, salps, siphonophores, jelly like substances, larval forms like alima larvae, decapod larvae, Phyllosoma larvae etc., and micro nektons such as pelagic shrimps, crabs, cephalopods, leptocephalus, fish juveniles and mesopelagic fishes belonging to families myctophidae, photicthyidae, gonostomidae, sternopthydiae, bregmacerotidae, melanostomidae, stomidae, astron esthidae, nemicthydae, trichiuridae, idiacanthidae etc. (Menon 2002). Daniel et al. (1969) made a preliminary study of the faunal components of the DSL in the Bay of Bengal in India waters. Majority of the zooplankton groups of DSL

which form food for several crustaceans, molluscs, fish and marine mammals are known to make extensive diurnal vertical migrations in response to light and other physico-chemical characteristics of the environment (Madhupratap et al., 1996). The first major attempt to study the quantitative distribution and abundance of zooplankton of the Indian Ocean was by the International Indian Ocean Expedition (IIOE) during 1960-65. Apart from the IIOE, many other intensive but localised surveys for zooplankton have been carried out. *R. V. Varuna* investigated the shelf and oceanic waters off the southwest coast of India (Ramamirtham and David Raj, 1981). Mathew et al. (1990a) conducted a detailed study of the zooplankton biomass and secondary and tertiary production of the EEZ of India. The purpose of this study is to show DSL variation, in South Eastern Arabian Sea (SEAS) with time as a wide ranged oceanic phenomenon.

Materials and Methods:

The sampling sites represent 45 stations (2012-2016) across the South Eastern Arabian Sea (figure 1) open ocean with spatial and depth variability depending up on the thickness and Day and night migration of Deep scattering layer.Acoustic surveys using SIMRAD EK 60 with either 38 KHZ or 120 KHZ depending up on the depth of DSL. Trawl depth were identified parallel to the Deep scattering layer depth and thickness, either during day or during night and scanning was performed prior to all operations in order to get a clear picture about the Deep scattering layer. All the cruises during the survey were multidisciplinary in nature and therefore Continuous monitoring of Deep scattering layer was not possible due to the operations of RDI 75kHz Ocean Surveyor Acoustics Doppler Current Profiler (ADCP) causing frequency interferences with EK-60.

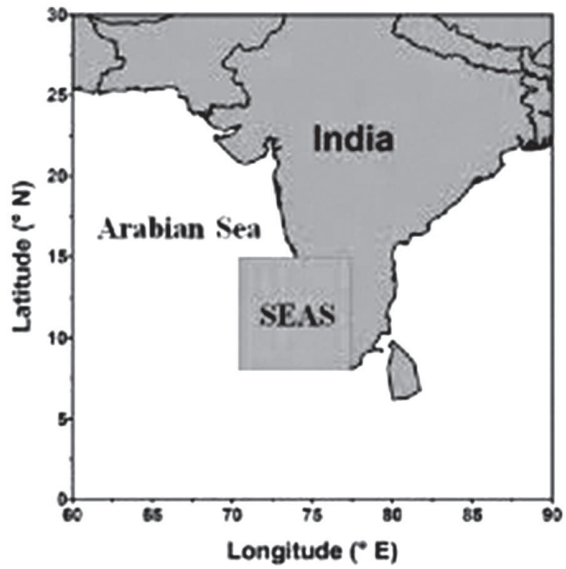

Figure 1: South Eastern Arabian Sea

Results:

Echo sounder records show that many myctophid species aggregate in compact layers, especially during daytime when they are relatively quiescent. These aggregates are the primary component of the dense DSL. Their densities, which correspond to concentrations of five-ten individuals per cubic meter and trawl catches of up to 10 to 20 t/hr have led to commercial fishery feasibility trials (FAO 1997a).

Depths of DSL at various trawl stations in SEAS are given in Figure 1. At all such depths the thickness of the primary DSL was estimated from echograms. Most of the trawl operations were conducted in the primary DSL during the mornings/ evenings and night, when the DSL was relatively shallow and trawl operations were much easier and efficient. Maximum density of myctophids was recorded in the depth ranges 200-600m during day and 20-90m during night, in SEAS. In SEAS after sunset between 18.00hrs to 6.00 am dense thick layer of DSL (N1) was seen below surface which extended up to 120m. During day time one to two and rarely three layers were observed at three to four stations. The D1 layer was below 100m and D2 layer was below 200m. However at the remaining stations the DSL layer was seen below 200m which extended up to 600m in some stations (figure 2).

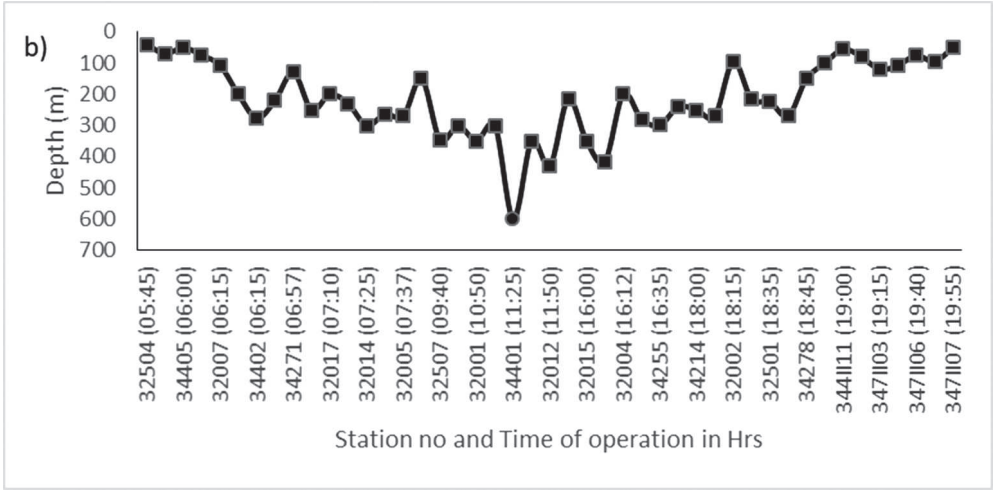

Figure 2 : Occurrence of DSL in each depth with regard to time in b SEAS

Discussion:

Diel vertical migrations might have developed as a means of escaping predation by surface predators and/or vertical migration may have evolved simply because there is not sufficient food at mesopelagic depths to support the organisms that live there. They must return to the photic zone to take advantage of higher levels of primary production. In addition, by feeding in surface warmer waters and then returning to the deep colder waters to digest food, energy is conserved because metabolic rates are slower at low temperatures (Catul et al., 2011; Dypvik et al.,2012 a,b).Migrating patterns of DSL organism vary with species, size groups,

life history stages, sex, latitude, time and season. Nektons form part of the Oceans Deep Scattering Layer (DSL) that are vertically narrow (hundreds of m) but horizontally extensive (continuous for thousands of km) layers containing fish and zooplankton and are readily detectable by echo sounders (Fay and Mckinley 2014). Nektons play significant roles in the marine food web, acting as both prey and predator. As predator mesopelagic nektons primarily feed on crustaceaous zooplankton like copepods, ostracods and euphasids etc and in turn lantern fishes form the prey of numerous fishes, seabirds and marine mammals (Schneider *et al.*,1984; Pearcy *et al.*,1988; Ohizumi *et al.*,1998; Beamish *et al.*,1999).

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Waste management in campus

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Abstract

Campus solid waste management is always a problem in every campus. The proper awareness among the teaching and non teaching faculty is a need to prevent epidemics. Waste collection,disposal methods must be adopted to reduce this problem. Green audit is also an important measure to overcome this.

Keyword: - Green audit, Solid waste management

Introduction

Waste is often regarded as consisting of materials that are no longer considered valuable and which are subsequently disposed of (Tchobanaglous, 1993). Solid waste is something that is to be disposed off. Anything like paper, garbage, food waste, plastic etc can be classified under solid waste .Nowadays campuses are filled with these wastes and their proper disposal is a problem faced by every management. Very few of the campuses concentrate on the proper disposal of these wastes.

The best way to combat this problem is to educate and give awareness to the faculty and students regarding the waste management .Green audit an example of such a measure. The aim of green audit is to review the measures taken by the institution to combat pollution. Green Audit can be defined as systematic identification, quantification, recording, reporting and analysis of components of environmental diversity. The 'Green Audit' aims to analyze environmental practices within and outside the college campus, which will have an impact on the eco friendly ambience.

Waste management strategies

Sources:

The first step in waste management is to gain an understanding of the waste types being generated in order to design appropriate collection and disposal strategies. The types of waste generated from various is as follows:

1. Class rooms

   Paper, plastic (Polythene covers, PET bottles, Wrappers-chocolate and chips), aluminum foil, pens, disposable cups, metal cans, Charts, Cardboards, thermo cols.

2. Laboratories

   Paper, plastic (polythene covers, plastic bottles), Glass (slides, cover slips, glass bottles, blotting papers, tissues, syringes, organic wastes such an tissue remains, blood, plasma, culture media, electrophoretic gels, chemicals etc

3. Staffrooms

   Paper, plastic (polythene covers, plastic bottles, disposable containers)
4. Office

   Paper, plastics and e-waste
5. Canteen

   Paper, plastic, wrappers, paper boxes, disposal cups, PET bottles, metal cans, glass bottles.
6. Library – Paper, plastics, e-waste
7. Toilets -Paper, plastic, and sanitary napkins

Collection:

The next step is segregation of wastes. The wastes from different sources must be collected according to the type and sorted as wet waste, dry waste, plastic waste, metal wastes, recyclable waste, and biodegradable waste such as food waste. The waste can be collected in different bins ie. Different coloured bins can be used for sorting out the wastes. After segregation the types of waste is treated.

Treatment methods:

Treatment schemes can be adopted according to the type of waste. Dry waste like paper can be recycled converted into books with sheets can be made available in the stores. Use of reusable pens can be adopted. Vermi composting pots can be equipped in the college for treating the food wastes, organic wastes etc. The resulting compost can be used in the gardens of college campus. The waste water produced from labs and hand washing etc can be treated using fluidized bed reactors and spent to gardens of the campus.

Incinerators can be equipped for burning out napkins and non reusable wastes. Plastics can be recycled to usable working scientific study models, pen stand, chalk box, key holders. E-wastes can be either reused after repair or can be handed to scrap pickers,

Reduce ,Recycle and Reuse must be the campus strategy for management of wastes .Reducing at source is the best way to control .Hence the government should take initiatives to create awareness and sensitize people about waste segregation and promote recycling or reuse of segregated materials. It is the responsibility and obligation of all the citizens to adopt proper waste management strategies and ensure a hygienic city and country. (Sreedevi S, 2015)

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Seasonal distribution of meroplankton in and around the Barmouth region of Cochin Estuary.

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Abstract

The backwaters of Kerala along with its network of canals spread and extend almost throughout the coastline and form important areas of fisheries and other human use. Cochin Backwaters is a typical nursery ground for a variety of fishes, which in the larval and juvenile stages are voracious plankton feeders. In the present study, the abundance and distribution of meroplankton along three selected stations were studied during premonsoon, monsoon and postmonsoon period. Zooplankton biomass was relatively high during the post and pre monsoon periods and low during monsoon. Consequent to the heavy rainfall and the resultant large influx of freshwater to the estuarine system, many marine organisms migrate from the environment. Following the monsoonal decline the marine components gradually get established in the estuarine system during the post monsoon season with the invasion of seawater from the bottom zone towards the surface. An important aspect brought to light through these observations is the remarkable influence of the seasons on the occurrence and abundance of different groups of zooplankton. Nauplius larvae constituted the majority of the invertebrate larvae with more than half of the contribution in all the three stations during pre monsoon and post monsoon. Fish eggs and larvae are abundant in this area during post monsoon and early premonsoon indicative of active breeding of fishes during this period.

Keywords: Cochin Estuary, Zooplankton, Meroplankton.

Introduction

The Cochin Backwaters is a shallow semi enclosed body of water of the tropical zone with characters of a tropical estuary. A narrow gut, about 450 m wide forms its main connection with the Arabian Sea and this region is subjected to regular tidal influence. Apart from the tides, the seasonal outbursts of the monsoon have great bearing in controlling the environmental factors and thereby distribution of the organism of the estuary (Madhupratap and Haridas, 1975). It is a catchment basin for several rivers such as Periyar, Pamba and Muvattupuzha which empty either into the Vembanad Lake or into the Cochin Backwaters which extends into the form of shallow brackish water lagoons. The continual discharge of freshwater and the inflow of seawater into the estuary bring about dynamic conditions which make the backwater extremely interesting and ecologically an intriguing environment (Silas and Pillai, 1975). The major hydrological variable in the Cochin Backwaters is salinity, similar to situations encountered in estuaries with a gradual declension of salinity from 30

ppt at the entrance of the estuary to 0.2 ppt at the point of entry of the rivers. The changes in the hydrology controlled by the seasons play an important role in regulating the migrant fauna of the estuary (Menon et al., 2000). More than 75% of the rainfall is recorded during the South West monsoon period which occurs during late May or in June to September. The rainy season also extends from late October to early December, which is the period of North-East monsoon. As the freshwater influx gets reduced, the stability also decreased. From February onwards, the stability is very low indicating a situation of vertically homogeneously mixed estuary (Silas and Pillai, 1975).

Salinity gradient in the Cochin backwaters supports diverse species of flora and fauna depending on their capacity to tolerate oligohaline, mesohaline or marine conditions. These are highly productive ecosystems and provide substantial support to the inhabitants of many coastal communities through their role in sea food production and nurturing of many valuable marine organisms. These areas are also rich in biodiversity and act as breeding and nursery grounds for fin and shell fishes. Plankton biomass is considered as an index of fertility of the aquatic ecosystem. In addition, they are important as they form the base of food web upon which larger organisms including fishes ultimately depend.

The objectives of the present study are to determine the distribution and population density and percentage composition of the meroplankton in and around Barmouth region of Cochin Backwaters.

