Scientia (Annual)
Number 1
Jan - Dec 2012
Volume 8
ISSN: 0976-8289

SCIENTIA

A National Science Journal

PUBLISHED BY
MERCY COLLEGE
PALAKKAD - 678 006, KERALA, INDIA
Govt. Aided Arts and Science College
Affiliated to University of Calicut, Accredited with 'A' grade in 3rd cycle
by the National Assessment and Accreditation Council

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SCIENTIA

A National Science Journal Published Annually in January

Volume 8 ● Number 1 ● Jan - Dec 2012 ● ISSN: 0976-8289

Editorial Board

Statement of ownership and other particulars

Place of publication : Mercy College Palakkad

Periodicity of publication : Annual

Printers Name and Address : Dr. Sr. Annamkutty (Sr. Kripa) Principal, Mercy College, Palakkad

Phone : 0491-2541149

Fax : 0491-254681

Website : http//:www.mercycollegepkd.com

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editorial

2012

Greetings to all our contributors and readers

Scientia is an interdisciplinary national science journal publishes annually to inculcate and share scientific knowledge among researchers, scientists and learners among various institutes. The year 2012 marks the 8th year of publication of Scientia (ISSN: 0976-8289), an annual science journal from Mercy college, Palakkad. We are happy to bring out this issue of Scientia which features 18 articles from various areas of science. Scientia publishes finest peer-reviewed research in all fields of science and technology on the basis of its originality, importance, interdisciplinary interest, timeliness, accessibility, elegance and surprising conclusions. Scientific journals represent the most vital means for disseminating research findings and are usually specialized for different academic disciplines. Often, the research challenges common assumptions and/or the research data presented in the published scientific literature in order to gain a clearer understanding of the facts and findings. Depending upon the policies of scientia journal, articles may include review, original research article and short communication.

Scientia covers frontier areas like Physics, Chemistry, Mathematics, Computer science, Biotechnology, Bioinformatics and Life sciences. Let the 8th volume of Scientia nurture the diversity with 2 review papers, 14 full papers and 2 short communications. Scientia congratulates all our contributors and readers for your achievement of 2012 and wishes all of you a Happy New Year.

With warm personal regards.

Dr. S. Jayasree
(Chief Editor)

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Vol. 8, No.1, Jan - Dec 2012, pp 7 - 20

ISSN: 0976-8289

The significance of Juvenile Hormone and Juvenile Hormone esterase in embryonic and post embryonic development of insects

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Abstract

Juvenile Hormones (JHs) synthesized in the Corpora Allata (CA) of insects regulates embryonic development, repress metamorphosis and induce vitellogenin synthesis and pheromone production. Metamorphosis comprises dramatic transformation in shape and function of organs, tissues and individual cells.JH being a morphogenetic hormone during nymphal stages, its titer at particular life stages determines which type of molt the developing insect has to undergo. JH acid is an inactive precursor and metabolite of JH which actually induces cells to become competent to undergo metamorphosis, whereas ecdysteroid merely stabilizes this commitment and facilitates the expression of this state of development program. In the final instar stage, Juvenile hormone must be cleared effectively if successful molting to adult is to occur. One pathway of this degradation is through juvenile hormone esterase (JHE), which cleaves the JH ester bond to produce methanol and JH acid. JHE a selective enzyme that hydrolyzes the methyl ester of insect Juvenile hormone plays an important role by regulating metamorphosis in nymphs and reproduction in adults. It appears that action of JHEs is indeed an important mechanism in the regulation of JH titer. A hypothetical decrease in the rate of JH biosynthesis alone is not ample for rapid JH clearance but prevention of natural degradation of JH by JHE is also vital. It is apparent that the JH degradation system in the adult acquires particular importance in this regard .This study analyzes the role of JH and JHE in development using a heteropteran insect *Dysdercus cingulatus*.

Key words: Juvenile Hormones, Juvenile Hormone Esterase (JHE), Metamorphosis, *Dysdercus cingulatus*.

Introduction

Biology of JH is an illustration of one of the most elaborate results of the evolution of control and response mechanisms. Juvenile hormone (JH) produced in the Corpora allata (CA) of insect plays a pivotal role in an insectan life. The most wonderful and interesting aspect of JH is the exceptionally diverse range of functionality that JH or JH metabolites have on the insect life cycle including their roles in development, metamorphosis, reproduction, diapause, migration, polyphenisms and metabolism [1,2] .The diverse functionalities of JH suggest that not only the numerous target sites for JH, but also its biosynthesis, transport, and degradation must be carefully regulated in the endocrine control of embryogenesis, molting, metamorphosis and reproduction. Metamorphoses being the most important among these adaptations, precisely timed changes of JH titer are essential for unfolding the cascade of events in insect development and reproduction.

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It involves all the tissues and cells of an insect and comprises dramatic transformation in shape and function of organs, tissues and individual cells. Both ecdysone and juvenile hormone, regulate the morphogenetic changes of insect metamorphosis [3]. According to the classical theory of the hormonal control of insect metamorphosis, ecdysteroid initiates a molt independent on the titer of JH. However a few observations earlier [4] indicate that tissues must first acquire competence in the presence of JH acid alone is not sufficient for the metamorphic response to ecdysteroid. JH acid is an inactive precursor and metabolite of JH actually induces cells to become competent to undergo metamorphosis, whereas ecdysteroid merely stabilizes this commitment and facilitates the expression of this state of development program.

Juvenile hormone esterase (JHE) is a member of the α/β –hydrolase family of enzymes that is thought to be the key enzyme responsible for the formation of JH acid metabolite[1]. The scavenging role of JH specific enzyme, JHE is responsible for the changes brought in the rate of degradation at critical times of insect development and metamorphosis. The catalytic mechanism of of JHE involves a nucleophilic attack on the substrate, leading to the release of methyl alcohol and the formation of an acyl-enzyme intermediate *via* a tetrahedral transition state intermediate. This acyl-enzyme intermediate is then hydrolyzed, leading to release of JH acid and regeneration of the enzyme. Biological criteria for the term 'JH specific esterase shows that JH hydrolyzing esterase is essential for the clearance of JH from an insect's own body and its titer correlates with suspected decline in JH titer. Also, the JH enzyme should have a low apparent K_m for the substrate JH and therefore hydrolyse JH with a high K_{cat}/K_m ratio. Biochemically esterase is capable of rapidly hydrolyzing JH in the presence or absence of carrier proteins. The rapid appearance and disappearance of JHE are important factors in the regulation of JH titer during the process of commitment and metamorphosis. Both JHE and carrier proteins are important in the regulation of JH level in the haemolymph during larval/nymphal development. In the early instars, where high levels of JH are required the binding protein is necessary to protect JH from degradation by general esterase. Because of the continuous presence of these esterase, only JH complemented to the binding proteins can reach the target tissues and thus the binding proteins is the true carrier in the haemolymph.

JHE has been purified and characterized from several lepidopteran insect species like *Trichoplusia ni*[5], *Manduca sexta* [6], *Lymantria dispar*,[7] *Heliothis virescens* [8], *Tenebrio molitor* [9], *Bombyx* mori [10], *Choristoneura fumiferana*[11].

As the continuous use of pesticides adversely affected the environment leading to the emergence of Integrated Pest Management (IPM), strategy which is an ecologically compatible methodology that

produce endocrine imbalance by disrupting molting and metamorphosis. Biological control of agricultural pests has gained importance in recent years primarily due to increased pressure to reduce the use to increased pressure to reduce the use of agrochemicals and their residues in the environment and food. The current millennium demands that the pest management studies should be biointensive. Most of the attributes like ecofriendly, safe, compatible approaches and tactics for pest management systems reduce pest inputs and conserve natural fauna.

Biological insect control agents are an alternative to chemical pesticides since they do not pose the environmental and health risks associated with chemical residues. New approaches to the development of insect control agents have been revealed through the molecular description of neuropeptides, their biogenesis, action and degradation. Purification, amino acid sequencing, and gene cloning provide the molecular tools necessary for the studies on neuropeptide synthesis processing, secretion, receptor binding, and inactivation. Each of these areas consists of a number of amino acid sequence and enzyme-dependent steps, which may be considered as targets for the development of highly specific control agents. These agents will include antagonists, and superantagonists, peptidomimetics, recombinant peptides delivered through the baculovirus technology, receptor blockers and enzyme inhibitors.

With the advent of recombinant DNA technology and the recent development of rapid action recombinant baculovirus, interest in the field potential of these viruses for insect control has increased dramatically. Several major companies and various academic and government laboratories are currently contributing to this area. Wild type baculoviruses are an integral component of the natural biological control of many pest species, and application of wild type viruses has been very effective for the pest management in several cases.

Because of the profound role that JHE plays both physiologically and endocrinologically, its usefulness as a biopesticide has been explored. If this JHE inhibited *in vivo*, JH titer remains high enough to keep the larvae in the feeding stages resulting in giant insects. Thus if sufficient quantities of JHE were expressed *in vivo* at an early stage of development, the reduction of JH titer should cause the affected insect to stop feeding. *In vivo* expression of JHE may help in the improvement of genetically engineered viral insecticides, which work by reducing insect feeding [12,13].

A homology based molecular model of JHE from the agricultural crop pest, Heliothis virescens12 is being used as a predictive basis to design biopesticides. The alteration of specific residues of JHE that disrupted lysosomal targeting13, increased the insecticidal activity of this protein. JHE produced in vitro by baculovirus –infected Spodoptera frugiperda cells, showed anti –JH effects when infected into M. sexta

larvae12. The coding sequence for JHE from the tobacco bud worm, Heliothes virescens was expressed in various baculovirus constructs13. JHE has been expressed in Ac NPV under control of the polyhedrin p10 and basic protein promotors13. Recombinant JHE, cloned from H.virescens, was injected into A.aegypti larvae resulting in a dose dependent decrease in survival and also resulted in impaired ovaiole maturation. The expressed recombinant JHE, acting as an anti-JH enzyme, is potentially useful for mosquito control. JHE expressed by an appropriate mosquito vector would be useful for control both as a mosquitocide and through deleterious effects on reproduction. The recombinant baculovirus expression modified form of JHE named as Ac-JHE-SG, has enhanced activity against lepidopteran larvae[13].

The vast majority of work concerning the regulation of JH titer by Juvenile hormone esterase has been demonstrated only in lepidopteran[1]. Some aspects of JH titer regulation have been documented in Diptera [10,11] Coleoptera [14] and Orthoptera[15] as well. Relatively little is known about similar or divergent mechanisms in the Order Heteroptera. This review explores similar mechanisms in a heteroptan insect, *D.cingulatus*.

The Morphostatic action of Juvenile hormone in *Dysdercus cingulatus*.

Juvenile hormone act as special repressor agent which inhibit morphogenetic process for a determined time at any stage between the fertilized egg cell and fully differentiated adult and is hence responsible for the existence of polymorphic immature stages so characteristic of insect development. To understand the embryological roles of JH, the approaches selected are chemical allatectomy using antiJH Precocene and application of exogenous JH or a JH agonist.JH applied shortly after oviposition has little effect on embryogenesis, but applied after that has a profound effect on development, including disruption of blastokinesis, or to complete ecdysis. Studies on the effect of exogenous JH in *D.cingulatus* embryos of diverse ages showed that the presence of this hormone during the early stages of embryogenesis (cleavage, blastoderm formation, and grastrulation) is deleterious to its normal development. Depending on dosage and the time of JH application, embryonic development is blocked either at germ band stage, or at blastokinesis stage. JH has an important role in embryonic morphogenesis at the germband formation or at blastokinesis. This further suggests that JH plays a crucial role in late embryogenesis[16].

In the fifth instar nymphs of *D.cingulatus*, the critical period (when tissues are much sensitive to JHa) was found to be between just after emergence and the third day. The presence of mosaic forms has one rather important endocrinological implication that an insect target cell of JH is programmed to go or the other way but it can never proceed through both the pathways simultaneously[18].The effect

produced by a given juvenoid in insect development should always be a result of two variables: one represented by developmental timing and the other by changes in the effective concentration of the

juvenoid. Morphologically perfect extra-nymphal instars are formed when the effective concentration of the juvenoid has been maintained or surpassed during the whole duration of the critical sensitive period to JH. Different intermediate forms developed when the effective juvenoid concentration has been reached for just a part of the critical period. The mode of action of JHa lies in their ability to block the depression, transcription, or utilization of fresh genetic information[17]. Juvenilizing activity of JHa is dependent on the stage of development at the time of application. Studies with Juvenile hormone analogues on to 5th instar nymphs and adults of *D.cingulatus* suggests that metamorphosis is a sequence of events which are each dependent on the absence of JH. Intervention of multi level endocrine system at appropriate life stage point provides excellent opportunities for combating insect pests A high titer of JHa at the initial period of fifth instar nymphs appears to stimulate release of ecdysone hormone from PG cells. Presence of a high titer of JHa in the hemolymph and a very low level of ecdysone liberated from PG cells induces the insects to moult into supernumerary nymphs. Accumulation of JH due to an exogenous source of JHa resulted in an extra- nymphal moult. If exogenous JH application mimics endogenous buildup, then blockage of JH degradation, conserving the endogenous JH and allowing it to accumulate should also result in supernumerary moult [18]

Electrophorectic protein profile studies were done with haemolymph and fat bodies of methoprene-treated *D.cingulatus adults*, 48 hours after administration of JH analogue. These studies showed that there is a reduction in both fat body and haemolymph protein levels after treatment with Methoprene

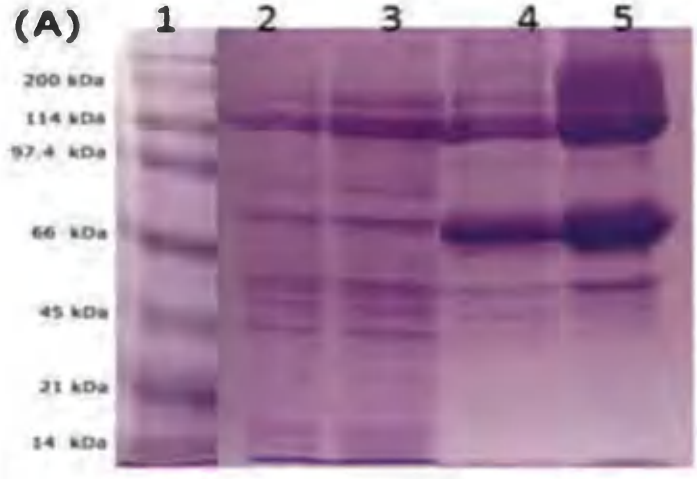

Fig.1A.Electropherogram showing changing protein profile in the hemolymph and fatbody after methoprene treatment (Lane 1-Marker, Lane 2-Treated fat body, Lane 3- control fat body, Lane 4-treated hemolymph , Lane 5-control hemolymph)

These juvenoids may have a possible direct effect on fat body, which is the major source of haemolymph JHE[18]. In hemimetabolous and holometabolous insects, the cellular constituents of the adult fat body are directly descended from the larval tissue. JH regulates the synthesis of specific larval and adult proteins in this tissue. These proteins reveal a pattern of qualitative and quantitative changes during development, which is regulated by hormones. JHa affect the synthesis of proteins at least in part by destabilizing pre-existent mRNAs. So the system appears to be post- translational[19] .In *Manduca*, changes in protein synthesis occur at least in part as a consequence of changes in the presence of translatable mRNAs, suggesting that these metamorphic changes at least partly result from differential gene transcription[20].

In the beginning of last nymphal instar, the target cells of JH gradually lose the sensitivity to the hormone and increasingly larger doses are required to affect them The mode of action of JHa lie in their ability to block the depression, transcription, or utilization of fresh genetic information [17]. Juvenilizing activity of JHa is dependent on the stage of development at the time of application. Studies with Juvenile hormone analogues on to 5th instar nymphs and adults of *D.cingulatus* suggests that metamorphosis is a sequence of events which are each dependent on the absence of JH.

The magnitude of JHE in D.cingulatus.

Control of JH degradation is achieved by regulatory enzymes of JH catabolism . JH is present throughout most of the larval instars, although not in constant amount. JH titers tend to be high early in an instar and low towards the end. In the last instar, there is an abrupt disappearance of circulating JH that allows metamorphosis to occur. In the adult, JH titer rises again, depending on the physiological state or the stage of the reproductive cycle. The degradative pathway involves the esterase-catalyzed hydrolysis of the methyl ester to generate the carbolic acid to 'JH-acid' followed by the opening of the epoxide ring by an epoxide hydrolase to generate 'JH acid diol'. Both the esterase and hydrolase are members of the / hydrolase fold family and have homologous mechanism although they are widely separated in evolutionary history. The acid diol and JH acid have been found to be essentially devoid of biological activity. They are further metabolized by gluconoride glucoside to sulphate formation. Either of these products is further metabolized to JH acid diol and a number of polar metabolites. It is proposed[4] that JH acid specifically is required as a hormone in order to induce metamorphic competence in some larval tissues. Ester hydrolysis appears to be the major route of metabolism in most insects so far studied and JH esterase recognition of JH is very specific[1]

Application of Juvenile hormone esterase inhibitor 3-octylthio-1,1,1- trifluropropan-2-one (OTFP) to 5th instar nymphs and virgin females of *Dysdercus cingulatus* revealed the profound role played by Juvenile

hormone esterase (JHE) in metamorphosis and reproduction. OTFP a potent JHE inhibitor was used for *in vivo* demonstration of the need for hormone-degrading enzymes. OTFP-treated 5th instar nymphs of *D.cingulatus* exhibited similar physiological symptoms as JHa treated nymphs. The ability of OTFP to cause a similar delay and the formation of malformed nymphs, suggests that inhibition of JHE *in vivo* is maintaining a higher than normal haemolymph JH titer. It is obvious that OTFP does inhibit the *in vivo* JHE activity in late instar nymphs, it is also possible that inhibition of other esterase such as acetyl choline esterase could in some way also contribute to the formation of apparent supernumerary nymphs and adultoids.

The OTFP experiments to inhibit JH degradation also demonstrate that endogenous JH accumulated to abnormal levels of biological importance with respect to the nature of the moult when *in vivo* degradation drops below normal. Thus the available evidence appears to favour endocrine, rather than nervous disruption as the mechanism by which OTFP acts to form the nymphal adult intermediates. The study also demonstrated that under the circumstances of suppressed JHE activity, an extra nymphal molt can occur.

JHE activity is likely to be one of the three main factors that regulate JH titer dynamics in females, along with JH biosynthesis in CA and JH transfer by males. Inclining JH titer may have a role in regulating egg development. It has been demonstrated in many insects earlier that mated females exhibited significantly higher titers of JH than did virgin females. Most interestingly upon uncoupling mated females showed JH titers as shown by virgin females of same age [21].

JH titer continues to increase significantly in mated females, while because of the inhibition of JHE in mated females it is hypothesized that JHE was involved in the mating induced gonadotropic effect and was tested using JHE inhibitors. Analysis of the available data on JH titer reveals that gonadotropic effect in various insect species seem to require different levels of JH . JHE inhibitor OTFP to given virgin females of *D.cingulatus* demonstrates that such substituted trifluropropanones can indirectly stimulate egg development by inhibiting JHE activity in virgin females [22].

The co-occurrence of transfer of JH from male to female by the mating-resulted activation of female CA to the biosynthesis of JH and also JH degradative system is inhibited and these three mechanisms accounts for the post-mating increase in hemolymph JH titers of mated females, resulting in the stimulation of egg production[21].It is apparent that the degradation system of JH in adult acquires particular importance in this regard. It appears that JHEs are indeed an important mechanism in the regulation of JH titer and further demonstrate that prevention of natural degradation of JH by JHE will block metamorphosis and that a hypothetical decrease in the rate of synthesis of JH alone is not sufficient for rapid clearance of JH. This

study with JHE inhibitor to virgin females provides support for the nexus between mating, inhibition of JHE activity, and stimulation of egg development

Characterization of JHE

All lepidopteran and dipteran JHEs reported so far are monomers with M_rs ranging from 50- 68 kDa [7,23,24,25]. The JHE proteins from *H. zea* comprises bands with molecular weights of 66 kDa and that from *L. dispar*[7] showed 50 kDA band. Estimated molecular weight of JHE from *D.melanogaster* was 54 kDa band [26].By contrast, JHEs characterized from two coleopterans exist partially or completely as dimers with subunit molecular masses of 57-71 kDa [7]. The native molecular mass of JHE from *G.assimilis* was 92 kDa. Surprisingly, closely related species *G. rubens* showed M_r of 188 kDa[27]. All these studies suggest that JHEs of different quaternary structure may exist even within the same species .It has been shown that JHE can exist in an active state as dimers or monomers. The pattern of molecular weight shows that a wide range of JHE forms occur even within the same species. Haemolymph of *D.cingulatus* when subjected to electrophoresis, two JHE bands analogous to ~50 kDa and 66 kDa enzyme were found to be present in late 5th instar nymphs. Molecular weight of JHE already reported from other insect forms also laid in this range Fig I (B).

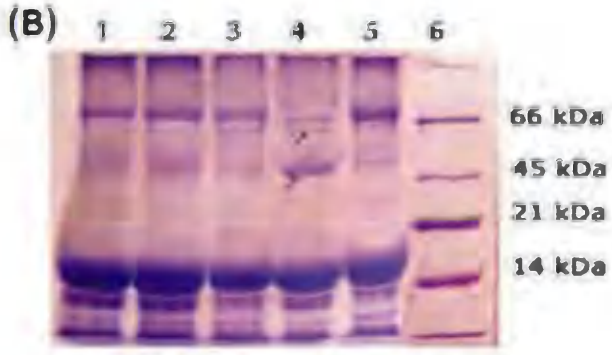

Fig. 1 B . Molecular mass determination of the putative Juvenile Hormone esterase on 10%Acrylamide gel from the haemolymph of *D. cingulatus*. Lane 1 -Third instar nymphs, Lane 2 - Fourth instar nymphs, Lane 3 - Fifth instar nymph (1stDay) Lane 4 - Fifth instar nymph (6th Day), Lane 5- Adult , Lane 6-Markers

Fig.1C. Esterase substrate (α -Naphthyl acetate) selectivity in the haemolymph of *Dysdercus cingulatus*. Lanes 1to4 ,5th instar nymphs of 0- ,2nd-,4th- and 6th-day,respectively; and lane 5,adult - 0-day 5th instar nymph

In *D.cingulatus* two-isozyme pattern were seen[28] Fig I (c). The first band noticed is as evident from the present study having a molecular mass of 66 kDa was weakly stained in 3rd, 4th, and in early 5th instars, but showed as a prominent band in late 5th instar. The second band having a molecular weight of 50 kDa was also prominent in 5th instar and was actively stained. Studies on the pattern of the esterase isozymes in different lepidopteran species varied dramatically among developmental stages even within a species[29]. Four different esterase isozymes could be stained in *I. typographus*[30].Three isozyme patterns are detected in the haemolymph of 5th instar larvae of *B.mori*[10]. Multiple JHEs have been reported in the heamolymph of *Hyalophora gloveri* [30], *M .sexta*[31], and in *G. mellonella* [32].The pyralid moths representing families Liparidae, Archidae, Gelechidae, and Saturnidae all displayed multiple JHEs. But some pierids like *C. eurytheme, E. elutella* all showed only a single JHE form. This again illustrates that differences are possible within a single family[29].