About the study area

Cochin backwaters, situated at the tip of northern Vembanad lake, is a tropical positive estuarine system extending between 9Ú14' and 10Ú12'N and 76Ú10' and 76Ú30'E with its northern boundary at Azhikode and southern bounder boundary at Thannermukkam bund. The lake has a length of 80 km and width varies between 500 and 4000 km. The lake has two permanent openings into Arabian Sea, one at Cochin gut through a channel 450 m width. The major portion of the estuary has a depth range of 2-7 m, but the shipping channels are maintained at a depth of 10-13 m. Waters from six river drain into the estuary. On the southern half rivers, Murinjapuzha, Manimala, Menachil, Pamba and Achancoil join the lake while the Periyar river join at the Northern half. During South west monsoon, the estuary is virtually converted into freshwater basin even in areas around Barmouth, where salt water penetration occurs below 5 m depth only. The cycle of events leading to the fluctuations in the physico chemical factors in the Cochin Backwater is fairly regular and the year can arbitrarily be divided into three seasons of four months each: (i) a premonsoon season (Feb- May) of stable hydrographic parameters showing typical marine conditions; (ii) a monsoon season (June- Sept) associated with pronounced changes in the environmental features, and (iii) a period of recovery during the post monsoon season (Oct- Jan) when the marine components

being to develop in the estuarine system (Silas and Pillai ,1975). Samples were collected from 3 stations, Station 1, Tenth Buoy ($9^0$58'26'N $76^0$14'18"E) which is situated close to the mouth of the estuary. So the tidal currents are influencing estuarine biota. Station 2, Fishing harbour $9^0$56'35.30'N, $76^0$15'50.67"E where the fishing and processing unit in this area has been recognized as potential resources limiting factors. Station 3, Barmouth, $9^0$58'4.84"N $76^0$14'00.71"E where the marine estuarine waters are closely interacting.

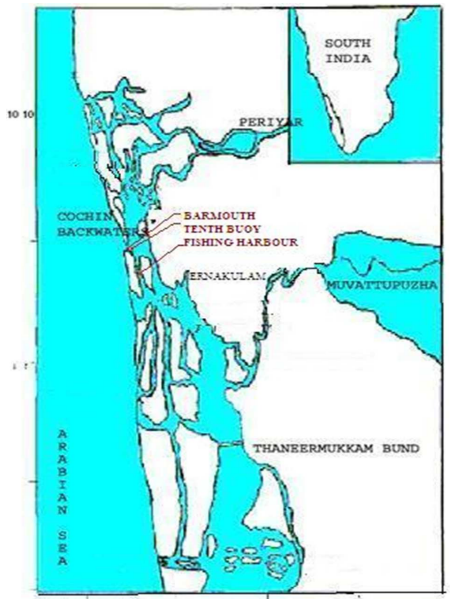

Figure 1: Map showing the Cochin backwaters and sampling stations.

Measurement of physico chemical parameters

As part of the study, data on various physico chemical and biological parameters were collected during premonsoon, monsoon and postmonsoon seasons from the study area. The monsoon collections were taken under Cruise No: 1 on 21-08-2007 by R V Malabar. The post monsoon and premonsoon collections were taken onboard R V Treesa under Cruise No: 2 and Cruise No: 3 on 28-11-2007 and 26-2-2008 respectively. Water samples were collected from the surface water using Nansen bottle and analysed for physico chemical parameters. The depth of the station was measured with the help of Nansen bottle. Light penetration depth of the water column was measured by lowering the Secchi-disc from the board using a twine containing knots at an interval of 25 cm. Salinity of the water was measured using an electrode less induction salinometer (DIGI-AUTO model 3G, Tsurumi, Seiko, Japan). Temperature of the water was measured with an accuracy of +/_ 0.1^0C using precision thermometer. pH of the surface water was measured by using a digital pH meter (Perkin Elmer, Accuracy+/_ 0.01). Surface zooplankton samples were taken by hauling the boat for 15 minutes at 0.5km/ hr speed in a horizontal fashion using Bongo net of mesh size 200 microns and a diameter of 60 cm. Zooplankton sample thus obtained was preserved in 4% formalin for later analysis.

(Tait, 1981 and Omori et al., 1984). Biomass was calculated using the displacement volume method. The value thus obtained is expressed as biomass in ml /m^3. The composition of plankton is expressed in numerical abundance and in number of individuals per volume of water.

Results

The physico chemical parameters of the water samples showed considerable variation during the different seasons. The light penetration depth was recorded highest during the monsoon (139 – 90cm) and was more or less same during pre monsoon and post monsoon (90 -50 cm) seasons. Seasonal changes in temperature were not pronounced in the estuary. In general the temperature ranged between 26^0 C - 30^0C. The lowest temperature was recorded during the monsoon period which varied between 26^0 C - 28^0C. During the post monsoon and premonsoon period the temperature comparatively increased and varied between 29^0 C - 30^0C. Wide range of salinity values were observed in the estuary during different seasons. During the monsoon season, the surface salinity exhibited lower values ranging between 0-7 ppt. During the postmonsoon, the salinity values increased to 21 – 29 ppt and during the pre monsoon period the salinity varied between 24 – 25 ppt. pH of the three seasons varied between 6.5- 8.2. The highest value of 8.2 was recorded during postmonsoon. The lowest value of 6.5 was recorded during monsoon.

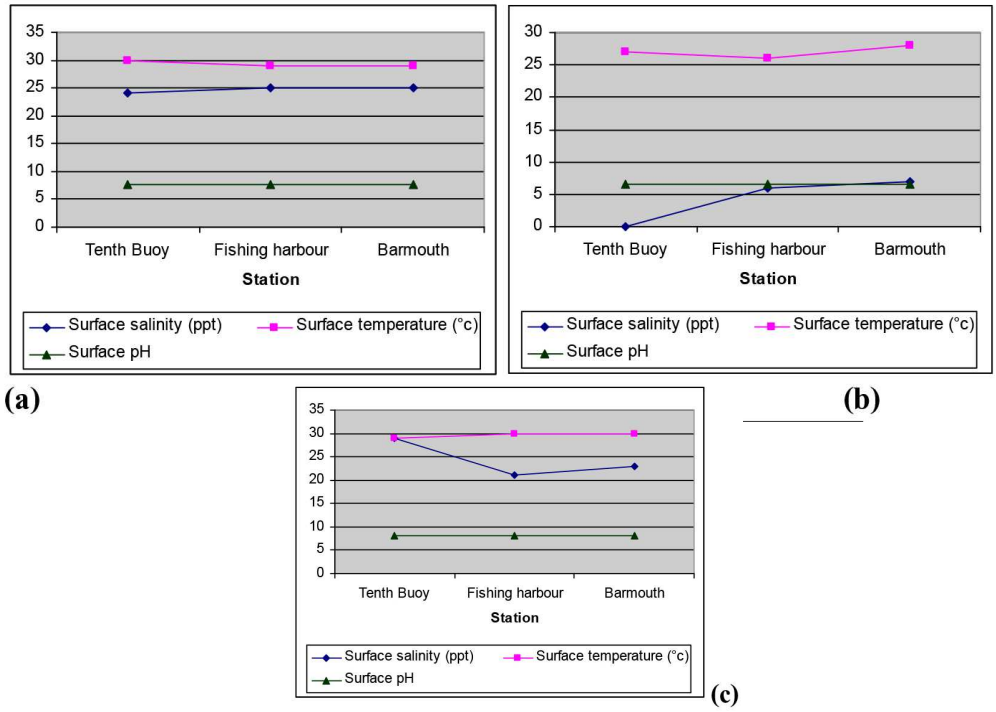

Figure 2 : Surface salinity (a), temperature (b) and pH (c) along the three stations during the pre monsoon, monsoon and post monsoon.

Zooplankton biomass

The highest biomass was recorded in the postmonsoon season followed by premonsoon and monsoon. In the pre monsoon season the highest biomass was recorded in station-2 (Fishing harbour) with a value of 0.16985 ml/m^3 followed by station-3 (Barmouth) and station-1 (Tenth Buoy) with the values 0.11323 ml/m^3 and 0.05661 ml/m^3 respectively. In monsoon season the highest biomass of 0.09908 ml/m^3 was recorded in station-3 (Barmouth) followed by station-2 (Fishing harbour) with 0.08492 ml/m^3 and station-1 (Tenth Buoy) with a value of 0.05661 ml/m^3. During the postmonsoon season a general increase in the biomass was found in all the three stations. The highest recorded biomass was in station-1 (Tenth Buoy) with 0.16985 ml/m^3 followed by station-2 (Fishing harbour) and station-3 (Barmouth), both having the same biomass of 0.11323 ml/m^3. The difference in the value of the biomass was found to be proportionate to the salinity.

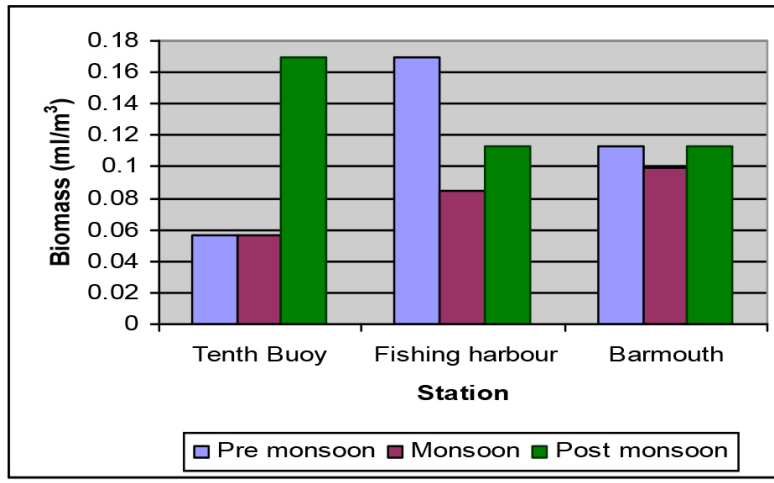

Figure 3 : Biomass distribution along the three stations during premonsoon, monsoon and postmonsoon.

Seasonal distribution of the population density of meroplanktonic groups.

The population density of polychaete larvae was recorded highest during monsoon in all the three stations. It declined during the postmonsoon and premonsoon and was completely absent during the premonsoon period. The peak value of 57 no:/m^3 was observed in station- 3 (Barmouth) during the monsoon period followed by 13 no:/m^3 in station- 2 (Fishing harbour) and 4 no:/m^3 in station-1 (Tenth Buoy). Only lesser number was present during the postmonsoon in station- 2 and station- 3 and was completely absent in station-1 during the postmonsoon and all the three stations during premonsoon. Nauplius larvae constituted the majority of the invertebrate larvae. They were present throughout the year but only in small numbers during the monsoon period. A tremendous increase in population density was shown

during the premonsoon period with a peak of 190 no:/m^3 in station- 2 followed by station- 3 with 181 no:/m^3 and station-1 with 132 no:/m^3. A relatively high value was recorded during postmonsoon in all the three stations.Protozoea was found abundant during the postmonsoon season in all the three stations. A minor population density was recorded during the monsoon and premonsoon periods. The highest value of 48 no:/m^3 was recorded in station-1 followed by station -2 with 39 no:/m^3 and station -3 with 28 no:/m^3during the postmonsoon period. Zoea larvae were common and abundant during the premonsoon period. The highest density of 79 no:/m^3 was recorded in station-2 during the premonsoon period. Mysis constituted an important group which was distributed throughout the estuary in all three seasons. It showed the peak abundance during premonsoon. They were evenly distributed during monsoon and postmonsoon. A highest density of 37 no:/m^3 was recorded in station-2 followed by 15 no:/m^3 in station -1 and 14 no:/m^3 in station -3.Megalopa larva was observed scarcely in all the three seasons in the stations. It was observed only in station-3 during the postmonsoon with a very low density of 3 no:/m^3.Bivalve larva was present in all the three stations during monsoon. Fish eggs were recorded maximum during the postmonsoon period in all the three stations followed by premonsoon and monsoon. They were found in small numbers during the premonsoon and monsoon periods. A highest value of 57 no:/m^3 was recorded in station-1 followed by station -3 with a value of 23 no:/m^3 and station -2 with a value of 22 no:/m^3 during the postmonsoon period. They were found in all the three seasons in all stations. The fish larvae were also recorded maximum during the post monsoon period followed by premonsoon and monsoon. A highest value of 75 no:/m^3 was recorded in station-2 followed by 48 no:/m^3 in station -3 and 17 no:/m^3 in station -1 during the postmonsoon period. During the premonsoon and monsoon period also the highest value of the corresponding season was recorded in station-2 with a value of 42 no:/m^3 and 30 no:/m^3 respectively.

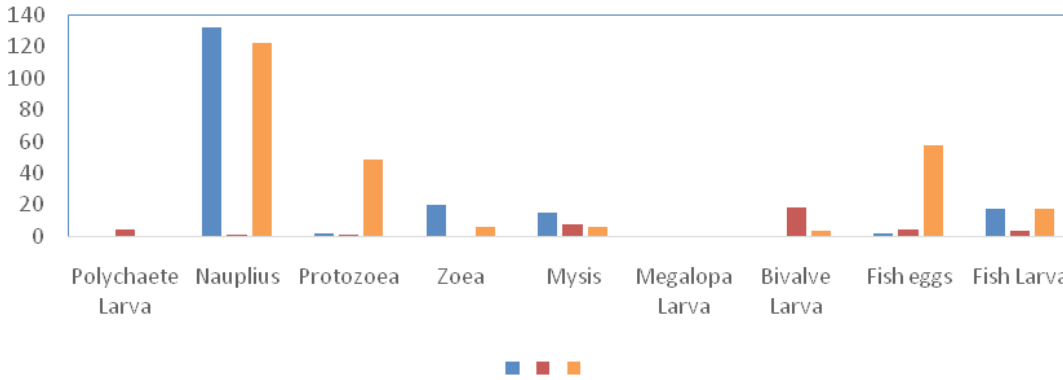

Figure 4 : Population density of meroplankton groups at station -1 (Tenth Buoy) during pre monsoon, monsoon and post monsoon seasons.

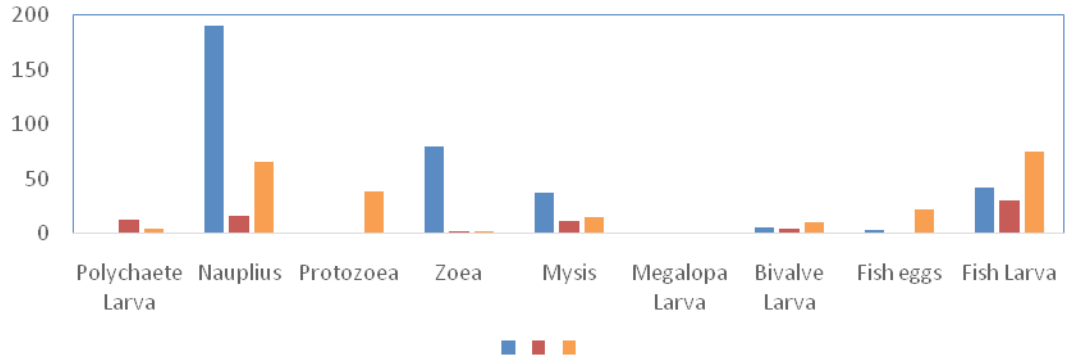

Figure 5 : Population density of meroplankton groups at station -2 (Fishing harbour) during pre monsoon, monsoon and post monsoon seasons.