The protein band of M_r 50kDa was electro-eluted and stained for esterase substrate selectivity and it showed positive staining. This esterase band was immunoblotted with *B.mori* antibody, but no cross reactivity was found. This indicates that JHE is very species specific and contains some immunological determinants that differ from the species to species. There must be a strong correlation between immunological differences and amino acid because JHEs of different insects from same Order also show wide differences in their molecular mass, amino acid substitution and nucleotide sequences. The antibody raised against the JHEs of *H. Zea* showed high reactivity to its own antigen but low-cross-reactivity when the enzyme from *H. virescens* was tested as a coating antigen .The same pattern was true for the antibody raised against JHE from *H.virescens*[2].

Characterization of the JHE gene from the last day 5th instar nymphs of *D. cingulatus* (FigI D and E) and was done with mRNA isolated from fat body as the starting material. The PCR products obtained after amplification of cDNA using degenerate primers showed four bands showing molecular size varying from 1000 bp, 900 bp, 400 bp and 200 bp[28]. These bands seem to be JHE isoforms since 1.3kb PCR product obtained from *L. decemlineata* and 800bp band generated after PCR in *T. molitor* showed analogous base pair configuration in the present observations. 400 bp and 200 bp bands may be JHE proenzyme, JHE gene precursors or JHE products after post translational modifications[28]. The existence of multiple JHE isoforms as is evident in the electrophoretic form and cDNA in *D.cingulatus* is typical of most insects[15]

Differences of electrophoretic mobility of JHE isoforms from different insect species may be due to small regions of the proteins, which possess either potentially different sequence or different post translational

modifications. Differential processing and /or post-translational modifications are an inherent property of JHE gene within an individual cell type [24,25] .

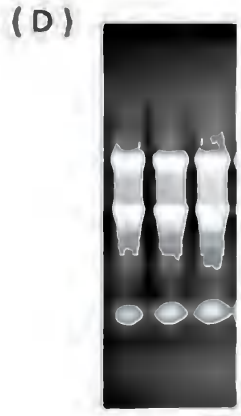

Fig.1D Formaldehyde-Agarose gel showing total RNA isolated from the fat body of last day fifth instar nymph.

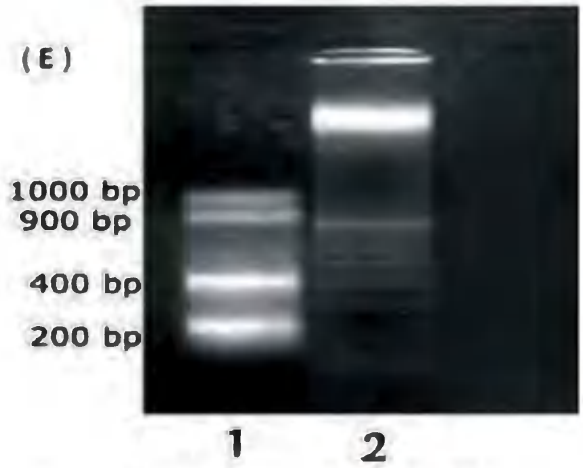

Fig. 1E. 1% Agarose gel showing PCR products. Lane 1-JHE cDNA Lane 2- Marker (100bp ladder)

The purified JHE protein of *Gryllus assimilis*[15] using Isoelectric focusing was separated into four isoforms. N-terminal amino acid sequences of the isoforms differed from each other, suggesting that the isoforms are products of same or similar genes. The results indicate that either more than one catalytic site/ protein or that closely related forms of the JHE gene exist[25]. The existence of JHE isoforms in *G.assimilis*, which differ by several unclustered amino acid N-terminal substitution within the 20 amino acid N-terminal portion of the enzyme, raises the possibility that JHE in *G.assimilis* may be encoded by a single highly variable locus that contains alleles that differ at multiple sites. JHE also exists as a single copy gene in *T.ni* [33]. This is an important issue for both evolutionary genetics of JHE as well as JHE regulation in the number of loci that encodes the enzyme. A dimeric form of JHE from *L.decemlineata* [14] by native gradient PAGE and cloning of two JHE-related genes was reported from this insect. The sequence of this protein is quite different from that of *H.virescens* .It does not contain the characteristic GQSAG sequence that has the active site serine. This raises the question of whether JHE evolution has a monophyletic or polyphyletic origin.

The JHE enzymes are stored in the fat body in an inactive form (pro- enzyme) and are converted and released in response to JH activity .So the 200 bp band obtained after cDNA PCR amplification in *D.cingulatus* may be a proenzyme. JH stimulates fat body mRNA protein synthesis, which then indirectly

causes the activation or modification of the pre existing protein proenzyme and modification is either by attachment or deletion of small molecules [34] .The possibility exists that changes in esterase enzymology may represent control points for hormone action. It is known that in some proteins there are exposed peptide segments usually without secondary structure, free to move about in solution and probably more sensitive to biochemical cleavage.

Future implications.

Knowledge on the basis of occurrence of the isoforms would show that they are primarily due to two genes, which if the case would offer two more promoters for comparison of conserved sequences. Post-translational modification can give rise to different isoforms from the same gene product. JHE from *T.ni* and *M. sexta* were differentially glycosylated [35]. But the basis for the isoform diversity has not been identified for JHE from *G. assimilis* [15]. However, it is unknown whether isoform diversity results from variations in the site of glycosylation or type of sugars attached as opposed to some other causes such as variation in primary amino acid sequence. If post-translational modification is the cause, elucidation of how a consistent percentage is converted to each isoform may reveal previously unknown regulatory mechanism controlling isoform ratios. The significance of additional forms of JHE activity remains uncertain. Characterization of each isoform would be feasible to determine whether these are isoform of the same proteins, or different gene products of completely independent genes. It will also be interesting to assess the role of each form and to examine their prominence throughout development. Partial characterization of a different JH form (bug JH)[36] from *D.cingulatus* may reveal the elucidation of JHE isoforms in this insect. At the present time the genomic structures of only JHEs from *H. virencens* and *D. melanogaster* are completely known. Sine gene expression may be coordinately regulated by domain control elements a knowledge of neighboring genes may provide new insights into the regulation of JHE and potential molecular cascades in which the gene is involved[37].

Juvenile Hormones (JHs) of insects are a group of structurally related acyclic sesquiterpenoids that regulate critical physiological processes including metamorphosis and reproduction in most insect species.CA of *D.cingulatus*[36] secrete an active compound that is different from other known JHs. Chemical identification of the CA products would be the next step in this research and it is under way.The other area of investigation is the molecular mode of action of JH. The hormonal key to regulation of metamorphosis is Juvenile Hormone with its classic "Status Quo' action. JH acid is an inactive precursor and metabolite of JH which actually induces cells to become competent to undergo metamorphosis, whereas ecdysteroid merely stabilizes this commitment and facilitates the expression of this state of development program. Evidences[2] has implicated that transcription factor Broad, a member of the Broad-

Tramtrack-Bric-a- Brac family act as a key component involved in mediating some of the actions of JH. In the context of complete metamorphosis , broad expression occurs as morphogenesis starts to make the adult form, but then is absent during the transition when tissue maturation then occurs. Broad expression begins during embryogenesis and is prominent during the nymphal molts, but disappears during the nymph-adult molt.

Acknowledgements

The studies on *Dysdercus* were carried out in the laboratory of Dr. D. Muraleedharan, CSIR Emeritus Professor, Centre for Arthropod Bioresources and Biotechnology, University of Kerala.

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Scientia
Vol. 8, No.1, Jan - Dec 2012, pp 21 - 27

ISSN: 0976-8289

Perceptual Computing

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Abstract

In 1996, Zadeh proposed the paradigm of *computing with words* (CWW). A specific architecture for making subjective judgments using CWW was proposed by Mendel in 2001. It is called a *Perceptual Computer.* Perceptual computing is not a rigorous basic science, it is an engineer's approach to building devices based on sensory models and sensory substitution models. These models, therefore, lay the ground work for what should be shown to be high fidelity engineered devices for Immersive Systems.

Keywords: *Perceptual computing, codebook.*

Introduction

"Perceptual" implies the use of natural and immersive interaction with devices and includes touch, facial recognition and voice. Intel thinks voice will emerge as a mega trend over the next few years, completely changing the way users currently operate a personal computer. Perceptual computing will be built around voice commands, facial recognition, eye-tracking and gestural controls. People love the way they can interact with machines using voice and gesture. This will be more natural and intuitive.

Perceptual computer

The *perceptual computer – Per-C* – an instantiation of perceptual computing – has the architecture that is depicted in Fig:1 It consists of three components: encoder, CWW engine and decoder. Perceptions – words – activate the Per-C and are the Per-C output (along with data); so, it is possible for a human to interact with the Per-C using just vocabularies.

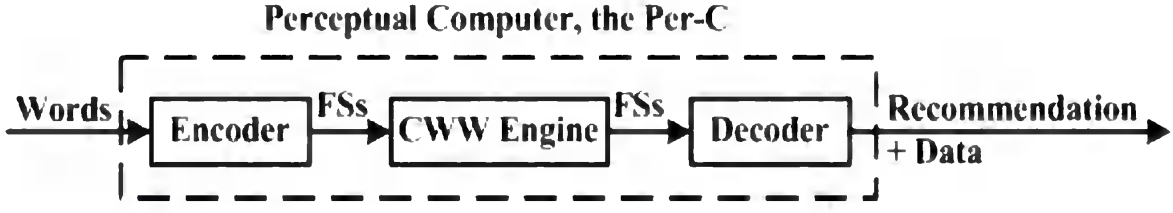

Fig.1. Architecture of perceptual Computer.

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A vocabulary is application (context) dependent, and must be large enough so that it lets the end-user interact with the Per-C in a user-friendly manner. The encoder transforms words into fuzzy sets (FSs) and leads to a *codebook* – words with their associated FS models. The outputs of the encoder activate a Computing with Words CWW) engine, whose output is one or more other FSs, which are then mapped by the decoder into a recommendation (subjective judgment) with supporting data. The recommendation may be in the form of a word, group of similar words, rank or class.

Fig.2. Perceptual Computer

Although there are lots of details needed in order to implement the Per-C's three components – encoder, decoder and CWW engine . It is when the Per-C is applied to specific applications, that the focus on the methodology becomes clear. Stepping back from those details, the *methodology of perceptual computing* is:

1. Focus on an application (*A*).
2. Establish a vocabulary (or vocabularies) for *A*.
3. Collect interval end-point data from a group of subjects (representative of the subjects who will use the Per-C) for all of the words in the vocabulary.
4. Map the collected word data into word-FOUs by using the *Interval Approach*. The result of doing this is the *codebook* (or codebooks) for *A*, and completes the design of the encoder of the Per-C.
5. Choose an appropriate CWW engine for *A*. It will map IT2 FSs into one or more IT2 FSs. Examples of CWW engines are: IF-THEN rules and Linguistic Weighted Averages
6. If an existing CWW engine is available for *A*, then use its available mathematics to compute its output(s). Otherwise, develop such mathematics for the new kind of CWW engine. The new CWW engine should be constrained so that its output(s) resemble the FOUs in the codebook(s) for *A*.

7. Map the IT2 FS outputs from the CWW engine into a recommendation at the output of the decoder. If the recommendation is a word, rank or class, then use existing mathematics to accomplish this mapping. Otherwise, develop such mathematics for the new kind of decoder.