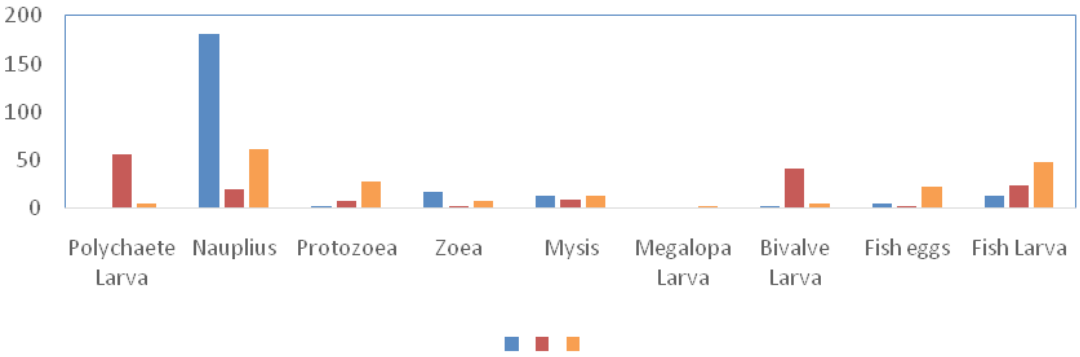

Figure 6 : Population density of meroplankton groups at station -3 (Barmouth) during premonsoon, monsoon and postmonsoon seasons.

Discussion

A general picture of the seasonal variation in zooplankton biomass of the area can be summarized as it is relatively high during the postmonsoon and pre monsoon periods and low during the monsoon months. During the post and premonsoon period the entire water column shows stable and uniform hydrographic conditions and the estuary becomes virtually an extension of the adjoining sea with high salinity and temperature values. Heavy rainfall during the monsoon months has significant effect on the zooplankton distribution of this area since the dominant species constituting the bulk of the zooplankton are marine in origin. A very low value ofnumerical counts and biomass of meroplankton was obtained during the monsoon season. A gradual rise in salinity was noted during this period. This was reflected in the biomass distribution of the zooplankton also. Generally the estuarine zooplankton is volumetrically abundant but limited in species composition. During the post monsoon and premonsoon period, the rainfall is negligible and the salinity of the area is uniformly high. Temperature values remains high during this period, and this condition is maintained until

the onset of south west monsoon. Conditions prevailing in the estuary during the premonsoon period closely approximate to those in the near by inshore areas, allowing the migration of a large portion of marine components into the estuary and their effective propagation in the area. The reduction in the salinity during the south west monsoon does not seem to affect the brackish water forms seriously, it is detrimental to the marine forms which enter the estuary during the premonsoon period.

The mesohaline conditions prevailing in the post monsoon period allow rapid multiplication of brackish water forms and other groups which prefer reduced salinity and temperature for their propagation. Evidence of such occurrence and abundance was found in groups such as benthic invertebrate larvae and larvae of many estuarine fishes. The gradual increase in salinity which occurs during the latter half of the post monsoon period attracts many littoral forms and brings about an effective recruitment of the larvae of many marine species into the estuary from the adjacent inshore waters.

After the monsoonal decline in the number and the biomass, different groups recorded a recovery during the period of rapidly increasing salinity and other environmental parameters. Thus of all the environmental features which typify the Cochin backwaters, the most significant appears to be the salinity variation which exerts considerable influence on the type of fauna find in estuary. The normal salinity ranges for a particular species may vary considerably and salinity plays a major role in balancing interspecific competition.

The maintenance of local populations in the Cochin Backwaters and the rate of intrusion by coastal marine forms seem to be dependent mainly on the flushing rate, circulation patterns and other biological features such as predation. Oligohaline forms can propagate in the less saline environment but the marine forms should alleviate the problem either by adapting the benthic mode of life or they should physically disperse themselves to the nearby inshore waters during the monsoon period. In a system where the salinity is completely reversed during the seasonal cycle, this must be the main factor which governs the adaptation of the lifecycle of the organism to the changing hydrographic patterns.

Three factors are taken into consideration for discussion on the diversity of zooplankton fauna in the Cochin Backwaters. They are (i) salinity,(ii) temperature and (iii) availability of food. According to Pillai et al. (1973) a definite correlation exists between temperature and salinity and the total abundance of different species. The temperature and salinity was found increased during the post and premonsoon seasons which also bears a considerable increase in the biomass. Food supply seldom acts as a limiting factor in the estuary and does not seem to govern the seasonal distribution and abundance. The faunal composition shows that the secondary peak of zooplankton during the post monsoon months is largely constituted by eggs and larvae of different species of fishes. The high diversity of fauna which is noticeable

during the premonsoon seems to be derived partly from the post monsoon peak of these populations and partly induced by the reestablishment of favorable hydrographic conditions prevailing in the estuary during this monsoon. According to Mohammed and Rao (1972) With the exception of *Parapenaeopsis stylifera*, the larvae and juveniles of all other commercially important species of prawns of this area represented in the estuary. The causative factor of such immigration of larvae into the Cochin Backwaters has been ascribed to the congenial hydrobiology conditions prevailing in the estuary, availability of food especially phytoplankters and detritus for the larvae and the conditions which offer them refuge from predators help them to maintain themselves in the estuary. The optimum conditions in which most of the estuarine species flourish seem to be somewhat away from the mouth to the middle reaches as their densities were higher at these stations. This area represents a more stable environment when compared to the mouth which is subjected to disturbances due to tidal mix. High values of heavy metals concentration, sewage disposal and petroleum hydrocarbons have been recorded in the Cochin Barmouth areas. Nauplius was found to be lower during the monsoon period and a tremendous increase was found during premonsoon. This might be due to the seeds of zooplankton and the possible resting stages which may occur in the backwaters when the environmental conditions are adverse and spring back to the activity when the salinity regains, breed and repopulate in the estuary during the succeeding high salinity period.

It is the nature and diversity of the plankton community that determines or controls the fish population of that area. Many members of the meroplankton community are the larvae of economically important organisms like prawns, crabs, bivalves and fishes. Some other members of the meroplankton are the larvae of benthic invertebrate which play a dominant role in the estuarine ecosystem trophic level. The data of the distribution of meroplankton groups can be of use in the valuation of fish nursery grounds (estuary) and helps the better management of the future fisheries and traditional aquaculture like prawn filtration practiced in the Cochin backwaters.

Acknowledgement

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Distribution of Nematodes along the Southwest Coast of India: A review

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Abstract

Studies on meiobenthic community along the South West Coast of India is comparatively less when compared to that of macrobenthos.The most prominent meiobenthic community found in Kerala coast were nematodes. Most of the meiobenthic studies were concentrated on seasonal variation and species diversity. Diversity indices were maximum during pre-monsoon and post-monsoon season and the most abundant species reported include *Anoplostoma, Mesacanthion, Anticoma, Psuedocella, Halalaimus, Oxystomin,* and *Viscosia.* Here an attempt has been made to evaluate the previous studies on nematodes along the south west coast of India.

Key words: Meiobenthos, Nematodes, South West Coast of India

Introduction

Kerala is a state with ample number of brackish waters, lakes, rivers, estuaries, lagoons etc. These backwaters are connected by canal system for easy transportation. Kerala is known for its wetlands and the major wetlands of Kerala include Sasthamkotta, Ashtamudi and Vembanad (Ramsar sites). These backwaters play a prominent role in the socio-economic and cultural history of Kerala (Udayavarma, 1983). The topography of the Kerala's coastline is distinctive and changes occurs abruptly as one proceeds from north to south. It is prone to severe erosion due to natural processes like waves, currents and winds (Mallik et al., 1987).

Meiofauna comprises organisms having a size between 63µm to 500µm.They are larger than microfauna and smaller than macrofauna (Somerfield & Warwick, 2013).The term 'interstitial fauna' introduced by Nicholls (1935), is used to denote the animals living in the interstitial spaces between all types of sediment particles.Meiofauna are chiefly represented by nematodes, foraminiferans, harpacticoidcopepods etc. (Kumar Chakraborty & Kumar Datta, 2018).

Distribution of nematodes

The study of benthic fauna of the Saphala Salt Marsh by Ingole et al., (1897) showed that the faunal composition comprised 10 taxonomic groups dominated by nematodes, harpacticoids, turbellaria, crustacean naupli, polychaetes, gastrotrich, oligochaeta, Tardigrada, Decapoda and Ostracoda of which more than 60 % occurred in the top 5 cm sediment layer and showed

clumped distribution and their distribution was influenced by salinity, dissolved oxygen and availability of food. In the survey conducted from areas with different mangrove cover from Cochin, Southwest coast of India,seven major taxa were recorded comprising nematodes as the most dominant taxon contributing 51.2-97.3% of the total faunaby Chinnadurai and Olivia (2006). Anila Kumary (2008), made a diversity study in Poonthura estuary in Thiruvananthapuram district covering a distance of 4.5km for a period of one year from February 1995 to January 1996. Nematode species were limited to 9 species belonging to 9 genera of 7 families. *Desmodora extensa* was the dominant species among nematodes. Feebarani (2009) investigated the spatial,temporal and vertical distribution of nematodes in Cochin backwaters. Sajan et al., (2010), conducted a meiobenthic diversity study in the regions of south west coast of India during a period of 16-02-1998 to 06-03-1998 and 20-02-2001 to 28-02-2001. 154 species of nematodes belonging to 28 families were recorded. The nematode species were categorised into 3 groups; 'restricted', 'widespread' and 'ubiquitous. Restricted species are those that are found only in one depth range, widespread species are found in at least two depth ranges whereas ubiquitous species are found in all depth ranges. *Desmodora spp*, *Dorylaimopsis spp*, *Tricoma spp*, *Theristus spp* and *Halalaimus spp* were the dominant nematode species.

A total of 10 meiofaunal groupscomprising nematodes, foraminifera, copepoda, polychaeta, kinorhynchs, gastrotrichs, ostracods, turbellaria, cladocerans and Acari reported from the South West continental shelf of India by Smitha (2011). The community composition, density, richness, evenness and diversity of meiobenthic fauna of Manakudy estuary, west coast of India, during February 2010 to January 2012 recorded thirty four species of nematodes (Kannappan et.al, 2016). The second dominated population group of nematodes were represented by *Daptonema conicum, Desmoscolex* sp., *Halalaimus* sp., *Theristus* sp. *and Viscosia* sp. Free-living marine nematode diversity was analysed by K.G. Mohamed Thameemul and Ansari et al., 2014 between the mangroves of Vellar Estuary. Nematode diversity was most abundant in the sandy sediments than the muddy sediments. Maximum nematode diversity was shown during dry months when diversity indices were calculated. The predominant species found were *Metachromadora spp*, *Viscosia spp*, *Sphaerolaimus spp* and *Theristus spp*.

A quantitative and qualitative study of interstitial fauna and environmental variables was carried out on five selected sandy beaches (Cherai, Fort Kochi, Arthungal, Shakthikulangara and Veli) of the west coast of India by Priyalakshmi and Menon (2014) and they reported that the bulk of the population were constituted by nematodes, harpacticoid copepods, turbellarians, and polychaetes. Bhadury et al. (2015) made a checklist of free-living nematodes that includes 33 species of marine nematodes that mainly belonged to 20 genera and 13 families. Microlaimidae, Camacolaimidae and Ironidae were the three families exclusive to

the sandy sites. Comesomatidae, Anoplostomatidae and Linhomoeidae were families exclusively found across muddy sites. Free-living marine nematodes dominant across all sites include the genera Viscosia, Halalaimus, Ptycholaimellus, Oncholaimus and species such as *Sphaerolaimus balticus* and *Metachromadora suecica*.A study was conducted by J Varghese and Miranda (2015) along the coast of Arthungal during the period 2013-14. Samples were collected and analysed seasonally. Meiobenthos belonging to 15 taxa were recorded with faunal abundance maximum being nematodes recorded during pre-monsoon period. Anila Kumary (2016) made a study on meiobenthic community along the Thiruvananthapuram coast of Kerala at two distinct beaches. Fauna found in the two selected beached come under 11 taxa which includes Nematoda, Foraminifera, Turbellaria, Kinorhyncha, Oligochaeta, Polychaeta, Archiannelida, Ostracoda, Copepoda, Amphipoda and Arachnida. In both beaches predominant species found were nematodes. Nematode diversity was found to be maximum during post-monsoon period followed by pre-monsoon period. The meiobenthic abundance in general was independent on sediment granulometry and physicochemical characteristics of water prevailing along the coast.A study on nematode diversity in the Sundarbans was conducted by Kapuli Gani Mohamed Thameemul Ansari & Bhadury, 2017. This study provides a checklist of nematode species from 1950s till 2016. The list comprised of 179 species in 84 genera and 29 families. Species of genus Anoplostoma, Mesacanthion, Anticoma, Psuedocella, Halalaimus, Oxystomin, and Viscosia were abundantly found.

Conclusion

In most of the studies, seasonal diversity is analysed. It is seen that diversity is maximum during the dry period. Themeiobenthiccommunityis mainly represented by nematodes. It is the time to draw the attention of meiobenthic ecologists to make regular studies on other areas apart from the diversity and distribution of nematodes.

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Rapid assessment of Molluscan diversity of Chettuva backwaters Post-2018 Kerala flood

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Abstract

Molluscan diversity of severely flooded areas (Ethayi, Munikkakkadavu and Koorikkadu) of Chettuva backwaters was studied. Altogether 51 species of molluscs were identified. Of 31 species of gastropods identified, Nassaridae family excelled in number. Among bivalves, family Arcidae were dominant. There was a drastic reduction in species diversity in post monsoon season. The numerical density of *Crassostrea madrasensis* and *Perna viridis* reduced considerably during post monsoon season. The dominant species of monsoon season like *Sunetta donacina, Turritellia javena and Cantharus spiralis* was not recorded in post monsoon season. Cataloguing the biodiversity and generation of baseline data will enable to adopt management strategies towards conservation of molluscs.