Features:

- **Usage-Mode Coordination**: With the Perceptual Computing SDK, applications can easily implement more than one perceptual computing usage mode simultaneously such as close-range tracking, facial analysis, and speech recognition.
- **Multi-Application Coordination:** The SDK manages sharing of the Creative camera and other system resources so that users can switch seamlessly between perceptual computing applications.
- **Privacy Notification:** To protect the privacy of end-users the SDK includes a privacy notification function that notifies end-users when the RGB and depth camera are switched on.
- **Extensibility**: The Perceptual Computing SDK was designed with the ability to easily add more usage modes in the future.

Applications of Per-C

To-date a Per-C has been implemented for the following four applications:

(1) Investment decision-making,

(2) Social judgment making,

(3) Distributed decision making, and

(4) Hierarchical and distributed decision-making.

A specific example of the fourth application is the so-called *Journal Publication Judgment Advisor* in which for the first time only words are used at every level of the following hierarchical and distributed decision making process:

n reviewers have to provide a subjective recommendation about a journal article that has been sent to them by the Associate Editor, who then has to aggregate the independent recommendations into a final recommendation that is sent to the Editor-in-Chief of the journal. Because it is very problematic to ask reviewers to provide numerical scores for paper-evaluation sub-categories (the two major categories are *Technical Merit* and *Presentation*), such as importance, content, depth, style, organization, clarity, references, each reviewer will only be asked to provide a linguistic score for each of these categories. They

will not be asked for an overall recommendation about the paper because in the past it is quite common for reviewers who provide the same numerical scores for such categories to give very different publishing recommendations. By leaving a specific recommendation to the associate editor such inconsistencies can be eliminated.

How words can be aggregated to reflect each reviewer's recommendation as well as the expertise of each reviewer about the paper's subject matter is done using a linguistic weighted average. Although the journal publication judgment advisor uses reviewers and an associate editor, the word "reviewer" could be replaced by judge, expert, low-level manager, commander, referee, etc., and the term "associate editor" could be replaced by control center, command center, higher-level manager, etc. So, this application has potential wide applicability to many other applications.

In summary, the Per-C (whose development has taken more than a decade) is the first complete implementation of Zadeh's CWW paradigm, as applied to assisting people to make subjective judgments.

Current developments

Perceptual Computing: Critical to Intel's Future

Fig. 3. Intel's SDK Camera

At Intel's recent IDF (Intel Developer Forum), Intel formally announced their 2013 Beta. This SDK (software development kit) enables developers to build applications that enable users to interact with computing devices via multi-modal interfaces, combining voice and machine vision with keyboard, mouse, and direct touch. While having the potential to fundamentally change the way users interact with their phones, tablets and PCs, it is also the key to Intel's future as it soaks up high degrees of computing resources, very important to Intel's future.

Today, users interact with their computing devices like phones, tablets and PCs in a myriad of ways through direct touch, keyboard, mouse and trackpad. As we have seen with early machine vision and speech investments made by Microsoft via Kinect and Apple via Siri there is a lot more than can be done to improve the user experience. Both speech recognition and machine vision are in extensive use by the military and received significant boosts in the last decade with government funding since 9/11, but isn't a part of the mainstream everyday use.

Speech Recognition: Includes capabilities for voice command and control, short sentence dictation and text to speech synthesis. Speech recognition is a common way to add perceptual computing interactivity to an application. Examples of this might be direct PC commands, dictation, or translation .For speech recognition, when a user speaks to the Creative camera, the speech recognition algorithm

in the SDK interprets the speech, recognizes that the user has spoken a command pre-programmed into the application, and passes the command on to the application.

Fig. 4. Speech Recognition

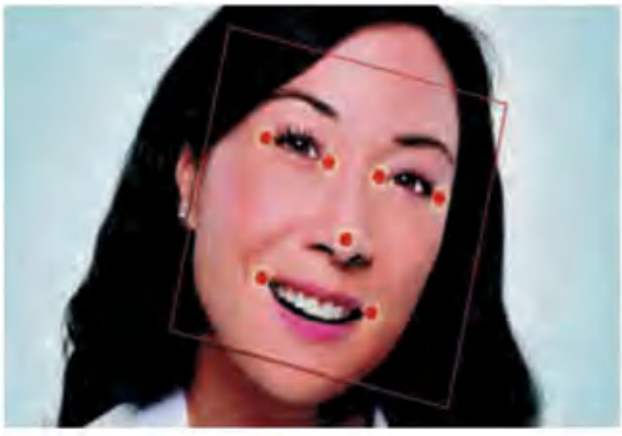

Fig. 5. Face Analysis

Face Analysis and Tracking: Includes the ability to do face detection and recognition, six and seven point landmark detection, and attribution detection such as smiles, blinks and age groups. Face tracking can be used as a perceptual computing component in games or other interactive applications. The Intel Perceptual Computing SDK supports facial recognition, facial tracking, gender and age determination, attribution detection such as smile.

Close-Range Tracking (6 in to 3 ft): The SDKs unique close-range finger-tracking mode provides developers with the ability to define innovative usages that recognize the positions of each of the user's hands, fingers and joints and allow the user to grasp things like control knobs or switches on a UI. Close-range tracking additionally supports recognition of static hand poses and moving hand gestures.

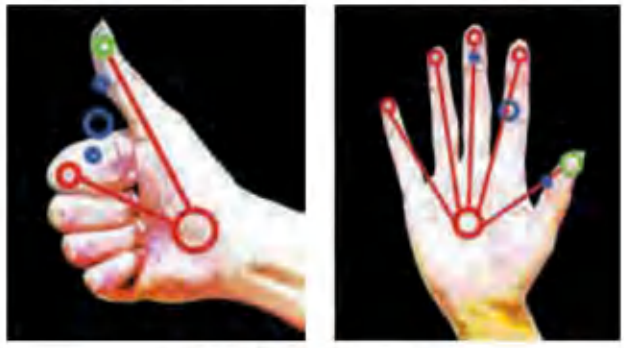

Fig.6. Close Range Tracking

Fig.7. 2D/3D Object Tracking

2D/3D Object Tracking: 2D/3D Object Tracking is a compelling augmented reality usage that allows developers to combine real-time images from the RGB camera and close-range tracking from the depth sensor with 2D or 3D graphical images to create innovative, immersive experiences. The SDK supports marker-less object tracking so that pre-defined 2D or 3D objects can be inserted seamlessly into a live scene.

"These applications were created with the Intel® Perceptual Computing SDK. If you meet the system requirements below, you can download the applications, install them and check them out.

Who uses it?

Users of 2nd generation Intel Core processor based systems or later including Ultrabook devices, laptops, All-In-One devices and desktops will benefit from the new usage modes offered by perceptual computing. In addition, Intel believes perceptual computing will open up new market segment opportunities for software application developers, middleware developers, as well as PC OEMs and device OEMs.

Why Intel's SDK?

- Presenter at a business meeting uses gestures to move around the slides without the need of a "clicker". The presenter should just wave their hands.
- A cook with flour on his hands waves his hand from side to side to turn the page of a recipe book.

- A designer uses their own hands, arms and torso to fit a pair of shoulder pads designed on their computer.
- By intonations in your voice, your home "computer" knows to limit distractions because you are annoyed. Soft music and low lighting awaits you at home.
- By the panic in your voice, your car's computer knows you are in trouble and asks you if you want to call 911.
- Your home computer senses someone is using your computer that it doesn't recognizes and texts you with a photo of that person.
- Dictation reaches near 100% accuracy with the combination of speech to text and lip reading.
- Your TV recognizes and alerts you that there are five people in your living room after you told your kids they could only have two guests over the house.
- Replacing the physical mouse or track pad with a "Hand-mouse" where the hand can rest anywhere on a flat surface and can be tapped and swiped like a physical device. A camera is mapping your hand, joints, and finger tips in real time.
- Meeting transcription where everything is recorded in the meeting and transcripted and separated, person by person. Action items and "parking garages" are automatically "sensed", too with a running tally in a pane of the computer.

Conclusion

Perceptual Computing is about bringing exciting user experiences through new human-computing interactions such that devices sense and perceive the user actions in a natural, immersive, and intuitive way.

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Scientia
Vol. 8, No.1, Jan - Dec 2012, pp 28 - 44

ISSN: 0976-8289

Statistical Modelling of Epidemilogical Data using Marshall-Olkin Bivariate Pareto Distribution

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Abstract

Epidemiological data on various diseases like AIDS, cancer, dounge fever, H1NI influenza, chikungunya etc. pose challenges to statisticians and health workers. For modeling such data accurate and reliable data is to be collected. There are many gaps in the proper collection, analysis and modeling of such data. In this paper, the author considers a Marshall-Olkin bivariate Pareto model and uses it to construct survival indices that measure the survival mechanism associated with such diseases. Bivariate survival functions are developed and some characteristic properties are explored. The model is applied to a data on cancer incidence collected from internet resources regarding Regional Cancer Centre Thiruvananthapuram and Amala Cancer Centre, Thrissur.

Key words: Epidemiological data; Marshall-Olkin bivariate Pareto model; cancer disease; bivariate survival functions

Introduction

Generally the reliability of a component or device is assessed at the manufacturing stage through suitable experiments conducted in the laboratory environments. However, when the device goes out of the factory, it works in an environment that may not be identical to that of the laboratory. The changed environment may be milder, harsher or same as that was originally available in the laboratory. This means that the original model of life times prescribed for the device is subject to change under the new conditions. This change can be accommodated by assuming that the failure under the new premises is a multiple of the original rate, where the multiplying quantity is considered as a random quantity which is $> 1 (< 1, = 1)$ for harsher (milder, or same) environment. Lindley and Singpurwalla (1986) have made use of the above arguments in deriving the required bivariate Pareto distribution. The same situation can be assumed to occur in the case of humans subjected to environments conducive to the occurrence of cancer.

In section 2, we consider the Survival characteristics of Marshall-Olkin bivariate Pareto (MO-BP) distribution. Some distributional characterizations are also investigated. In section 3, we consider application to analysis of cancer data. In section 4, we consider the findings of age distribution related to various types of cancer.

Survival characteristics of MO-BP distribution

Let (X, Y) be a random vector with joint survival function $\bar{F}(x, y)$. Then

$$\bar{G}(x,y) = \frac{\alpha \bar{F}(x, y)}{1-(1-\alpha)\bar{F}(x, y)} ; x, y \geq 0, 0 < \alpha < 1. \qquad (2.1)$$

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is a proper bivariate survival function. The family of distributions of the form (2.1) is called Marshall-Olkin bivariate family of distributions.

In view of the importance, Pareto distributions are enjoying recently in the context of survival of cancer patients, survival analysis and life testing, we investigate the survival properties of MO-BP distribution.

In the following section we consider the special case namely bivariate Pareto distribution having the survival function

$$\bar{F}(x, y) = \frac{1}{1 + x^{\beta_1} + y^{\beta_2}}; \; x, y \geq 0, \beta_1 > 0, \beta_2 > 0.$$

From (2.1) the new survival function is

$$\bar{G}(x, y) = \frac{1}{1 + \frac{1}{\alpha}(x^{\beta_1} + y^{\beta_2})}; \; x, y \geq 0, \beta_1, \beta_2 > 0, 0 < \alpha < 1,$$

which is known as Marshall-Olkin bivariate Pareto(MO-BP) distribution.

In view of the importance, Pareto distributions are enjoying a significant role in the context of survival analysis and life testing.

When (X_1, X_2) are viewed as the life times of a two component system in which the component i is certain to survive for a mission time p, the survival of the system can be modeled by the MO-BP in terms of the survival function

$$R(x_1,x_2) = P(X_1 > x_1, X_2 > x_2)$$

$$= \frac{1}{1 + (1/p)(x_1^{\alpha_1} + x_2^{\alpha_2})}; \; x_1, x_2 \geq 0, \alpha_1, \alpha_2, p > 0. \qquad (2.2)$$

In this section the precise physical conditions under which (2.2) may serve as a model of life lengths will be discussed.