Keywords: Bivalve, gastropod, diversity, Chettuva backwaters, flood 2018

Introduction

Molluscs forms an important link in food chain from primary to tertiary level leading to fish production and also an edible source for coastal population. They are used for ornamental trade, pharmacological products and manufacture of cement and lime (Jaiswar et al., 2005). Diversity and abundance of molluscs is a potential biological indicator of changing ecosystem. The rich and diverse molluscan fauna is a characteristic feature of coastal environments like sea, estuary, brackish water, wetlands and mangroves. Chettuva estuary is a part of Vembanad – Kol wetlands cited under Ramsar sites of Kerala. Commencing on 15th August 2018, severe floods affected Chettuva, due to abnormally high rainfall during the monsoon season. The coastal areas, backwaters and canals of Chettuva were flooded. An understanding of the molluscan diversity of backwaters of Chettuva is an essential prerequisite for implementation of sustainable utilisation of molluscan resources and for adopting suitable conservation measures. The present study focuses on assessing the molluscan species diversity of flooded near shore areas of Chettuva backwaters. This study will reveal the shifts in diversity, abundance and distribution of molluscs as an indicator of flood response.

Materials and Methods

Of Chettuva backwaters, Ethayi (10.5123^{0}N, 76.0563^{0}E), Munikkakkadavu (10.5102^{0}N, 76.0366^{0}E) and Koorikkadu (10.543578^{0}N, 76.06001^{0}E) were selected as study sites, which were severely flooded in 2018 and had prevalent molluscan diversity. The molluscan were

collected by handpicking and identified. The gastropods and bivalves were identified to species level as far as possible using standard references and published literature (Mohan Joseph 2007; www.gastropods.com). The different species of molluscs identified were catalogued. The number and biomass of each species was noted. The abundance, distribution, diversity and seasonal variations of gastropods and bivalves were analysed.

Results and Discussion

Altogether 51 species of molluscs were identified. The 31 species gastropods identified belonged to 19 families and the 20 species of bivalves belonged to 7 families. Among gastropods, Nassaridae family excelled in number, with 137 number of specimens in different 4 species. Among bivalves 7 different species of family Arcidae were dominant (Table 1).

During the monsoon season, *Cantharus spiralis* belonging to Pisaniidae family with total 40 numbers and *Sunetta donacina* belonging to Veneridae family with a total of 189 numbers represented the dominant species in Gastropoda and Bivalvia respectively. During the post monsoon season, *Nassarius* sp. Belonging to family Nassaridae consisting of total 99 numbers and *Perna viridis* of family Mytilidae with a total 74 numbers represented the species of Gastropoda and Bivalvia respectively. According to Laxmilatha et al 2006, the most dominant species of Chettuva estuary were *Meretrex casta* and *Villorita cyprinoides*. But these species were not recorded in the present study. During monsoon season, 25 different molluscan species were recorded in Ethayi. There was a drastic reduction in species diversity at Ethayi in post monsson season. The variation can be attributed to variations in hydrological parameters and sediment characteristics.

The numerical density of *Crassostrea madrasensis* reduced considerably during post monsoon season at Ethayi and Koorikkadu (Figure 1). The abundance of *Perna viridis* also reduced considerably during post monsoon season at Munikkakadavu and Koorikkadu (Figure 1). The fishermen and residents of nearshore areas of Chettuva backwaters have reported a decline in density of *C. madrasensis* and *Perna viridis* after flood 2018, which has been ascertained in this study. The dominant species of monsoon season of study area like *Sunetta donacina, Turritellia javena and Cantharus spiralis* was not recorded in post monsoon season (Figure 1*). Donax cuneatus* and *Turritella* spp. dominated the flood affected regions of Marina beach post-Chennai flood 2015 (Singh et al., 2019). In Central European floodplain, gastropod communities show a high species diversity as they have developed resilience strategies to survive in highly variable and frequently disturbed floodplain habitats (Iig et al., 2011).

At Ethayi, the total number of gastropods increased during post monsoon (172 nos) when compared to monsoon season (156 nos). At Munikkakadavu also, the total number of gastropods increased during post monsoon (38 nos) when compared to monsoon season (24 nos). The total number of bivalves decreased at Munikkakadavu from 475 nos (Monsoon) to 130 nos (post monsoon). A reduction in number of bivalves was also noted at Koorikkadu.

In studies conducted in European waters, the density of large gastropods declined and the percentage of gastropods with strong calcification increased after flood (Reckendorfer, 2006). Earlier studies in European waters have indicated that extreme flood events favour floodplain mollusc diversity. The flood favoured the colonisation of aquatic species and led to a shift of the community towards more hydrophilic composition. Both diversity and abundance increased significantly in the first year following the flood but decreased gradually to the pre flood levels (Ilg et al., 2009). A continuous monitoring of molluscan biodiversity and generating a baseline data will aid in assessing the consequences inflicted by flood.

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Category C
Biotechnology and Pharmacology

A review on peptidomic profiling of venom from spiders

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Abstract

Venom based research have great importance for the development of innovative pharmacological tools, drug candidates and in cosmetic and agrochemical industries. Spider venom contains varying number of immense novel molecules with different pharmacological properties. Venom peptides are also involved in antiarrhythmic, antimicrobial, analgesic, antiparasitic, cytolytic, haemolytic and enzyme inhibitory activities. These physiological effects raise the possibilities of effective treatment by the application of antivenoms. In order to enhance the effectiveness of such treatments, the knowledge of venom composition is vital.The opportunity for drug discovery is increasing recently as the knowledge about venom peptides were obtained through bioinformatic tools.The venom components are found to be diverse in between spider species and also the limited availability of genome sequences makes the peptidome profiling of venom a challenging task. This paper presents a review on the methodology of profiling of peptidomes present in the spider venom having diverse pharmacological applications. The presence of huge amount of venom constituents and its boundless applications are awaiting to discover and explore it. The main focus of this review is the methodological aspects of venom profiling. More studies were conducted on spider venom through certain methodologies mainly focuses on RP-HPLC, SDS-PAGE and Mass spectrometry.

Keywords: Spider venom, drug discovery, RP-HPLC, SDS-PAGE, Mass spectrometry.

Introduction

Spiders are one of the most fascinating and successful organisms in the world. Their efficient life strategies through the significant evolution made them a successful organism in the world. The efficient strategies mainly include the production of silk and the complex venom. We know that, venom is the excellent adaptation of some organisms that helps them as an anti-predatory mechanism and also for paralyze or kill their prey. All spiders have venom glands except for the families Uloboridae and Holarchidae (Hu et al., 2014). Most of the spider venoms have only milder effects on human, but some species, eg. *Loxosceles* sp. may be deadly (Nagaraju & Kemparaju, 2013). Spider venoms are complex mixtures of biologically active compounds including salts, small organic molecules, peptides and proteins (Saez et al., 2010).

Even though the venom profiling of spiders is found to be a challenging task as there is a little availability of sample venom and lack of peptidome sequences, there are numerous studies were conducted on spider venom. Pioneer works on venomous spiders were started

in the 20th century itself. According to Brazil and Vellard in 1924, they conducted a study about symptomatology and treatment of accident. They made a comprehensive review of the published articles on arachnidism and concluded that knowledge on venom effects was not only imperfect but also less complete when compared to clinical studies. Later, in 1952, because of the onset of advanced technologies, the work was continued through the methodology of obtaining pure venom by using electrical stimulation of spiders or by dissection of venom glands. This excellent study was conducted by Bucherl and he also developed a method to obtain pure venom from *Phoneutria*species, using two long glass pipettes joined by a thin elastic rubber tube in 1953. However, the major work done was on a few medically important genera like the black widow spider, *Latrodectus* by Mccrone (1964), Maretic (1966), Mccrone & Hatala (1968), the brown recluse spider, *Loxosceles* by Smith & Mick (1969), Morgan (1969) and Mowis & Russel (1975), the Sydney funnel web spider *Atrax* by Wiener (1957, 1961) and Gilbo& Coles (1964) and the wolf spider, *Lycosa* and the banana spider, *Pheneutria* by Fischer & Bohn (1957), as they possess potent venoms which can produce severe envenomation in man. Spider venom has received considerable attention by a number of workers because of its potency and their clinical manifestations in man (Margaret & Phanuel, 1988).On the beginning of 21stcentury, a considerable attention on the composition and pharmacology of spider venoms with emphasis on polypeptide toxin structure, mode of action and molecular evolution were extensively studied (Escoubas et al., 2000). In 2010, Saez et al. conducted a detailed studyon spider venom cocktails and its extensive usage for pathophysiological conditions including cardiovascular disorders, chronic pain, inflammation, and erectile dysfunction. Oldrati et al. (2017) described about venomics for collecting detailed data about venom composition based on NextGen mRNA and DNA sequencing of venom gland and LC-MS/MS technology by comparing toxin composition of one mygalomorph and three araneomorph spiders namely, *Heteropoda davidbowie* (Sparassidae), *Poecilotheria formosa* (Theraphosidae), *Viridasius fasciatus* (Viridasiidae) and *Latrodectus mactans* (Theridiidae).

In 2012, Liu et al., conducted a significant study on the venom of the spider *Macrothele raveni*and the study resulted that, the crude venom of this species has anti-tumour activity and the venom constituents potently suppressed cell growth in the myelogenous leukemia K562 cell line. This study reveals the wide range of applications of spider venom in pharmocology and its tremendous advantages in future life as well. Significant causes of envenomation of spider toxins in man led to the beginning of research on venomous spiders in India. The preliminary studies on three common spiders namely, *Pleisiophrictus collinus*, *Lycosa indaqastrix* and *Heteropoda venatoria* were conducted in Madras by Margaret & Phanuel (1988). It resulted that venom of the 3 common Indian spiders present unique and a distinct pattern of proteins and free amino acids which could probably account for the

differences in their clinical manifestation following envenomation by the spiders. In 2007, a significant study on *Hippasa sp.* by Nagaraju et al. and the results the extraction of a metalloprotease 'Partitagin' which has a role in tissue necrosis. The antimicrobial activity of purified toxins from *Crossopriza lyoni* against certain bacteria and fungi was studied by Gupta & Upadhyay (2016).

Santana et al. (2017) was tried to conduct the complete venom proteomics by analysing venom ontogeny based on the size of cephalothorax in different age groups of Australian tarantula *Phlogius crassipes.* Physiologically each venom component has specific targets. Usually they modulate neuronal ion channels and receptors. Moreover the venom peptides intervenes the voltage gated K+, Ca+, or Na+ channels. Most recently, in 2019, Saez et al., conducted a study on the specific action of venom peptides and it results the discovery of its action in certain channels like ligand-gated channels (e.g., purinergic receptors), acid sensing ion channels, mechanosensitive channels and transient receptor potential channels. Through these studies, it is obvious that the vast opportunities are still present to encounter the venom based studies of spiders.

Venom gland and its composition

Venom glands are one of the greatest adaptations of spiders, as there is significant evolution has occurred and well- developed glands are resulted now. Mygalomorph spiders (tarantulas) are found to produce more deadly venom than araneomorph spiders (true spiders). A pair venom glands are located in either in the chelicerae or under the carapace. Venom compounds are grouped into six categories (1618 venom compounds recorded yet): low molecular mass compounds (16 % of all compounds), acylpolyamines (11 %), linear peptides (6 %), cysteine-knotted mini-proteins (60 %), neurotoxic proteins (1 %) and enzymes (6 %). Low molecular mass compounds includes organic acids, nucleosides, nucleotides, amino acids, amines, polyamines, and some further substances, many of them acting as neurotransmitters (Kuhn-Nentwig et al., 2011).

Methodology of peptidome profiling

'Peptidomics' can be defined as the systematic analysis of the peptide content within a cell, organelle, tissue or organism. Peptides or small proteins, plays a major role in regulating various biological processes in the animal kingdom. And these biomolecules are the main focus of research for decades as it is present in almost all biological samples. The profiling methodology mainly includes liquid chromatography and Mass spectrometry. (Songping, 2010)

RP-HPLC (Reverse Phase- High Performance Liquid Chromatography)

Here extracted venom can be subjected to RP-HPLC using Vydac C4 column (55ØßM, 0.21 ×25cm) that had been pre-equilibrated with 0.1% TFA (Trifluoracetic acid) in water. The

column will be eluted using linear gradient from solution A (0.1% TFA in water) to 100% solution B (0.1% TFA in acetonitrile) for 40 min, and the protein will be eluted at a flow rate of 1mL/min and monitored at 280 nm (Nagaraju & Kemparaju, 2013). Separation occurs based on the interactions of the sample with the mobile and stationary phases.

SDS-PAGE (Sodium Dodecyl Sulphate- Polyacrylamide Gel Electrophoresis)

Separation of venom peptides can be done by using this analytical tool. It is widely used in proteomic analysis including protein size determination, protein identification, sample purity analysis, disulphide bonds identification and protein quantitation. Here the samples are loaded to the wells present on the top of the apparatus. Further steps ac be carried out using Tris (25mM), glycine (192 mM), and SDS (0.1%) as the buffer for 3h at 90V at room temperature. Tris is the buffer used for most SDS PAGE. (Laemmli, 1970) Its pKa of 8.1 makes it an excellent buffer in the 7-9 pH range. After electrophoresis, gels will be stained with 0.1% Coomassie brilliant blue R-250.

Mass Spectrometry

It is an analytical technique and is used to measures the mass-to-charge ratio of ions. The protein digests that is obtained in the SDS-PAGE can be applied to this apparatus and analyse the concentration and mass of peptides in the venom. TheVoyager DEPRO Matrix-Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry (MALDI-TOF MS) machine in positive ionization mode is a thriving technology, enabling used to the rapid detection of peptide/protein components that can provide comparative information (Boèánek, 2017*). De-novo*sequencing can be done using tandem mass spectrometry. Analyse the MS data using certain protein identification search engines, it includes, Mascot, Sequest or Proteome discoverer. Can also determine sequence homologies by using sequence obtained from literature data and searching the nonredundant protein databases, via the BLAST server (Songping, 2010).

Conclusion

In biology, the biomolecule, peptides has great importance as it has key role in various biological samples and it includes urine, hormones, cytokines and growth factors(Schulz-Knappe et al., 2001). The sequence availability of high molecular weight proteins are still in sparse. But development of technology which supports the profiling of peptides as it possesses diverse physiological properties. Through this revolutional attributes of peptidome profiling in science opened a novel opportunity in venom based studies and its relevant applications. Though a remarkable extent of information are available on spider venoms with a focus on structure and function of venom components and techniques of analysis, in our country, especially in Kerala such studies are lacking and hence the data generated will be helpful for future research.

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A review on the importance of spider silk protein

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Abstract

The strength, toughness, and elasticity of spider silk fascinate scientists worldwide, who wonder what gives this natural material its unusual qualities. Finer than human hair, lighter than cotton, and stronger than steel, silk tantalizes materials researchers seeking to duplicate its properties or synthesize it for large-scale production. Visions of wear-resistant shoes and clothes; stronger ropes, nets, seatbelts, and parachutes; and rust free panels and bumpers for automobiles all dance through researchers' minds. Hence the knowledge of silk protein genes in spiders will be of great significance in the scientific world. Spiders use silk in almost every part of their daily lives. Orb-weaving spiders produce up to seven mechanically distinct silk types that they use to make protective cocoons of offspring, as a safety dragline for locomotion and predator escapes, for ballooning locomotion, as adhesives to anchor other silks to each other and to substrates, and to provide their major source of nutrient uptake by entrapping airborne and terrestrial prey in intricately designed nets that function as complex mechanical systems. Spider silks are attractive biomaterials that are of particular biotechnological interest for industrial and medical purposes because of their unique physical and mechanical properties. Some studies described a method for the isolation of recombinant spider silk proteins based upon their unique stability and solubilization characteristics. Three recombinant silk proteins, (SpI)7, NcDS, and [(SpI)4/(SpII)1]4, were purified by extraction with organic acids followed by affinity or ion exchange chromatography resulting in 90-95% pure silk solutions. The protein yield of NcDS (15 mg/L culture) and (SpI)7 (35 mg/L) increased 4 and 5 fold, respectively, from previously reported values presumably due to a more complete solubilization of the expressed recombinant protein. [(SpI)4/(SpII)1]4, a hybrid protein based on the repeat sequences of spidroin I and spidroin II, had a yield of 12.4 mg/L. This method was found to be an effective, reproducible technique that has broad applicability for a variety of silk proteins as well as other acid stable biopolymers.

Keywords: Silk Protein, Dragline Silk, Spider Protein

Introduction

Spiders, unique among all organisms in their modes of silk production and usage and of reproduction, are common predatory arthropods in all terrestrial and many aquatic ecosystems. Among all organisms, spiders form the seventh largest order. Furthermore, this is the most diverse, female-dominated and entirely predatory order in the arthropod world. As such, spiders are key components of all ecosystems in which they live.

Spiders use silk for many purposes - to protect their young, catch food, make homes and move around. They are the only animals which use silk in almost every part of their daily lives. Orb-weaving spiders produce up to seven mechanically distinct silk types that they

use to make protective cocoons of offspring, as a safety dragline for locomotion and predator escapes, for ballooning locomotion, as adhesives to anchor other silks to each other and to substrates, and to provide their major source of nutrient uptake by entrapping airborne and terrestrial prey in intricately designed nets that function as complex mechanical systems (Vollrath, 2000).

Spider silks are characterized by remarkable diversity in their chemistry, structure and functions, ranging from orb web construction to adhesives and cocoons. These unique materials have prompted efforts to explore potential applications of spider silk equivalent to those of silkworm silks, which have undergone 5000 years of domestication and have a variety of uses, from textiles to biomedical materials. Recent progress in genetic engineering of spider silks and the development of new chimeric spider silks with enhanced functions and specific characteristics have advanced spider silk technologies.

Spider silks are attractive biomaterials that are of particular biotechnological interest for industrial and medical purposes because of their unique physical and mechanical properties (Scheibel, 2004). A range of material applications in biomedicine, textile technology and personal care products such as cosmetics are being targeted for these proteins. For instance, spider silks are being considered in the field of biopolymers, contact lens material, surgical threads, biomaterial membranes, scaffolds and tissue engineering (Foo & Kaplan, 2002; Scheibel, 2004).

Dragline silk

Silk from the major ampullate (MA) gland forms the primary dragline. It is an extremely tough material. MA silk reveals a tensile strength that is comparable to Kevlar (4 × 109 N/m2) along with a reasonable viscoelasticity (dragline 35 %, Kevlar 5 %). Spiders use dragline silk for various purposes like, a strong yet flexible structural element in the web, providing a framework to which other silks are attached, and also as a life line when a spider is dropping off to escape an enemy. Minor ampullate (MI) silk, used for structural reinforcement in construction of the web, has a similar high tensile strength in comparison to major ampullate silk but has little elasticity. Due to the low elasticity of MI silk it is irreversibly deforming when stretched. An orb web's capture spiral, in part composed of viscid silk produced by the flagelliform gland, has only half the tensile strength of major ampullate silk. The combination of strength and stretchiness gives the capture spiral a toughness greater than elastin, tendon, silkworm silk, bone, synthetic rubber, Kevlar, and high-tensile steel.

The dragline silk of the Golden Orb-Weaving spider is the most studied in scientific research. Spider silk is a natural polypeptide, polymeric protein and is in the scleroprotein group. The protein in dragline silk is fibroin which is a combination of the proteins spidroin 1 and spidroin 2. The exact composition of these proteins depends on factors including species and diet. Fibroin consists of approximately 42% glycine and 25% alanine as the major

amino acids. The remaining components are mostly glutamine, serine, leucine, valine, proline, tyrosine and arginine. Spidroin 1 and spidroin 2 differ mainly in their content of proline and tyrosine.

Molecular components of dragline silk

Biochemical experiments shows that dragline silk is a composite material largely composed of two structural proteins or spidroins called MaSp1 and MaSp2 (Xu and Lewis, 1990; Hinman and Lewis, 1991). Structural studies shows that the major ampullate spidroins form the core of the fiber that is wrapped inside a glycoprotein coat. Although the identities of the constituents of the glycoprotein layer remain unknown, experimental evidence shows that this layer is added in the ampulla prior to extrusion (Casem et al., 2002; Sponner et al., 2005). The molecular sequences coding for the dragline silk fibroin was first identified from N. clavipes (Xu & Lewis, 1990). Recently, the complete genetic blueprints for MaSp1 and MaSp2 were determined from L. hesperus (Ayoub et al., 2007). The predicted sequences for these fibroins encode large molecular weight proteins that are approximately 3,500 amino acids in length. These spidroins are highly modular, each containing internal repetitive block repeats that are flanked by N- and C-terminal nonrepetitive ends comprised of approximately 100 amino acids.

The internal block repeats are rich in glycine and alanine; these regions form polyalanine or polyalanine-glycine stretches that are interrupted by glycine-rich regions. The polyalanine segments form beta-sheet crystal domains and are responsible for the high tensile strength while the glycine-rich regions adopt 31-helix type structures and beta-turns that link the crystalline domains (Simmons et al., 1996). These interconnecting glycine-rich regions constitute the semiamorphous regions and have been implicated in the extensibility of the fibers. Extensibility of dragline silk fibers also has been attributed to glycine-proline-glycine-X-X (GPGXX) repeats within the MaSp2 protein sequence and the formation of beta-spirals. MaSp2 proteins have been shown to be tightly packed in certain core regions of fibers from N.clavipes, whereas MaSp1 appears to be uniformly distributed along the radial axis (Sponner et al., 2005). Interestingly, in natural fibers, MaSp1 and MaSp2 ratios have been shown to vary between different species (Tso et al., 2005); these differences have been linked to the diet and environment of spiders (Craig et al., 2000). As proline biosynthesis requires more energy, it has been suggested that the synthesis of MaSp2 is more energetically expensive. Therefore spiders synthesize cheap silk when resources are limiting, perhaps they produce fibers that contain predominantly MaSp1 molecules.

The advanced technologies in the scientific field, lead to many astounding discoveries in the area of spider silk. Scheller et al. (2001) disclosed the generation of transgenic tobacco and potato plants that express remarkable amounts of recombinant N. clavipes dragline proteins. They demonstrated the accumulation of recombinant silk proteins, which are encoded by

synthetic genes of 420-3,600 base pairs, up to a level of at least 2% of total soluble protein in the endoplasmic reticulum (ER) of tobacco and potato leaves and potato tubers, respectively. Spider silk proteins up to 100 kDa could be detected in plant tissues. When produced in plants, the recombinant spidroins exhibited extreme heat stability, a property that is used to purify the spidroins by a simple and efficient procedure.

Li et al. (2002) constructed and expressed synthetic gene monomer encoding recombinant spider silk protein based on the known repetitive protein sequence and partial cDNA sequence of dragline silk. DNA monomer sequences were multimerized to encode high molecular weight synthetic spider silks using a "head-to-tail" construction strategy. Multimer was cloned into pET30a(+), a prokaryotic high potency expression vector, and induced with IPTG. The protein from 8-unit repeat was produced in E. coli at levels up to 20 mg/L. The protein was easily purified with high recovery by using a metal ion affinity chromatography and purity was over 90%. The results of SDS-PAGE and Western blot suggested that the mass of the expression product was about 37 kD. This value and amino acid analysis were consistent with those of theoretic calculation.

Piruzian et al. (2003) obtained transgenic tobacco plants expressing recombinant analogs of spider dragline silk spidroin 1, artificial 1f5 and 1f9 coding for spidroin 1 analogs were 3'-fused in-frame with the reporter lichenase gene. The Tr2' weak constitutive promoter of Agrobacterium tumefaciens T-DNA and the strong constitutive promoter of the cauliflower mosaic virus 35S RNA gene were used as regulatory elements. The expression cassettes were used to transform agrobacteria and then introduced in tobacco leaf disks. On evidence of Southern hybridization, transgenic plants each carried a single copy of a hybrid gene, which corresponded in size to the constructed one.

Heiby et al., (2019) found that side chains of the amino acid methionine (Met), which were present in unusually high numbers in the core of the NTD, were responsible for conformational changes of the domain. They simultaneously mutated all core Met in the NTD of MaSp1 from the nursery web spider Euprosthenops australis to leucine (Leu) and made surprising observations. The mutant's ability to dimerize was considerably impaired. Conformational dynamics of the mutant were stalled, in contrast to the wild-type protein. The results showed that Met side chains in the NTD core facilitate structural plasticity, which tightens dimerization through shape-optimization of a mobile binding interface.

Conclusion

Spider silk is one of the toughest and strongest materials found in nature. It is stronger than the synthetic polymer, Kevlar. Since we have a lot of advanced techniques in genetic engineering and biotechnology today, the understanding of spider silk genes and proteins may lead to the creation of wonders in spider silk. There are numerous applications of spider silk, like in the manufacture of wear-resistant shoes and clothes; stronger ropes, nets, seatbelts,

and parachutes; and rust free panels and bumpers for automobiles and many more. Hence, the detailed knowledge about spider silk proteins and genes is very essential. Once the proteins and genes are understood, genetic engineering and recombinant DNA technology can be used to produce these in large amounts and it can be applied in various fields.

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The Olfactory imprinting in mice: Role of contact

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Abstract

Imprinting is a process that occurs during certain critical periods of the life of an animal. Bruce effect or the alien male–induced implantation failure is a well-studied phenomenon in mice. The presence of the stud male during exposure to an alien male protects the female from implantation failure. The pheromones of the stud male are imprinted in the female at the time of mating and it acts as a luteotrophic agent. Our earlier study revealed that, a nonpheromonal cue exposed to the female during pericopulatory sensitive period could protect pregnancy in newly inseminated females exposed to alien males. The present study examined the effect of direct contact of a nonpheromonal cue, which had been exposed to the female at the time of mating is found capable of protecting the female from Bruce effect. Virgin females were allowed to mate with its male in the presence of a cotton ball smeared with groundnut oil as a nonpheromonal cue on which the females had a direct contact. When these females were exposed to alien males in direct contact with groundnut oil, the majority of them retained their pregnancy. By contrast, when these females were exposed to alien males without direct contact with groundnut oil, the majority of them exhibited implantation failure. We evidenced that direct olfactory contact is necessary for imprinting of the nonpheromonal cue in the newly inseminated female and re exposure of the same nonpheromonal cue could protect the female from alien male-induced implantation failure.

Key words: Bruce effect, critical period, nonpheromonal cue, olfactory imprinting, pheromones

Introduction

Pheromones are substances that are secreted externally by an individual and elicit a specific response when perceived by another individual of the same species (Karlson and Butenandt 1959). Alien male-induced implantation failure is a well-studied pheromonal effect in mice (Bruce 1959; Dominic 1987; Archunan 2014). Exposing a newly inseminated female to an alien male results in implantation failure and revert the female to estrus stage (Bruce 1959). Re-exposure to the stud male 24 h after mating did not induce implantation failure (Parkes and Bruce 1961). Pregnancy failure was not observed when the newly inseminated female was exposed to alien male in the presence of the stud male or its urine (Thomas and Dominic 1987). It is suggested that inseminated mouse gets imprinted with the odor of the stud male and so responds differentially to a strange male than the stud male (Parkes and Bruce 1961; Thomas and Dominic 1988). This implies that each male has its signature odor to be imprinted on females. One of our study revealed that imprinting of the nonpheromonal cue will be

occurred only if we present it during the pericopulatory sensitive period (Thomas et al., 2018).

The present study focused on the role of direct contact with nonpheromonal cue for imprinting during the pericopulatory sensitive period.

The present study focused on the effect of direct contact of a nonpheromonal cue, which had been exposed to the female at the time of pericopulatory sensitive period in protecting the female from Bruce effect.

Methodology

36 adult virgin females and 14 adult males (10–20 weeks; BALB/c) were purchased from Kerala Veterinary and Animal Science University and kept as stock. 14 adult wild strains (*Mus musculus domesticus*) of male mice were collected from houses in and around Thrissur and kept in separate room undisturbed for few days, which were used as alien males.

All mice were housed individually in polypropylene cages of size 29 × 22 × 14 cm with rice-husk bedding. The temperature (23˚C) and reverse light-dark cycle of 12:12 (lights on at 18:00 h) were kept constant. All animals including alien males were fed on a standard diet purchased from Small Animal Breeding Station of Kerala Veterinary and Animal Sciences University and water was provided *ad libitum*.

Two drops (100 µL) of food-grade groundnut (*Arachis hypogaea*) oil (PRO PRIMIO Refined Groundnut Oil, R.R. Oomerbhoy Pvt. Ltd.) smeared cotton ball of 150 mg weight was used to provide a nonpheromonal cue.

Different stages of estrous cycle were monitored daily using standard vaginal smear technique (McLean et al. 2012).

30 adult virgin females from the stock with regular estrus cycle were selected and divided into 5 groups comprising 6 females in each group (Groups I–V). Their behavior was observed using an infrared camera connected to a monitor kept in the adjacent room.

Experiment

All females were monogamously paired with adult males and allowed to mate (Group I-V). In Group II and Group III, a cotton ball smeared with groundnut oil was provided at the time of pairing and females were allowed to have direct contact (non-volatile and volatile cues) with it during mating. In Group IV, a cotton ball smeared with groundnut oil was provided at the time of pairing on which the female had no direct contact with it. Females with vaginal plug (day 0 *post coitum*) (confirmation of pregnancy) were separated from the stud male and

housed individually in a new cage with fresh bedding. After 24 hours (day 1 *post coitum*) they were subjected to the following treatments:

Group I : Female was exposed to a confined alien male in the presence of confined stud male, allowing contact with urine and excreta of both males.