The concept of failure rate is fundamental in reliability modeling and there are various approaches in defining it. Basu (1971) defines bivariate failure rate as the scalar quantity

$$a(x_1, x_2) = \frac{f(x_1, x_2)}{R(x_1, x_2)}, \qquad (2.3)$$

at those points for which the denominator of (2.3) is positive. Corresponding to (2.2), we get

$$a(x_1, x_2) = 2\alpha_1\alpha_2 x_1^{\alpha_1 - 1} x_2^{\alpha_2 - 1} \frac{1}{p^2}\left(1 + (1/p)(x_1^{\alpha_1} + x_2^{\alpha_2})\right)^{-2}, \qquad (2.4)$$

which is a decreasing function in both the arguments.

where $b_i(x_1, x_2) = -\frac{\partial \log R(x_1, x_2)}{\partial x_i}$, i =1,2. (2.6)

Some simple calculations show that

$$b_1(x_1, x_2) = \frac{\alpha_1}{p} x_1^{\alpha_1 - 1}\left(1 + (1/p)(x_1^{\alpha_1} + x_2^{\alpha_2})\right)^{-1} \quad (2.7)$$

and $$b_2(x_1, x_2) = \frac{\alpha_2}{p} x_2^{\alpha_2 - 1}\left(1 + (1/p)(x_1^{\alpha_1} + x_2^{\alpha_2})\right)^{-1}. \quad (2.8)$$

Since p, $_1$, $_2$ > 0, b_1 and b_2 are decreasing in x_1 and x_2 the distribution belongs to the DFR class. The third failure rate, known as a conditional failure rate (See Nair and Nair 1991) is the vector

$$c(x_1, x_2) = (c_1(x_1, x_2), c_2(x_1, x_2)) \quad (2.9)$$

where $$c_1(x_1, x_2) = \frac{f(x_1 / x_2)}{P(X_1 > x_1 / X_2 = x_2)} \quad (2.10)$$

and $$c_2(x_1, x_2) = \frac{f(x_2 / x_1)}{P(X_2 > x_2 / X_1 = x_1)}. \quad (2.11)$$

From (2.2) we have

$$f(x_1, x_2; \alpha_1, \alpha_2, p) = 2\alpha_1.\alpha_2.x_1^{\alpha_1 - 1} x_2^{\alpha_2 - 1} \frac{1}{p^2}\left(1 + (1/p)(x_1^{\alpha_1} + x_2^{\alpha_2})\right)^{-3}; \quad (2.12)$$

x_1, x_2 0, $_1$, $_2$ > 0, 0 < p < 1.

In this density p acts as the scale parameter and $_1$, and $_2$ are the shape parameters. The marginal distributions of X_1 and X_2 are

$$f(x_1; \alpha_1, p) = \frac{\alpha_1}{p} x_1^{\alpha_1 - 1}\left(1 + (1/p)x_1^{\alpha_1}\right)^{-2};\ x_1 \geq 0,\ \alpha_1 \geq 0,\ 0 < p < 1 \quad (2.13)$$

and

$$f(x_2; \alpha_2, p) = \frac{\alpha_2}{p} x_2^{\alpha_2 - 1}\left(1 + (1/p)x_2^{\alpha_2}\right)^{-2};\ x_2 \geq 0,\ \alpha_2 \geq 0,\ 0 < p < 1 \quad (2.14)$$

$$f(x_1 / x_2) = \frac{2\alpha_1 x_1^{\alpha_1 - 1}}{p} \frac{\left(1 + (1/p)(x_1^{\alpha_1} + x_2^{\alpha_2})\right)^{-3}}{\left(1 + (1/p)x_2^{\alpha_2}\right)^{-2}}. \quad (2.15)$$

$$f(x_2 / x_1) = \frac{2\alpha_2 x_2^{\alpha_2 - 1}}{p} \frac{\left(1 + (1/p)(x_1^{\alpha_1} + x_2^{\alpha_2})\right)^{-3}}{\left(1 + (1/p)x_1^{\alpha_1}\right)^{-2}}. \quad (2.16)$$

$$E(X_1 / X_2 = x_2) = \frac{\int_0^{\infty} x_1 f(x_1, x_2) dx_1}{f(x_2)} \tag{2.17}$$

$$E(X_2 / X_1 = x_1) = \frac{2}{p^2} \frac{\left(p(1+(1/p)x_1^{\alpha_1}\right)^{\alpha_2}}{\left(1+(1/p)x_1^{\alpha_1}\right)} \beta(\frac{1}{\alpha_2}, 3-\frac{1}{\alpha_2}) . \tag{2.18}$$

From (2.15) $P(X_1 > x_1 / X_2 = x_2) = \int_{x_1}^{\infty} f(t / x_2) dt$

$$= \int_{x_1}^{\infty} \frac{2\alpha_1}{p} \left((1+(1/p)x_2^{\alpha_2}\right)^2 t^{\alpha_1 - 1} \left(+(1/p)(t^{\alpha_1} + x_2^{\alpha_2}\right)^3 dt$$

$$= \left(1+\frac{x_2^{\alpha_2}}{p}\right)^2 \left(1+(1/p)(x_1^{\alpha_1} + x_2^{\alpha_2})\right)^{-2} .$$

Here $c_1(x_1, x_2) = 2\frac{\alpha_1}{p} x_1^{\alpha_1 - 1} \left(1+(1/p)(x_1^{\alpha_1} + x_2^{\alpha_2})\right)^{-1}$ (2.19)

and $c_2(x_1, x_2) = 2\frac{\alpha_2}{p} x_2^{\alpha_2 - 1} \left(1+(1/p)(x_1^{\alpha_1} + x_2^{\alpha_2})\right)^{-1}$ (2.20)

are decreasing functions. It is therefore apparent that the distribution can model life times only when the failure rate is decreasing in nature.

Galambos and Kotz (1978) has shown that the failure rate $b(x_1, x_2)$ uniquely determines the distribution and hence if the functional forms (2.7) and (2.8) are realized in practice, the distribution can be identified as MO-GP form. We now prove that (2.19) and (2.20) determine the distribution (2.2) uniquely.

Theorem 2.1

A necessary and sufficient condition for the random vector (X_1, X_2) in the support of (p,) x (p,), p > 0 has the survival function (2.2) is that $c_1(x_1, x_2)$ and $c_2(x_1, x_2)$ have the form mentioned in equation (2.19) and (2.20).

Proof

The necessary part is already demonstrated. To prove the sufficiency, we note that by definition

$$c_1(x_1, x_2) = \frac{-\partial}{\partial x_1} \log P(X_1 > x_1 / X_2 = x_2) . \tag{2.21}$$

Integrating (2.21) from p to x_1,

$$\log P(X_1 > x_1 / X_2 = x_2) = -\int_p^{x_1} c_1(t_1, x_2)dt_1 + c(x_2)$$

As x_1 tends to p, $c(x_2) = 0$ since $P(X_1 > p / X_2 = x_2) = 1$.

This gives $P(X_1 > x_1 / X_2 = x_2) = \exp(-\int_p^{x_1} c_1(t_1, x_2)dt_1)$

or $f(x_1 / x_2) = \frac{-\partial}{\partial x_1} P(X_1 > x_1 / X_2 = x_2)$

$= c_1(x_1, x_2) \exp(-\int_p^{x_1} c_1(t_1, x_2))$.

Substituting $c_1(x_1, x_2)$ from (2.19),

$f(x_1/x_2) =$

$$2\frac{\alpha_1}{p} x_1^{\alpha_1 - 1}\left(1 + (1/p)(x_1^{\alpha_1} + x_2^{\alpha_2})\right)^{-1} \exp\left(-\int_p^{x_1} \frac{2}{p}\alpha_1 t_1^{\alpha_1 - 1}(1 + (1/p)(t_1^{\alpha_1} + x_2^{\alpha_2}))^{-1} dt_1\right).$$

The integral in the exponent has a value $\log\left(\frac{1 + (1/p)(p^{\alpha_1} + x_2^{\alpha_2})}{1 + (1/p)(x_1^{\alpha_1} + x_2^{\alpha_2})}\right)^2$.

Hence

$$f(x_1 / x_2) = \frac{2}{p}\alpha_1 x_1^{\alpha_1 - 1}\left(1 + (1/p)(x_1^{\alpha_1} + x_2^{\alpha_2})\right)^{-1}\left(\frac{1 + (1/p)(p^{\alpha_1} + x_2^{\alpha_2}}{1 + (1/p)(x_1^{\alpha_1} + x_2^{\alpha_2})}\right)^2. \qquad (2.22)$$

By similar arguments

$$f(x_2 / x_1) = \frac{2}{p}\alpha_2 x_2^{\alpha_2 - 1}\left(1 + (1/p)(x_1^{\alpha_1} + x_2^{\alpha_2})\right)^{-3}\left(1 + \frac{x_1^{\alpha_1} + p^{\alpha_2}}{p}\right)^2. \qquad (2.23)$$

Thus $$\frac{f(x_1 / x_2)}{f(x_2 / x_1)} = \frac{\alpha_1}{\alpha_2}\frac{x_1^{\alpha_1 - 1}}{x_2^{\alpha_2 - 1}}\left(\frac{1 + (1/p)(p^{\alpha_1} + x_2^{\alpha_2})}{1 + (1/p)(x_1^{\alpha_1} + p^{\alpha_2})}\right)^2. \qquad (2.24)$$

Abrahams and Thomas (1984) has proved that a bivariate joint density function is uniquely determined by its conditional densities if their ratio can be written in the form

$$\frac{f(x_1 / x_2)}{f(x_2 / x_1)} = \frac{h_1(x_1)}{h_2(x_2)},$$

where $h_1(x_1)$ and $h_2(x_2)$ are non-negative integrable functions with equal integrals. These conditions are satisfied by (2.24) and accordingly (2.22) and (2.23) uniquely determines (2.2).

Corollary

The conditional densities (2.22) and (2.23) characterize (2.2).

Application to analysis of Cancer Data

Considering the bivariate case, where (X_1, X_2) represent age of the cancer patient and the health index with an inherent dependency structure. Even though such interpretations are plausible, there does not seem to be much vigorous progress in developing mortality measures in multivariate cases compared to the one variable counterpart.

A basic inequality measure used in univariate analysis of incomes is the Lorenz curve. Extension of this idea in the multivariate case with a reasonable definition is provided by Arnold (1983) who defines the Lorenz surface as the function L(u, v) as

$$L(u.v) = \int_0^x \int_0^y uvf(u,v) / E(XY)$$

$$u = \int_0^x f_1(u)du \; ; \; v = \int_0^y f_2(v)dv .$$

A particular feature of this definition is that when X and Y are independent random variables L (u, v) is the product of the Lorenz curves corresponding to X and Y.

For the Marshall-Olkin Pareto form, these quantities can be computed as

$$u = \int_0^x \frac{\alpha_1}{p} u^{\alpha_1 - 1} \left(1 + \frac{u^{\alpha_1}}{p}\right)^{-2} du$$

$$= 1 - \left(1 + \frac{x^{\alpha_1}}{p}\right)^{-1} \qquad (3.1)$$

$$v = \int_0^y \frac{\alpha_2}{p} v^{\alpha_2 - 1} \left(1 + \frac{v^{\alpha_2}}{p}\right)^{-2} dv$$

$$= 1 - \left(1 + \frac{v^{\alpha_2}}{p}\right)^{-1} \qquad (3.2)$$

In terms of the Lorenz curve, the bivariate Gini index has the definition

$$G = 4\int_0^1 \int_0^1 (uv - L(u,v))dudv. \qquad (3.3)$$

where L (u, v) is given above. Similar situation occurs in the incidence of diseases with respect to living conditions, working atmosphere, nature of job, etc.