Group II : Female was exposed to a confined alien male allowing contact with its urine and excreta and exposed to a cotton ball smeared with groundnut oil on which the female had no direct contact.

Group III : Female was exposed to a confined alien male allowing contact with its urine and excreta and exposed to a cotton ball smeared with groundnut oil on which the female had a direct contact.

Group IV : Female was exposed to a confined alien male allowing contact with its urine and excreta and exposed to a cotton ball smeared with groundnut oil on which the female had no direct contact.

Group V : Female was left undisturbed (untreated control).

After 48 h, all females were returned to their cages and housed individually till the termination of the experiments (day 5 *post coitum*).

All females were housed separately in their home cages up to day 20 *post coitum* to ascertain their reproductive status. Vaginal smear was examined daily from all females. Presence of abundant irregular-shaped, nonnucleated, cornified squamous epithelial cells in the smear was taken as the indication of pregnancy block and return to estrus (Thomas and Dominic 1987; McLean et al., 2012). Vaginal smear with abundant leucocytes and mucus was taken as the indication of pregnancy. The number of females that were pregnant after showing estrous smear and the number of pups delivered by females that did not exhibit estrous smear were recorded.

Statistical analysis

Fisher's exact test was used for analyzing the data.

Results

P value of pair-wise comparison of the incidence pregnancy using Fisher's exact test revealed that there was a very statistically significant variation when Group III compared with Groups II, and IV; but Group III showed no statistically significant variation compared with Group I and V.

Table 1: Protective effect of nonpheromonal cue on alien male induced implantation failure-Role of contact

Groups	Treatment	No. of females (n)	Pregnancy n (%)	Results Pregnancy block n (%)	No. of pups delivered (n)
Group I	Exposed to alien male along with stud male	6	6 (100)	0 (0.0)	48
Group II	Exposed to alien male along with a cotton ball smeared with groundnut oil on which the female had no direct contact	6	1 (16.7)	5 (83.3)	3
Group III	Exposed to alien male along with a cotton ball smeared with groundnut oil on which the female had direct contact	6	6 (100)	0 (0.0)	50
Group IV	Exposed to alien male along with a cotton ball smeared with groundnut oil on which the female had no direct contact	6	0 (0.0)	6 (100)	0
Group V	Untreated control	6	6 (100)	0 (0.0)	51
P-value using Fisher's exact test of Group III with other groups Group III with Group I=1.0000 Group III with Group II=**0.0152**Group III with Group IV=**0.0022**Group III with Group V=1.0000					

The nonpheromonal cue, the groundnut oil on which the female had a direct contact is capable of protecting the female from alien male-induced implantation failure (Group III; Table 1). This is not, however, significantly different from Group I (exposed to the alien male in the presence of the stud male), or Group V that were left undisturbed during post-mating period. The female of Group II which had a direct contact with the nonpheromonal cue during mating and no direct contact with it during exposure to alien male and females in Group IV, which were exposed to alien male allowing free access to a cotton ball without nonpheromonal cue in the absence of the stud male or its urine showed significantly higher rate of implantation failure.

The females that exhibited cornified epithelial smear did not show any signs of pregnancy whereas those females that failed to show estrous smear continued their pregnancy and delivered varying number (7–9) of pups (Tables 1).

Discussion

This study on the protective effect of a nonpheromonal cue against implantation failure induced by an alien male indicates that the nonpheromonal cue the groundnut oil, which the females experience during pericopulatory sensitive period has the ability to act as a luteotrophic signal in inseminated mice. However, imprinting of the nonpheromonal cue is possible only if the female is allowed direct contact (the nonvolatile and volatile cues) with the nonpheromonal cue during pericopulatory sensitive period. In other words, exposure of the female, only to the volatile cues associated with the groundnut oil will not imprint the odor in the inseminated female. Similarly, contact with the nonvolatile cues of the nonpheromonal cue is essential for inducing protective effect against implantation block by alien male. To the best of our knowledge, this is the first report that demonstrates the effectiveness of an imprinted nonpheromonal cue in protecting pregnancy of a female against male-induced implantation failure (the Bruce effect). This study shows that ambient chemical cues or nongenetically determined odors that the female mouse perceives at the time of mating may blend with the genetically determined pheromonal cues of the stud male in forming a chemical signature of the stud male (Wyatt 2010). The female learns or gets imprinted with this chemical signature, rather than only with the pheromones of the stud male. It is important to note that the nonpheromonal cue, once imprinted, acts analogous to the stud male pheromones, and may form a memory in the olfactory system offering protection against the Bruce effect. However, the mechanism involved in the protective effect of nonpheromonal cue is presently unknown.

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Synthesis of Silver nanoparticles using *Bauhinia phoenicea* leaf extract and analysis of its Larvicidal activity against *Culex quinquefasciatus* larvae

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Abstract

Plants provide a better platform for nanoparticle synthesis as they are free from toxicchemicals and provide natural capping agents. In the present study, our aim is to investigate the larvicidal potential of biosynthesized silver nanoparticles against *Culex quinquefasciatus* larvae. It was found that *Bauhinia phoenicea* leaf extract reduced silver ions to silver nanoparticles, when mixed with silver nitrate solution in 1:9 ratio. This is due to the surface plasmon resonance property of silver ions. Using Ultraviolet-Visible Spectroscopy, X-Ray Diffraction Analysis and Transmission Electron Microscopy the biosynthesized silver nanoparticles were characterized. Nanoparticles serve as the fundamental building blocks for various nanotechnology applications. Silver nanoparticles are widely used because of their unique properties. When Culex larvae were treated with the biosynthesized silver nanoparticles, it caused the death of the larvae. This is due to Acetyl cholinesterase activity inhibition. When the enzyme is inhibited the neural transmission gets blocked and it results in the death of the larvae. These results suggest that the biosynthesized silver nanoparticle is known to possess significant larvicidal activity against *Culex quinquefasciatus* larvae. Thus it is highly beneficial to mankind in reducing the diseases caused due to mosquitoes.

Key words: Silver nanoparticles, Larvicidal potential,*Culex quinquefasciatus*.

Introduction

Nanotechnology deals with materials with dimensions in the nanometer range (<100nm). They play an ever increasing role in science, research and development as well as in everyday life, as more and more products based on nanostructured materials are introduced to the market. Of all the metals, silver nanoparticles (AgNPs) have gained tremendous interest due to its increasing commercial demand day by day (Mukherjee et al., 2002). Silver nanoparticles are reported to possess anti-fungal, anti-inflammatory, anti-viral, anti-angiogenesis and anti-platelet activity. The reason why plants work so well to synthesize silver nanoparticles is because antioxidants present in them act as reducing agents. Larvicidal activity of biosynthesized nanoparticles is determined by Acetylcholine esterase assay. Inhibition of acetyl cholinesterase (AChE), the key enzyme in the breakdown of acetylcholine, is considered as a promising strategy for the treatment of neurological disorders such as Alzheimer's disease, senile dementia, ataxia and myasthenia gravis. In the present study, the larvicidal activity of silver nanoparticles synthesized from *Bauhinia phoenicea* leaf extract is analyzed.

Materials and Methods

1. Collection of plant and preparation of plant extract

   The plant, *Bauhinia phoenicea,* was collected from St. Mary's college campus, Thrissur and authenticated by Dr. Sr. Meena K Cheruvathur of the Department of Botany. Fresh and healthy leaves were collected, washed thoroughly with tap water and used for the preparation of the plant extract (Marimuthu et al., 2010).

2. Synthesis of silver nanoparticles

   10ml of the plant extract is filtered and added to 90ml of 1mM $AgNO_3$ and used for the production of silver nanoparticles. The pellets formed after the final centrifugation were washed and stored at 4^0C. The formation of nanoparticles was confirmed and monitored with the help of the following analytical methods (Patel et al., 2015).

3. Characterization of the synthesized silver nanoparticles

   Using UV-Vis spectroscopy, the optical properties of AgNPs were determined, which specifies the time point of maximum production of silver nanoparticles by taking the absorption spectra ranging from 400 to 500nm.

   The crystalline structure of silver nanoparticles was determined by XRD. XRD patterns were recorded on Phillips PW-1710 automated diffractometer using a Cu tube operated at 40kV and 35mA.

   The synthesized AgNPs were used as the sample for TEM analysis to visualize the size and shape of silver nanoparticles. It was prepared by placing a drop of the silver colloidal solution on the TEM copper grid.

4. Acetylcholinesterase Activity Assay for Larvicidal Activity

   To determine the IC50 values, procedures from Ellman et al., 1961 were modified and applied. Yellowish or colourless solution was observed during reaction for 30 minutes at room temperature. Changes in absorbance were recorded by a microplate reader (Synergy H1 Hybrid MultiModeMicroplate Reader, USA) at 412 nm.

1. Inhibition=[(A con – A sam) / A con]/ 100 where, A con is the absorbance of the control and A sam is the absorbance of the test sample. (Hematpoor *et al*., 2016), (Bandyopadhyay *et al*., 2014).

Results and Discussion

1. Biosynthesis of silver nanoparticles

The fresh suspension of *Bauhinia phoenicea* leaves is slightly yellowish brown in colour. But after the addition of silver nitrate, the colour became dark brown due to the surface plasmon resonance property of silver ion (Figure 1). The plant extract reduced Ag+ ions to silver nanoparticles when mixed with silver nitrate solution in 1:9 ratio. The mixture was

kept at room temperature for 24 hours. The colour change in the reaction vessel is indicated rapidly by the formation of silver nanoparticles.

Figure1 : Photographs showing change in colour of the plant extract after addition of $AgNO_3$:
(a) before incubation,
(b) after incubation.

a. b.

2. Characterization of synthesized silver nanoparticles

The formation and stability of silver nanoparticles in aqueous colloidal solution is confirmed by UV-Visible spectral analysis. The solution showed an absorbance peak at 450 nm as shown in figure 2 due to the surface plasmon resonance of the synthesized AgNPs which is responsible for the unique and beneficial optical properties of nanoparticles.

The XRD patterns of the biosynthesized silver nanoparticles using aqueous extract of leaves of *Bauhinia phoenicea* shows large characteristic peaks indexed to the crystalline planes (Figure 3) TheXRD peaks at 2θ values of 32.460_0, 38.321_0, 44.391_0,64.713_0 can be attributed to (101), (111), (200) and (220) crystalline planes of face-centered-cubic (FCC) structure of AgNPs, respectively.The size of crystallites can be calculated using the Debye-Scherrer equation. $D = K\lambda / \beta \cos\theta$, where D is the mean size of ordered (crystalline) domains, K = 0.9 is dimensionless, θ is the X-Ray wavelength, β is the line broadening at half the maximum intensity (FWHM), in radians and θ is the Bragg angle. On substituting the values in the above equation, we get the value of D as 13.06 nm, which can be correlated with the values obtained from TEM analysis.

The particle size of the synthesized silver nanoparticles was characterized by TEM analysis (Figure 4). In our study, the TEM image was verified with multiple trials and evidenced that there is a variation in the size of the nanoparticle. The TEM analysis revealed that the size of silver nanoparticles ranged from 12.75 nm to 25.55 nm (Figure 4).

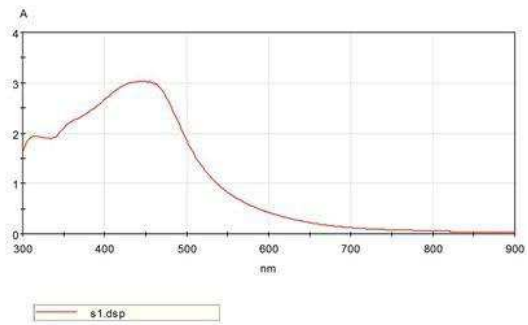

Figure 2 : UV-vis spectra of aqueous silver nitrate with *B. phoenicea* leaf extract at different time intervals

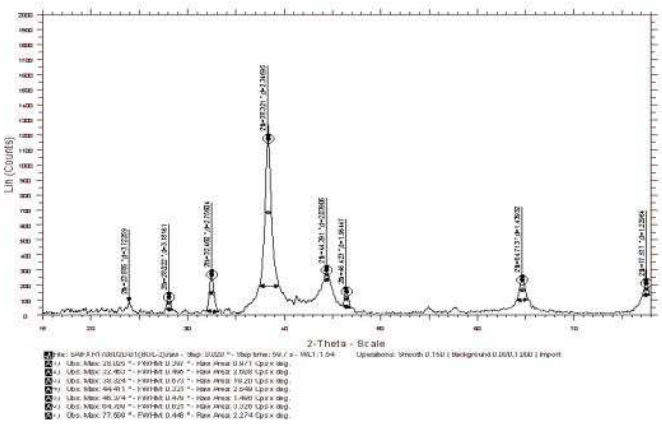

Figure 3 : XRD pattern of AgNPs synthesized using *B. phoenicea* leaf extract

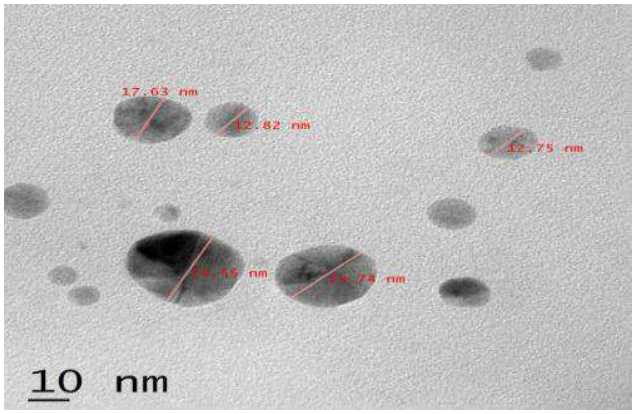

Figure 4 : Transmission electron microscopic image showing synthesized AgNPs from *B. phoenicea* leaf extract

3. Acetylcholinesterase Activity Assay for Larvicidal activity

When *Culex quinquefasciatus* larvae were treated with the silver nanoparticles synthesized using *Bauhinia phoenicea* leaf extract, it caused the death of the larvae. This is due to acetylcholinesteraseactivity inhibition due to the effects of AgNPs. When acetylcholinesterase enzyme is inhibited, the neural transmission gets blocked and results in the death of the larvae (Figures 5,6).