Theorem 3.1

A non-negative random vector $X = (X_1, X_2)$ has the Marshall-Olkin bivariate Pareto law specified by (2.2) if the conditional distribution of X_i given $X_j > t_j$ for all t_j 0 are of the form

$$f(X_i / X_j > t_j) = \frac{\alpha_i x_i^{\alpha_i - 1}}{\alpha_j t_j^{\alpha_j - 1}} (h_i(t_j))^2 \left(\frac{x_i^{\alpha_i}}{p} + h_i(t_j) \right)^{-2}.$$

where $h_i(t_j)$ are arbitrary functions for i, j =1.2, i ≠ j.

Proof

When the joint density is (2.2)

$$f(X_1 / X_2 > t_2) = -\frac{\partial}{\partial x_1} \frac{R(x_1, t_2)}{R(0, t_2)}$$

$$= -\frac{\partial}{\partial x_1} \frac{\left(1 + (1/p)(x_1^{\alpha_1} + t_2^{\alpha_2})\right)^{-1}}{\frac{\alpha_2}{p} t_2^{\alpha_2 - 1} \left(1 + \frac{t_2^{\alpha_2}}{p}\right)^{-2}}$$

$$= \frac{\alpha_1 x_1^{\alpha_1 - 1}}{\alpha_2 t_2^{\alpha_2 - 1}} \left(1 + \frac{t_2^{\alpha_2}}{p}\right)^2 \left(1 + (1/p)(x_1^{\alpha_1} + t_2^{\alpha_2})\right)^{-2}$$

$$= \frac{\alpha_1 x_1^{\alpha_1 - 1}}{\alpha_2 t_2^{\alpha_2 - 1}} (h_1(t_2))^2 \left(\frac{x_1^{\alpha_1}}{p} + h_1(t_2) \right)^{-2}$$

$$= \frac{\alpha_i x_i^{\alpha_i - 1}}{\alpha_j t_j^{\alpha_j - 1}} (h_j(t_j))^2 \left(\frac{x_i^{\alpha_i}}{p} + h_i(t_j) \right)^{-2} : \text{i, j =1,2, i} \neq \text{j.}$$

The models exhibit a rich variety with wider scope and application, which needs further study.

Applications

In this section we consider various forms of cancer and the age distribution of cancer patients.

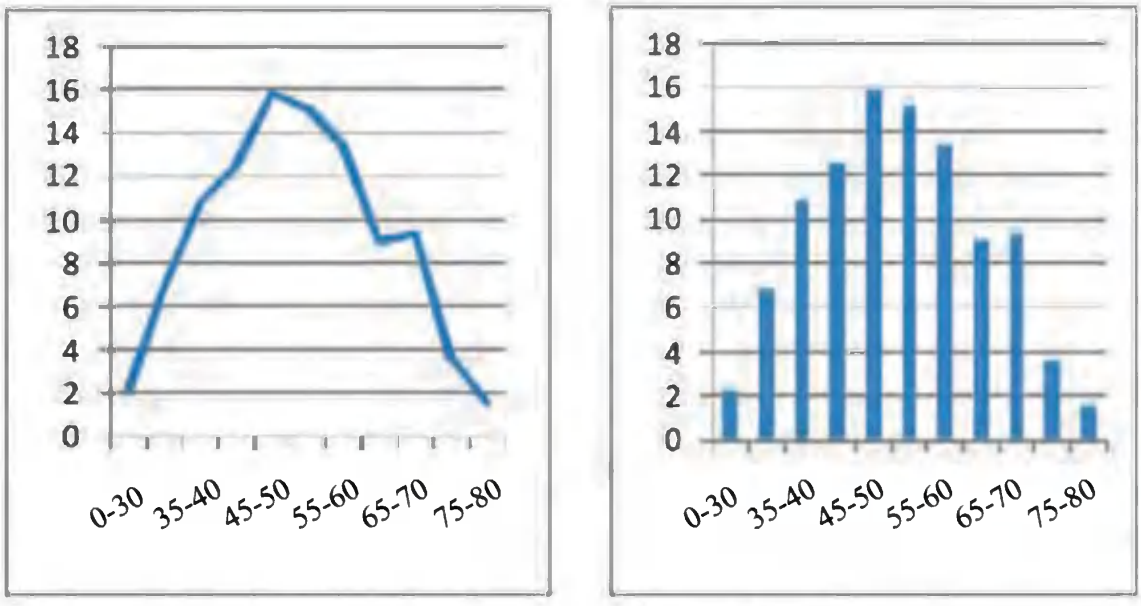

Fig. 1. Age Distribution of Ant. Tongue Cancer

This is moderately symmetric and can be modeled by logistic as well as Weibull distributions.

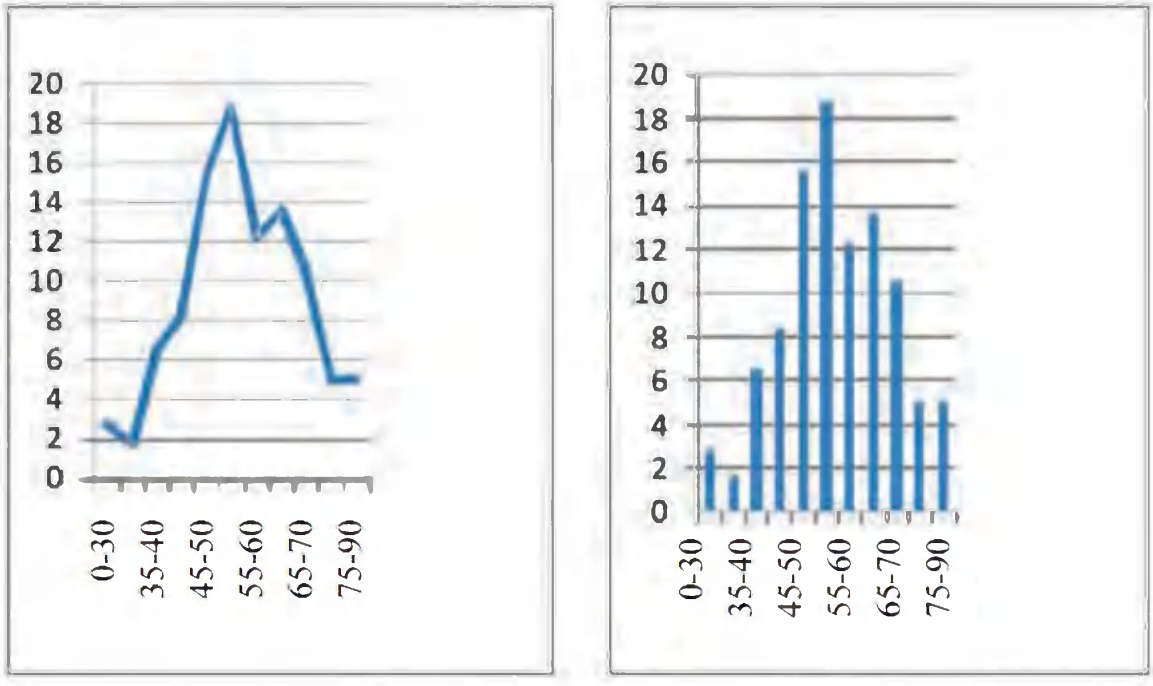

Fig. 2. Age Distribution of Lower Alveolus

An asymmetric Laplace distribution is more suitable to model this case.

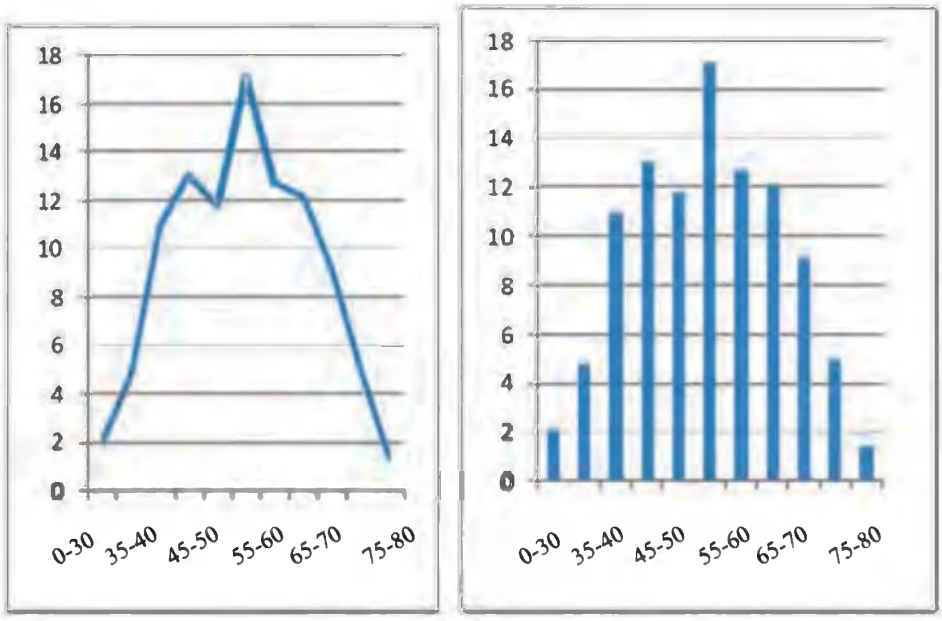

Fig. 3. Age Distribution of Buccal Mucosa cancer

This case is also approximately symmetric and can be modeled by Logistic as well as Laplace distributions.

It is clear from the Fig.4. that an extreme value distribution is needed to model this case. Weibull, Gumbul, Frechet etc distributions are possible candidates. Marshall-Olkin extended distributions are also possible alternatives. Other mouth, Oesophagus, stomach. lung, tongue, larynx, brain, Oropharynx cancers graphs shows that an extreme value distribution is needed to model these cases.