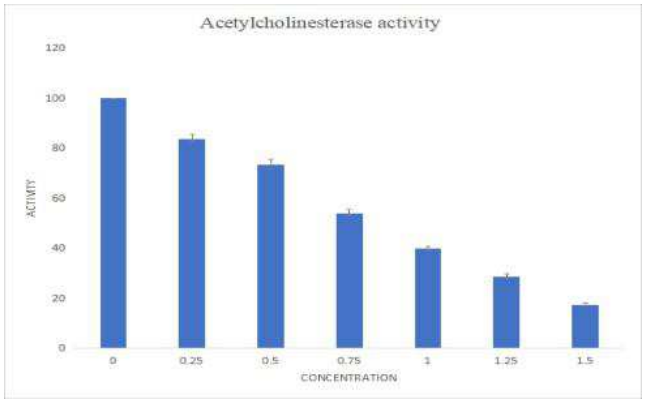

Figure 5 : Graph showing acetylcholinesterase activity of synthesized silver nanoparticles from *B. phoenicea* leaf extract against *Culex quinquefasciatus* larvae at varying concentrations

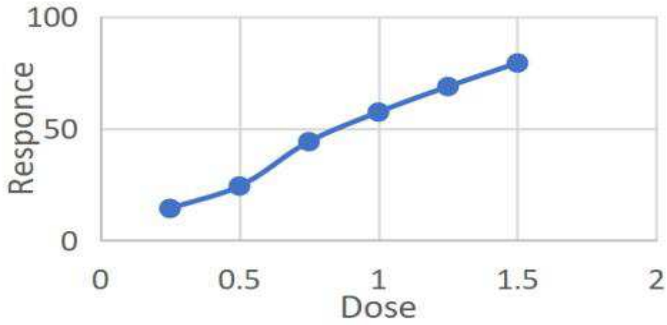

Figure 6 : Graphical plot depicting the response of the larvae at varying doses of the synthesized silver nanoparticles from *B.phoenicea* leaf extract (IC_{50}value analysis)

Conclusion

The biological method of synthesis of silver nanoparticles using *Bauhinia phoenicea* leaf extract appears to be an eco-friendly and cost effective way alternative to conventional chemical and physical methods. Silver nanoparticles were successfully synthesized by this method and were characterized using UV, XRD and TEM. In UV analysis we got a peak at 450 nm which was due to Surface Plasmon Resonance (SPR). XRD analysis revealed that the synthesized silver nanoparticles are semi-crystalline in nature and we got the size of nanoparticles (D value) as 13.06 nm. TEM analysis revealed that the size of silver nanoparticles ranged from 12.75 nm to 25.55 nm. Thus when we compare XRD results with TEM values, 13.06 nm falls within the range of TEM values, 12.75 nm to 25.55 nm, confirming our results. The green synthesized silver nanoparticle is also known to possess significant larvicidal activity and thus it can be used as an ideal eco-friendly approach for the controlof *Culex quinquefasciatus* larvae.

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Cardiac Tissue Engineering: Next-Gen Cardiac Care

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Abstract

The global incidence of ischemic heart diseases is increasing alarmingly where the conventional management strategies become insufficient. Tissue engineering emerged as an alternative which combines cells, biomaterials scaffolds and signaling mediators aiming to accelerate the myocardial regeneration. Promising outcomes have been obtained in small animal models and pre-clinical models suggesting the potential application of tissue engineering in regenerative cardiology. This article throws light into the overview of cardiac tissue engineering as a next-generation management strategy.

Key words: Cardiac tissue engineering, CVD, Myocardial infarction, Hydrogels, Synthetic polymers

Introduction

Ischemic heart disease and stroke are the major cardiovascular diseases (CVDs) causing serious health problems in developed and underdeveloped countries. CVDs account for 31% of global deaths and approximately, 422.7 million cases and 17.92 million deaths of CVDs were reported in 2015 throughout the globe. Alarmingly, it is estimated that CVDs would cause an economic burden of 1044 billion dollars by 2030 (Benjamin et al., 2017). Transition in the socioeconomic status of the people due to urbanization, industrialization and globalization bring about lifestyle changes accelerating the risk of CVDs. The major risk factors include physical inactivity, unhealthy diet, tobacco consumption, and alcoholism. These reports point out the severity and prognosis of CVDs (Amani and Sharifi, 2012)

Ischemic heart disease (IHD) arises primarily due to the blockage of coronary arteries due to cholesterol deposition or plaque formation. Myocardial infarction (MI), the principal complication of IHD, occurs due to the obstruction to blood flow to heart muscles leading to hypoxia and necrotic/apoptotic death of diverse cell phenotypes in cardiac tissue. MI results in the permanent loss of cardiomyocytes, the beating elements of heart, resulting in structural and functional alterations of surviving heart tissue. Cardiomyocytes are terminally differentiated; which hurdles the regenerative mechanisms following MI and promotes fibrosis. Conventional therapeutic approaches include pharmacological treatments (beta blockers, statins, ACE inhibitors, thrombolytic agents and others), medical devices and heart transplantation. Even though medications and transplantable devices improve the blood flow and cardiac function on demand; they fail to manage the progression of disease. Short life

span, potential risk of infection, and thrombosis are some other short comings of implants. (Vunjak Novakovic et al., 2014) Heart transplantation, in which the infarcted heart is replaced with a healthy heart from a donor, is found to be an effective treatment strategy in this context. However, limited number of organ donors and immunological rejections restricts cardiac transplantations. Thus novel therapeutic strategies for the effective management of cardiac tissue regeneration are urgent need of the hour.

Tissue engineering

Regenerative strategies based on stem cells and tissue engineering are gaining more attention as advanced and promising methods for the management of cardiac dysfunctions including MI. Earlier, researchers attempted to inject cells directly into injured myocardium to bring about regeneration. However, this strategy failed owing to poor integration at the site of injury and increased apoptosis. It was later proposed that cell retention could be improved by providing support to cells in the form of scaffolds and this marked the origin of tissue engineering. Tissue engineering approach includes isolation, expansion and culture of specific cardiac cell phenotypes in three dimensional scaffolds made from selected biomaterials, to act as a template to trigger regeneration. Regeneration is effected as the scaffold supports the seeded cells for proliferation, ECM synthesis and neo tissue formation. Being biodegradable, the scaffold ensures bioresorption, in tandem, with regeneration. The administration of cell-scaffold combination onto injured myocardium involves two approaches, viz injectable scaffolds or as implantable scaffolds. In the injectable scaffold approach, the scaffold seeded with cell is injected directly into the injured myocardium in a liquid or semi liquid form using a syringe. This, upon reaching the target site the components get cross linked to from a gel and aids in regeneration. Gelation can be effected by with the help of specific extrinsic or intrinsic cues such as chemicals or variations in the temperature, and pH (Peña et al., 2018). In the second method, the biomaterial is fabricated to form a porous scaffold and specific cell phenotypes are seeded into it. The cells are allowed to proliferate in vitro in the scaffold followed by tailoring onto infarcted heart (Thankam and Muthu, 2014). Injectable system has the advantage of minimally invasive administration procedures Kichula et al. (2013) attenuating wall stress following MI. For instance, the injection of hyaluronic acid hydrogel increased the stiffness of myocardium/hydrogel interface which in turn reduced global average fiber stress.

The different cell types used to seed cardiac tissue engineering scaffolds includes cardiomyocytes which are either neonatal, fetal, or adult in origin, skeletal myoblasts, bone marrow derived stem cells, embryonic stem cells, smooth muscle cells, adipose tissue derived stem cells, cardiac stem cells or induced pluripotent stem cells. The choice of cells is to made with utter care keeping several factors in mind including it availability and convenience (Rodrigues et al., 2018)

The success rate of tissue engineering approach depends solely on the performance of cells until a functional cardiac extra cellular matrix (ECM) is reconstructed by the surviving tissue and normal function is regained. For a scaffold to support cell survival, the ideal characters that are necessary include biocompatibility, biodegradability, presence of open and interconnected pores, mechanical strength, and native tissue mimicking features. The fabrication of a suitable scaffold for cardiac tissue engineering requires the careful the selection of candidate biomaterial, fine tuning of its properties, and proper designing strategies.

Biomaterials used in cardiac tissue engineering

Several polymeric biomaterials from synthetic or natural origin have been employed for the development of 3D scaffolds. Among the biomaterials proposed for cardiac application, the hydrogels subset has been considered to be ideal choice owing to their tunable features and ability to support cell adhesion and growth. Additionally, hydrogels can be programmed by using microfabrication techniques to induce vascularization of engineered cardiac constructs or to direct the alignment of cardiac cells to improve their functions. Hydrogels act as a provisional matrix for cardiac cells to deposit extracellular matrix (ECM) and facilitate neo-tissue formation as they degrade (Hofmann et al., 2005; Li and Guan, 2011). In addition, growth factors, signaling molecules and therapeutics can be incorporated within these hydrogels for accelerating repair and regeneration of injured heart (Davis et al., 2006)

Hydrogels based on natural polymers, have been explored extensively for applications in three dimensional cell cultures. Although promising results have been observed from cells cultured with these natural gels, complex, variable and ill-defined composition, poor mechanical properties, and immune system activation of these materials often offers hurdles to cardiac applications. In contrast, synthetic hydrogels with well-defined network and mechanical strength could provide a regular three dimensional platform for cell growth. However, uncontrolled degradation, toxicity of degradation products, lack of biological cues and immune-reactivity limits the application of synthetic polymers in regenerative medicine. (Li and Guan, 2011)Alginate, chitin, fibrin, collagen, and decellularized matrix, are the major natural hydrogels generally used for CTE while synthetic hydrogels include PVA, PEG, Poly(2-hydroxyethyl methacrylate), and polyacrylamide.

Even though several hydrogel types are being used for cardiac tissue engineering applications, sufficient information is limited in the literature regarding the application of hydrogels for taming the hostile environment and preventing the infarct zone expansion**.** McDevitt et al., (2009) found that elastomeric and biodegradable polyurethane film serves as scaffold material for the growth of cardiomyocytes. A surface modified cardiac tissue engineering scaffold was constructed using poly (2-hydroxy ethyl methacrylate methacrylic acid) hydrogel and found that embryonic stem cellderived cardiomyocytes survived and proliferated under *in vitro* conditions and observed the formation of an adult heart like tissue (Madden et al.,

2010). A panel of hydrogels were prepared by mixing porcine heart ECM and collagen type I to assess the ability of heart ECM to direct cardiac differentiation of human embryonic stem cells in vitro. A seminal study reported that native ECM hydrogels induce the differentiation of human embryonic stem cells towards cardiac lineage even without other signaling factors suggesting an attractive biomaterial system for cardiac regeneration (Duan et al., 2011).

Interestingly, the biological performance of natural biomaterials can be improved by reinforcement with biocompatible synthetic polymers. Such hybrid hydrogel system comprising natural and synthetic polymers could address the issues associated with their individual applications. They have been hailed to possess exceptional biocompatibility and appreciable mechanical strength for cardiac applications. It was demonstrated that polyethylene glycol interpenetrated cross linked hydrogel scaffold prepared from alginate and chitosan can function as an ECM mimic (Radhakrishnan et al., 2015).

Techniques for 3D scaffold fabrication

Several techniques are employed to fabricate scaffolds and to manipulate nano scale topographies such as porosity roughness etc. on the scaffold surface. This is very essential for the successful adherence, migration and proliferation of cells on scaffold surface. The different techniques include electrospinning, phase separation, etching, vapor deposition, self-assembly, lithography, solvent casting, and 3D printing (Chandrasekhar et al., 2020). A recent approach aims to obtain decellularized 3D structures as biomaterial scaffold for regeneration and also to make complete bio artificial organs. This can be achieved by decellularizing tissues using enzymes while maintaining the intact ECM (Rodrigues et al., 2018).

Conclusion and future prospects

Advancement in medical science, engineering, basic biology, and materials science have significantly motivated the field of cardiac tissue engineering. However, translation from laboratory to clinics warrants further improvements and groundbreaking discoveries. Multiple challenges including electrical integration, immunological hurdles and biological performance of post-implantation engineered tissues persist. Nonetheless, cardiac tissue engineering promise the potential as next-generation management strategy for cardiac complications offering pleasant hope for millions of sufferers across the globe.

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Ameliorative effect of Lac on Paracetamol induced hepatotoxicity in rats

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Abstract

Lac, a resin secreted by *Lacciferlacca*, possess moderate antioxidant activity in both *in-vitro* and *in-vivo* conditions. 'Hepatotoxicity' implies chemical-driven liver damage. In therapeutic dose, paracetamol (PCM) is metabolized by hepatic cytochrome p450 (CYP450) system to N-acetyl-p-benzoquinone imine (NAPQI), which is detoxified by glutathione (GSH). Over dose of PCM results in accumulation of NAPQI, generating reactive oxygen species (ROS) that cause hepatic necrosis. Liver function marker's level is also increased here. This study aims to evaluate the protective effect of lac on PCM mediated damage and lipid peroxidation in hepatic tissues.

Keywords: Antioxidants, Hepatotoxicity, Lipid Peroxidation

Introduction

Liver is involved in almost all the biochemical pathways, fight against disease, nutrient supply, energy provision and reproduction and thereby plays vital functions in maintenance, performance and regulating homeostasis of the body. Liver diseases are one of the major global health problems in developing countries. They are mainly caused by toxic chemicals (certain antibiotics, chemotherapeutics, peroxidised oil, aflatoxin, carbon tetrachloride, chlorinated hydrocarbons, etc.), excess consumption of alcohol, infections and autoimmune disorder (Rachna, 2018). 'Hepatotoxins' are chemicals which cause liver damage and this condition is termed as 'hepatotoxicity'. When taken in overdose or sometimes even in therapeutic dose, certain medicines may act as hepatotoxins.Paracetamol (PCM) is a widely used antipyretic or analgesic medication considered to be safe at therapeutic levels. In therapeutic dose, PCM is converted to water-soluble metabolites and excreted through urine. Excess PCM is metabolized by hepatic cytochrome p450 (CYP450) system into N-acetyl-p-benzoquinone imine (NAPQI), a toxic metabolite, which gets detoxified by glutathione (GSH) at normal conditions. However, toxic doses of PCM causes the depletion of GSH resulting in accumulation of NAPQI and forms NAPQI-protein adducts by covalently binding with cellular proteins (Saroj et al., 2012). This in turn results in the generation of reactive oxygen species (ROS) including the hydrogen peroxide (H_2O_2), superoxide anion and hydroxyl ($OH^{¨}$) radical that affect the cellular membrane and induce lipid peroxidation and also cause hepatic necrosis. The paracetamol induced toxicity model is widely used to study the potential

hepatoprotective activity of various extracts or compounds.In Ayurveda, plant materials have been used to protect liver injury. Animals and products derived from different organs of their bodies have constituted part of the inventory of medicinal substances used in various cultures since ancient times. Lac is one of the unexplored and only animal origin resin with naturally degradable characteristics. It is secreted by *Laccifer lacca*, a Hemipteran insect of family Kerridae, which is commonly known as Indian lac insect. Lac is obtained from host plants with animal remnants within it, called as stick lac. This stick lac on purification, obtains in pellet form is called as seed lac which on further purification gives shellac in the form of amber flakes (Sharma, 2017). In Ayurveda, Siddha and Unani system of medicine lac (luk) is used for treatment of a variety of diseases. In Ayurveda, lac is considered to balance pitta-kapha dosh and promotes strength. In Unani, lac is considered as tonic for the liver, stomach and intestine. Some preliminary studies shown that lac can act as scavengers of free radicals and effectively ameliorated the redox imbalance induced by sodium fluoride in mice. For instance, for ensuring the therapeutic values of lac the present study aimed to evaluate the protective effect of lac against paracetamol induced hepatotoxicity in rats.