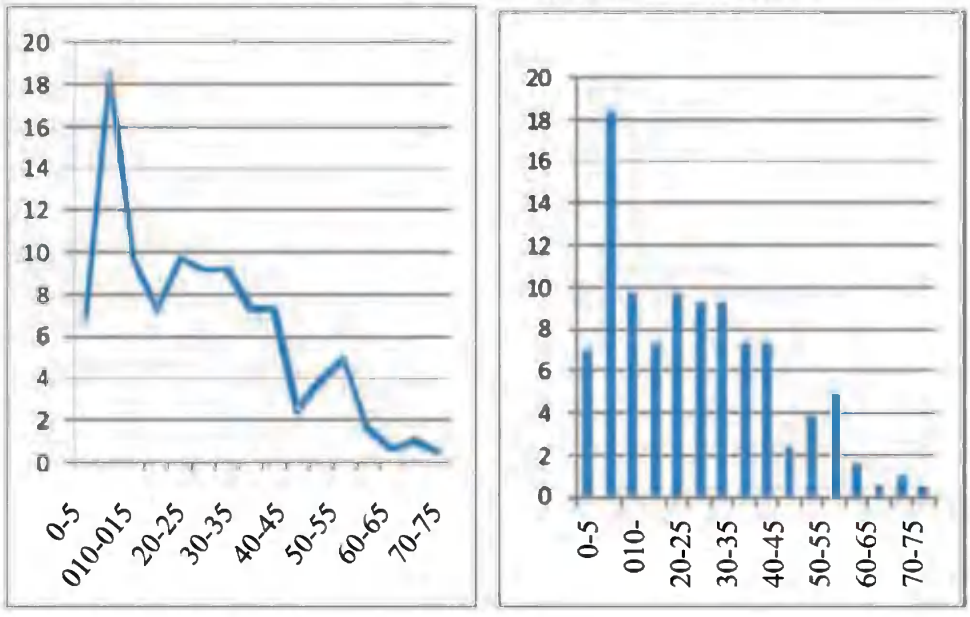

Fig. 4.
Age Distribution of Hodgkins cancer

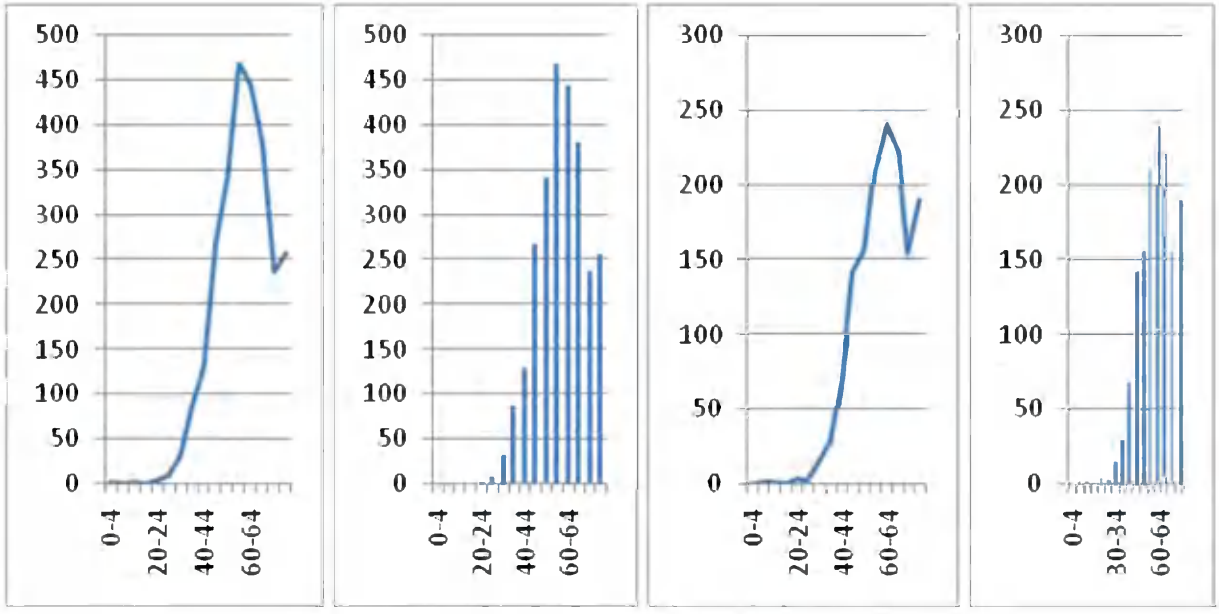

Fig. 5. Age distribution of Other Mouth Cancer

Male-Female

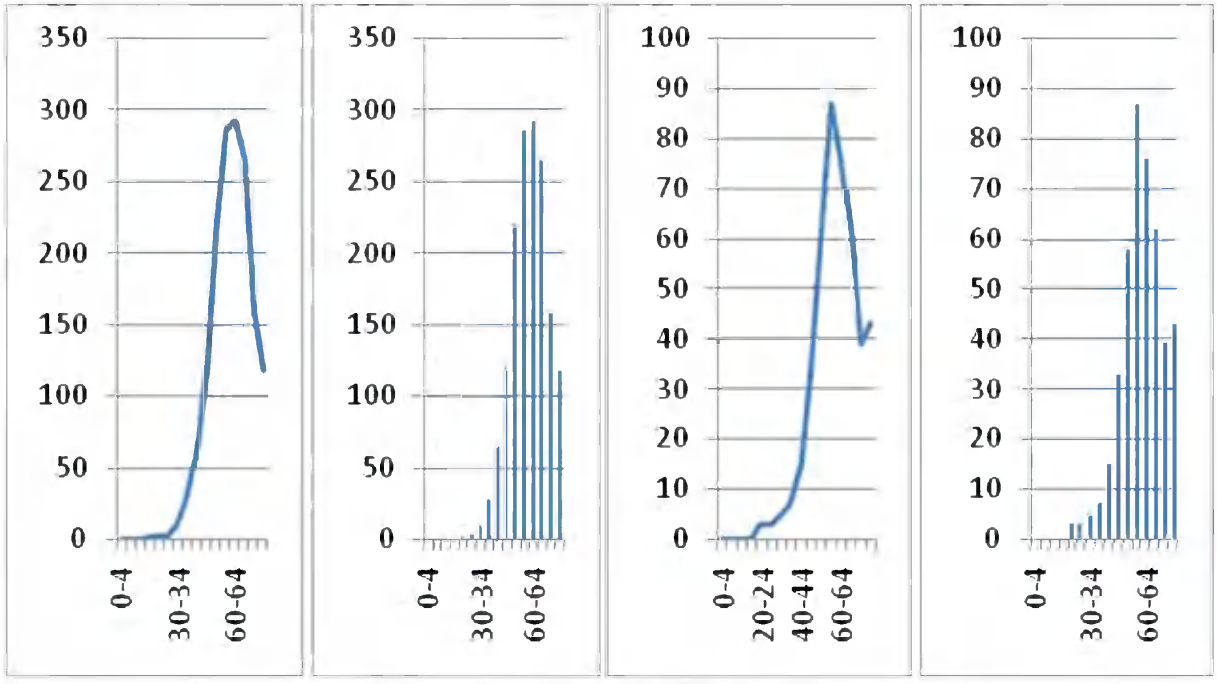

Fig. 6. Age distribution of Oesophagus Cancer

Male-Female

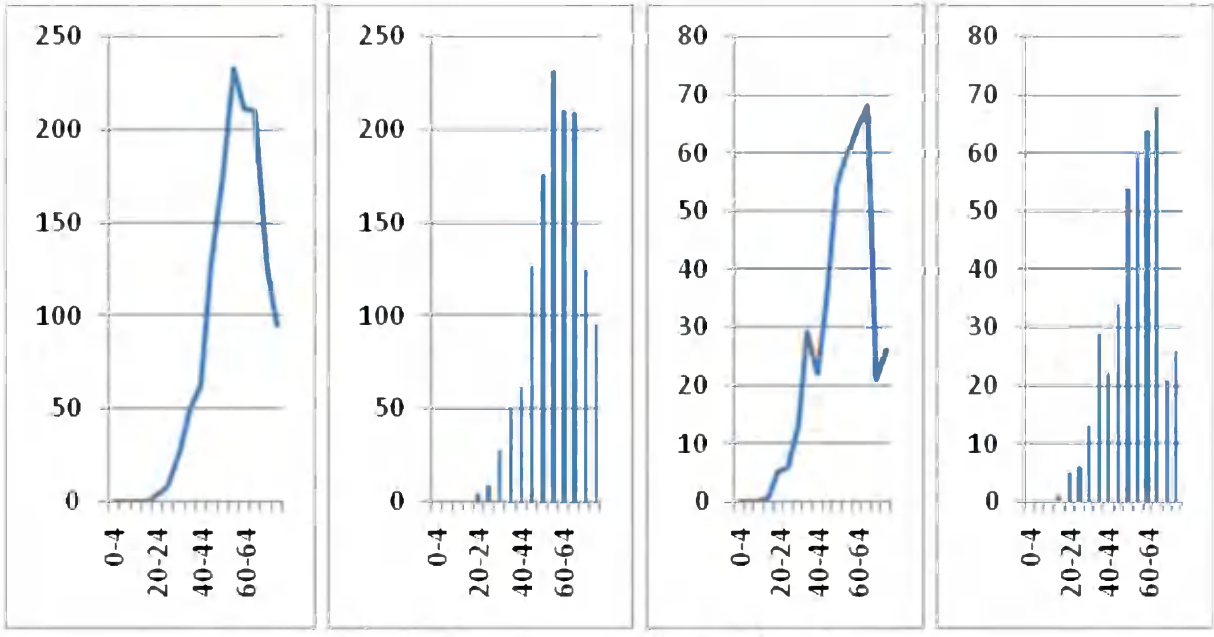

Fig. 7. Age distribution of Stomach Cancer

Male-Female

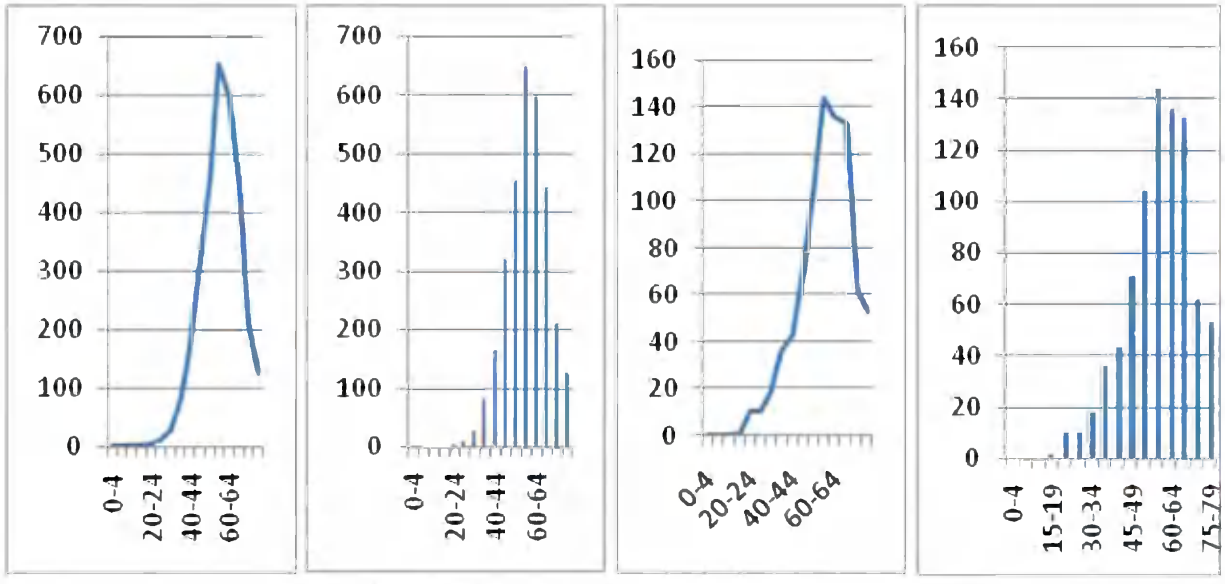

Fig. 8. Age distribution of Lung Cancer

Male-Female

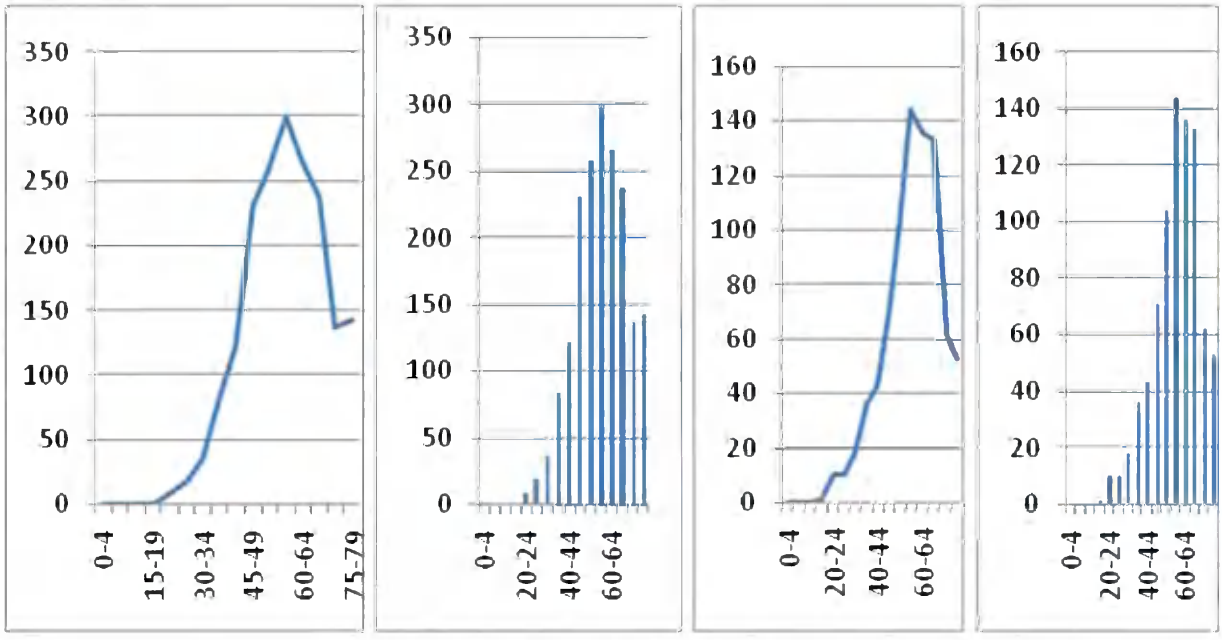