Materials and Methods

Preparation of lac extract

Lac was purchased in crude form from Ayurvedic shop. It was powdered and extracted using methanol.

Chemicals

Paracetamol and Silymarin were purchased from Sigma- Aldrich (USA). Euro Diagnostics Systems Pvt. Ltd (Chennai, India) supplied the biochemical kits for determining SGPT, SGOT, ALP, bilirubin and total protein. All other chemicals and reagents used were of analytical grade.

Animals

Female Wistar rats (150-180 g) were purchased from Small Animal Breeding Station, College of Veterinary, Agricultural University, Thrissur, Kerala. The animals were maintained under standardized environmental conditions (22-28^{0}C, 60-70% relative humidity, 12-hour dark / light cycle) and fed with standard rat feed (Sai Durga Feeds and Foods) and water *ad libitum*. All the animal experiments werecarried out in Amala Cancer Research Centre, Thrissur, by the prior permission of Institutional Animal Ethics Committee (IAEC).

Acute toxicity studies

Acute toxicity analysis of lac was done. Female Wistar rat weighing 150-180 g were divided into two groups. Group I was kept as normal and group II was treated with single oral administration of lac at a dose of 1 g/Kg b.wt. The feed and water were removed 4 hours before drug administration and replacing them 1 hour after the drug administration. All animals were carefully observed for 14 days for any toxicity symptoms. If mortality is not there, 1/5th and 1/10th of acute dosage was selected as high dose and low dose respectively.

Protective effect of lac against paracetamol induced hepatotoxicity in rats

Rats were divided into Group I (Normal without any treatment), Group II (Paracetamol-3 g/Kg b.wt.),Group III (Silymarin-100 mg/Kg b.wt.), Group IV (Vehicle control-1% propylene glycol) Group V (Lac-250 mg/Kg b.wt.), Group VI (Lac-500 mg/Kg b.wt.) with 5 animals in each group. Animals from Group III, V and VI were pretreated with silymarin and lac, respectively orally once daily for 8 days. Required doses of lac was dissolved in 1% propylene glycol for administration of animals. Group IV received propylene glycol once a day through out experimental period. On 9^{th} day, all animals except Group I were received a single dose of paracetamol (2 mL orally) to induce acute hepatotoxicity. All animals were sacrificed 24 hours after paracetamol administration.

Analysis of hepatic function

Blood was collected by cardiac puncture. Serum was separated and used to determine the activities of serum glutamate pyruvate transaminase/SGPT (IFCC method), serum glutamate oxaloacetate transaminase/SGOT (IFCC method), alkaline phosphatase/ALP, bilirubin (modified Jendrassik method) and total protein (Biuret method) photometrically using commercially available kits.

Determination of oxidative stress

The collected liver was washed with ice cold saline (0.9%) in order to remove blood contaminants. Then prepared 10% of liver homogenate using Tris HCl (0.1 M, pH 7.4). Lipid peroxidation *in-vivo* (TBARS assay, (Ohkawa et al., 1979), conjugated dienes in tissue homogenate (John and Steven, 1978), hydroperoxide in tissue homogenate (John and Steven, 1978) were determined.

Analysis of hepatic antioxidant enzymes

Liver homogenate (10%) was centrifuged at 8600 rpm for 30 minutes at 4! and the supernatant was used to estimate the levels of antioxidant enzymes. Superoxide dismutase/SOD (McCord and Fridovich, 1969), catalase activity in tissue (Beer and Sizer, 1952), glutathione/GSH (Moron et al., 1979), glutathione peroxidase activity/GPx (Hafemann et al., 1974) were estimated.

Histopathological analysis

A small piece of liver was excised, washed in ice cold saline and a small portion was fixed in 10% formalin for histopathological analysis using hematoxylin and eosin stain. The analysis was done at Polyclinic Medical Laboratory Pvt. Ltd, Thrissur, Kerala.

Statistical analysis

Values are expressed as mean ±SD and compared to control. The statistical analysis was done by one-way analysis of variance (ANOVA) followed by Dunnett's test using Instat software. P values - $p<0.05$* and $p<0.01$** were considered as significant and $p>0.05$ as non-significant.

Results and Discussion

Acute toxicity analysis of lac

Animals administrated with lac at a dose of 1 g/Kg b.wt. didn't show any toxic symptoms. Mortality was not observed in any animals, which lived up to 30 days after single administration of lac. All animals exhibited a normal increase in body weight without drastic difference between both normal and lac treated groups.

Effect of lac on liver function markers

GROUP	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	TP (g/dL)	TB (mg/dL)
Normal	87.59±4.03	45.39±9.44	57.12±5.75	7.64±0.3	0.29±0.1
Control	336.54±15.43**	296.38±8.02**	389.72±9.77**	4.58±0.24**	0.79±0.09**
VC	209.08±9.26**	160.63±19.75**	310.95±23.45**	5.07±0.93ns	0.64±0.16ns
Standard	95.59±3.09**	56.31±4.32**	66.34±1.95**	6.05±0.28ns	0.35±0.16*
LCLD	157.43±10.56**	82.06±15.88**	214.67±6.23**	6.94±0.30**	0.56±0.15ns
LCHD	139.09±7.53**	73.04±11.92**	135.89±11.08**	7.82±0.91**	0.38±0.1*

Table 1 : Effect of lac on hepatic function. Values are expressed as mean±SD for 5 animals. *$p<0.05$, **$p<0.01$ and $^{ns}p>0.05$

Liver damage is assessed usually by determination of serum enzyme levels of SGOT (AST), SGPT (ALT) and ALP. Due to paracetamol administration, these serum markers were released into circulation, by the loss of hepatocytes membrane integrity and cell damage (Gupta and Misra, 2006). The results demonstrated that the pre-treatment with lac low dose and high dose (250 and 500 mg/Kg b.wt., respectively) reduced serum ALT and AST activities raised by paracetamol, indicating preservation of structural integrity of hepatocellular membrane. Increase in serum ALP is due to increased synthesis, in presence of increasing biliary pressure. The result of this study shows that the lac significantly lowered the ALP and bilirubin level indicating an early improvement in the secretory mechanism of hepatic cells. This result was also observed in rats treated with silymarin (100 mg/Kg b.wt.), used as a positive control.

Effect of lac on oxidative stress

Tissue damage markers like MDA level, conjugated diene and tissue hydroperoxides were high in paracetamol alone treated group when compared with those of normal animals (figure 1 a, b and c). Administration of lac reduced these higher levels to nearly normal levels. Results were comparable with silymarin treated animals and have shown lower levels of oxidative stress.

Effect of lac on hepatic antioxidant enzymes

In paracetamol alone treated group, a significant reduction in hepatic SOD activity was observed, indicating a decrease in the antioxidant capacity. This may be attributed to the

consumption of SOD in ROS detoxification (Mladenovic et al., 2009). Pre-treatment with lac significantly enhanced the activity of SOD, catalase enzyme, GPx and GSH in LCLD and LCHD groups compared to control group (Figure 2 a, b, c and d). Silymarin treated animals also shown significant restoration in these enzyme activities. When the rate of NAPQI exceeds the rate of detoxification by GSH, it oxidizes the tissue macromolecules such as lipid or thio (-SH) group of protein after depleting GSH and inhibiting GSH synthesis (Gupta et al., 2004). It has been also found that paracetamol reduced the activity of GPx. The reduction of GPx effectiveness may be due to its inactivation by reactive oxygen species. Pre-treatment of rats with lac significantly increased the hepatic GSH content as well as GPx activities compared with rats administered paracetamol only, indicating decrease in oxidative damage. This may be due to the antioxidant capacity of lac.

Histopathological analysis

Histopathological examination of liver sections of normal animals showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein. In control group, there is disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis extending to mid-zone and sinusoidal hemorrhages and dilatation. There was chronic inflammatory cell infiltrate in the portal tracts.The standard group showed less vacuole formation, reduced sinusoidal dilation, and less disarrangement and degeneration of hepatocytes, indicating marked regenerative activity. In case of Lac treated rats showed least hepatocyte damage compared to control animals indicates its moderate hepatoprotective activity.

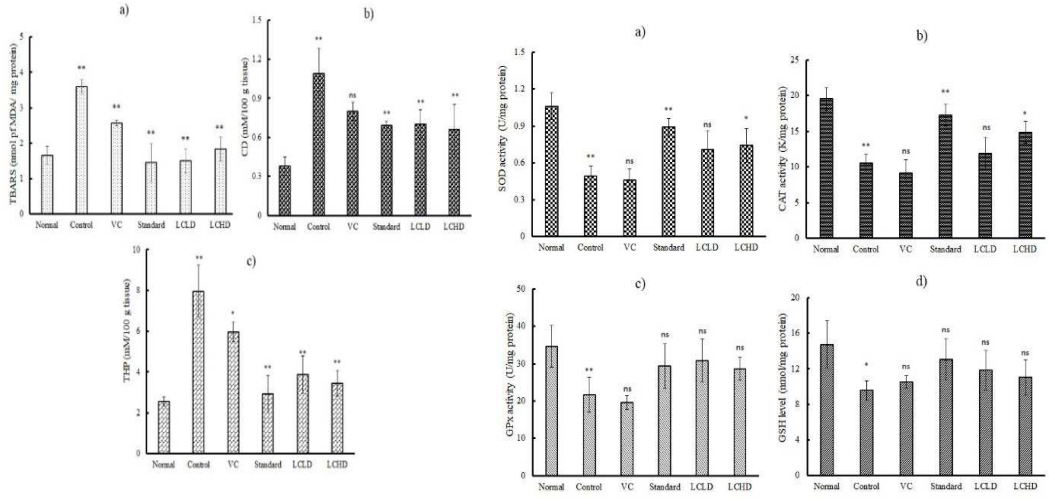

Figure 1: a) Effect of lac on hepatic lipid peroxidation level, b) Effect of lac on conjugated diene level, c) Effect of lac on tissue hydroperoxide level. **Figure 2 :** a)Effect of lac on superoxide dismutase (SOD) activity, b)Effect of lac on catalase (CAT) activity, c) Effect of lac on glutathione peroxidase (GPx) activity, d) Effect of lac on reduced glutathione (GSH). Values are expressed as mean±SD for 5 animals. *$p<0.05$, **$p<0.01$ and $^{ns}p>0.05$

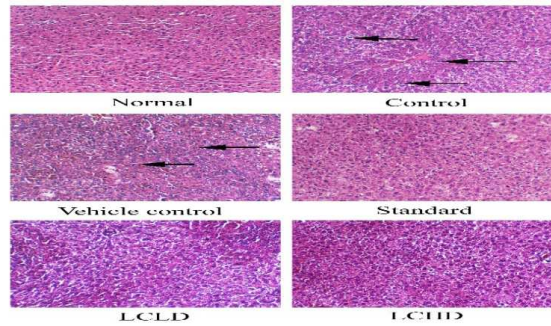

Figure 3 : Histopathological analysis

Results of previous studies shown that lac exhibited moderate antioxidant activity in both *in-vitro* and *in-vivo* conditions.This study revealed that lac holds modest protective property against paracetamol induced hepatotoxicity. Further studies should be needed to substantiate the use of lac for therapeutic purposes.

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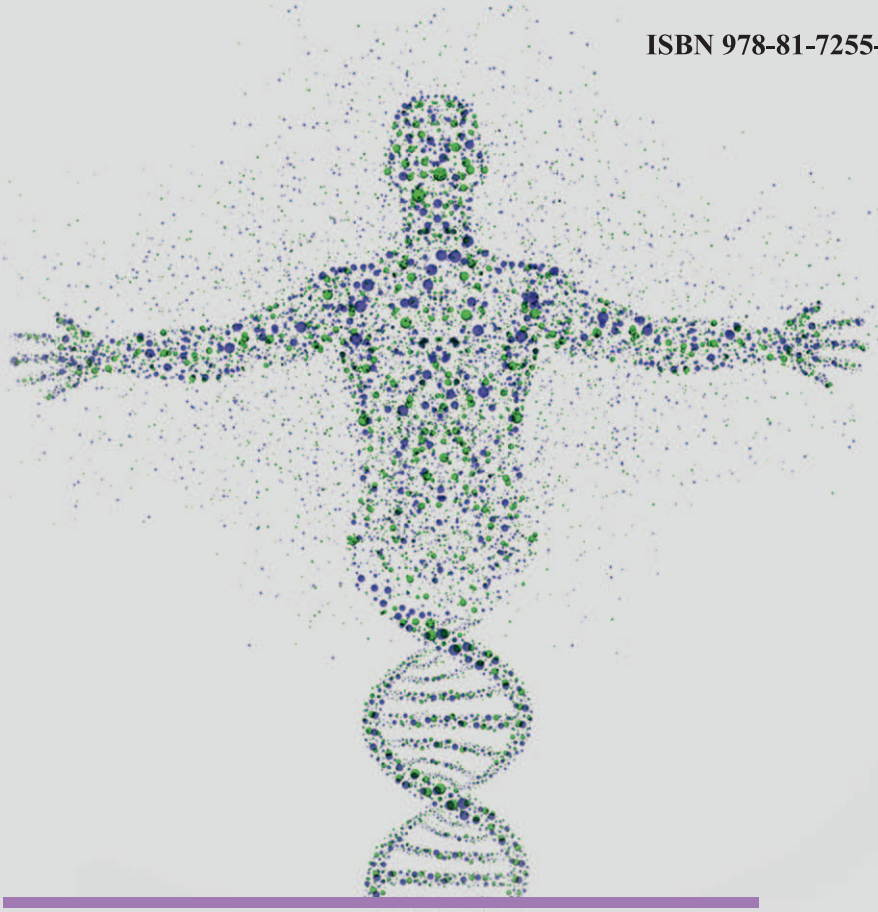

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