Fig. 9. Age distribution of Tongue Cancer

Male-Female

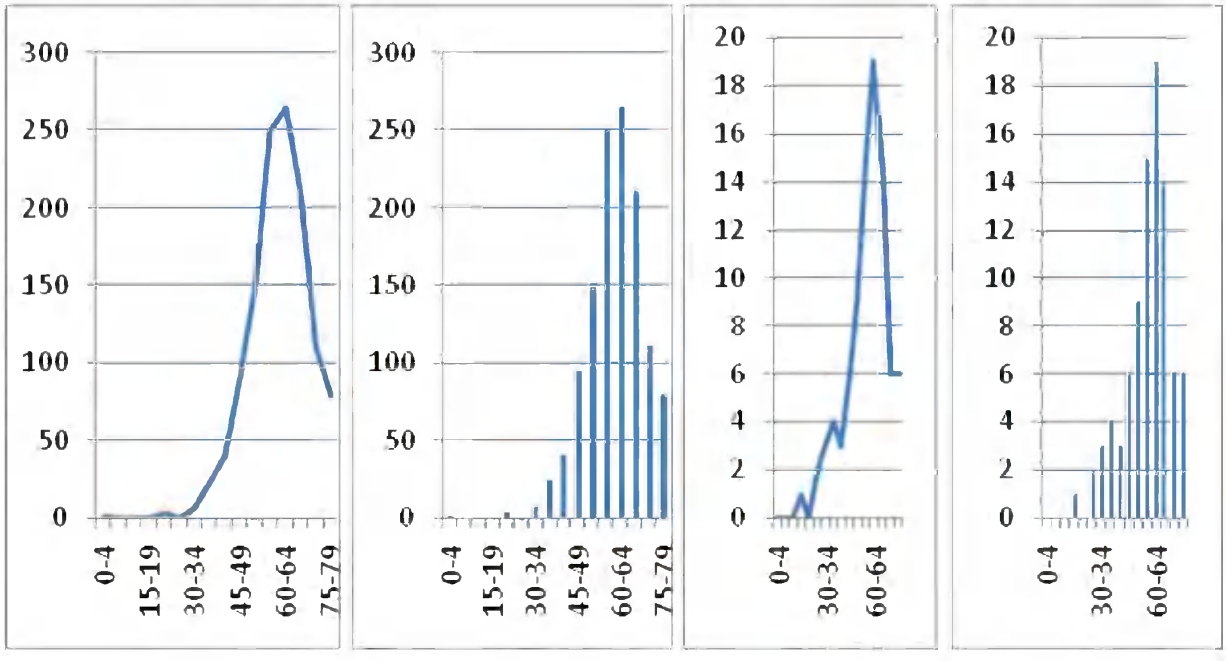

Fig. 10. Age distribution of Larynx Cancer

Male-Female

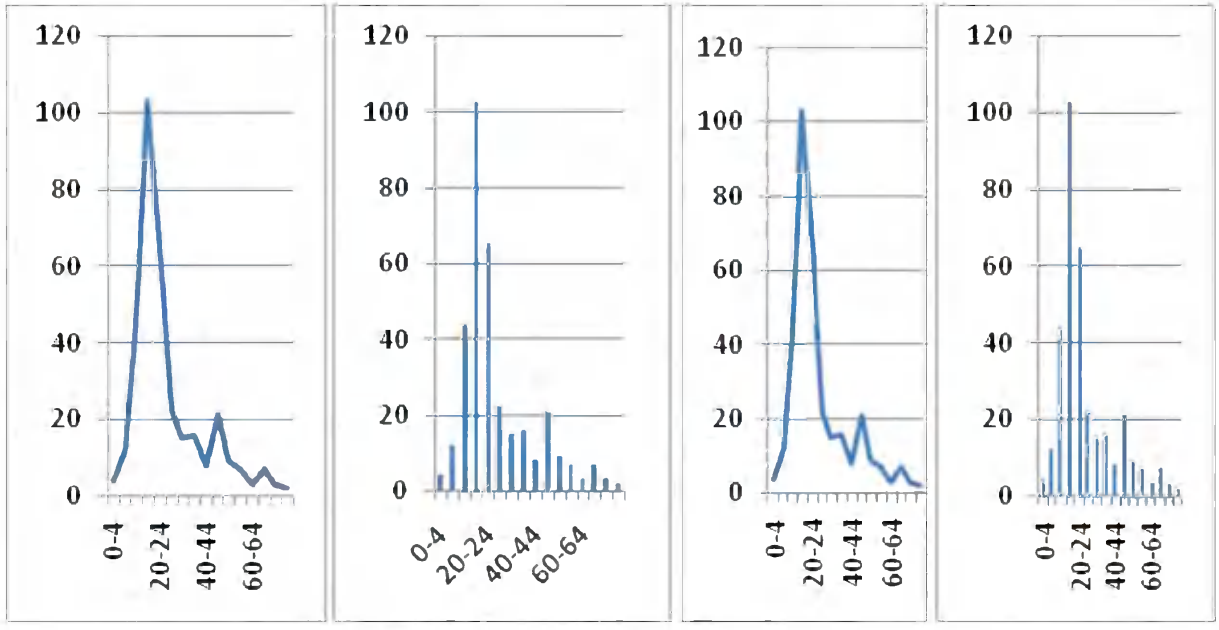

Fig. 11. Age distribution of Bone Cancer

Laplace distribution is more suitable to model this case.

Male-Female

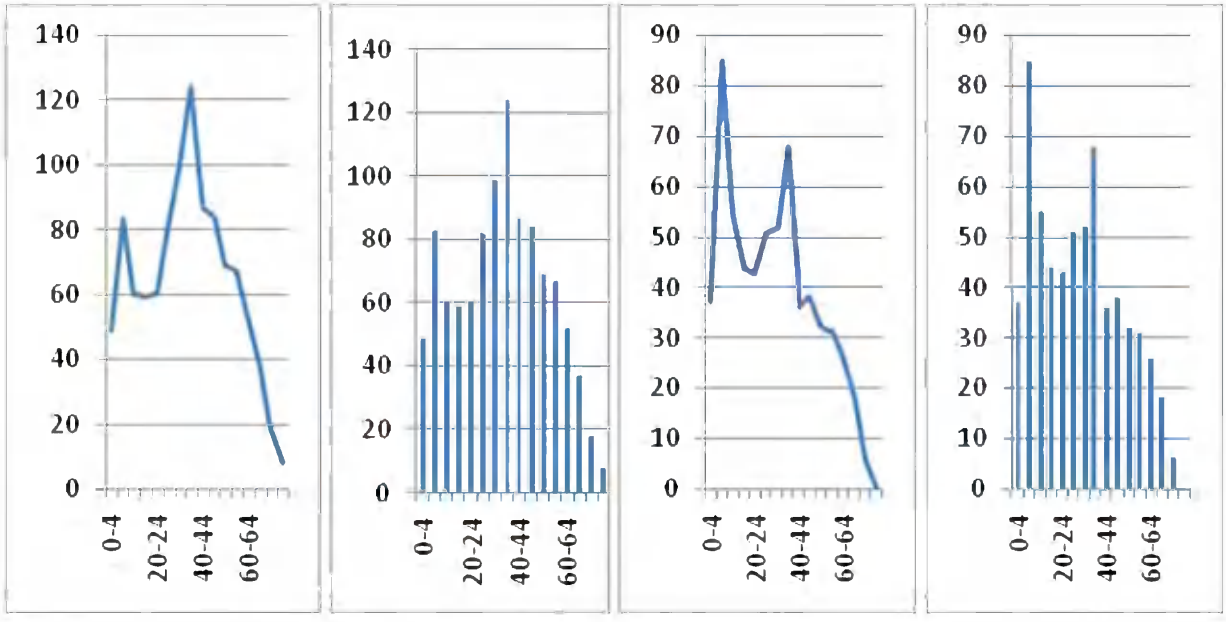

Fig. 12. Age distribution of Brain Cancer

Male-Female

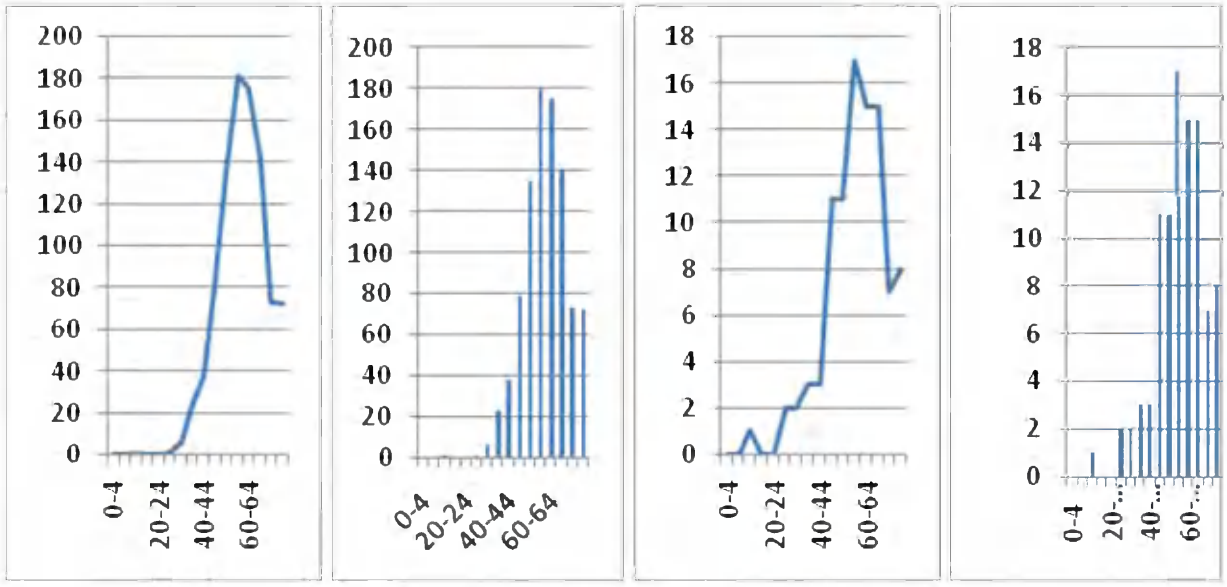

Fig. 13. Age distribution of oropharynx Cancer

Male-Female

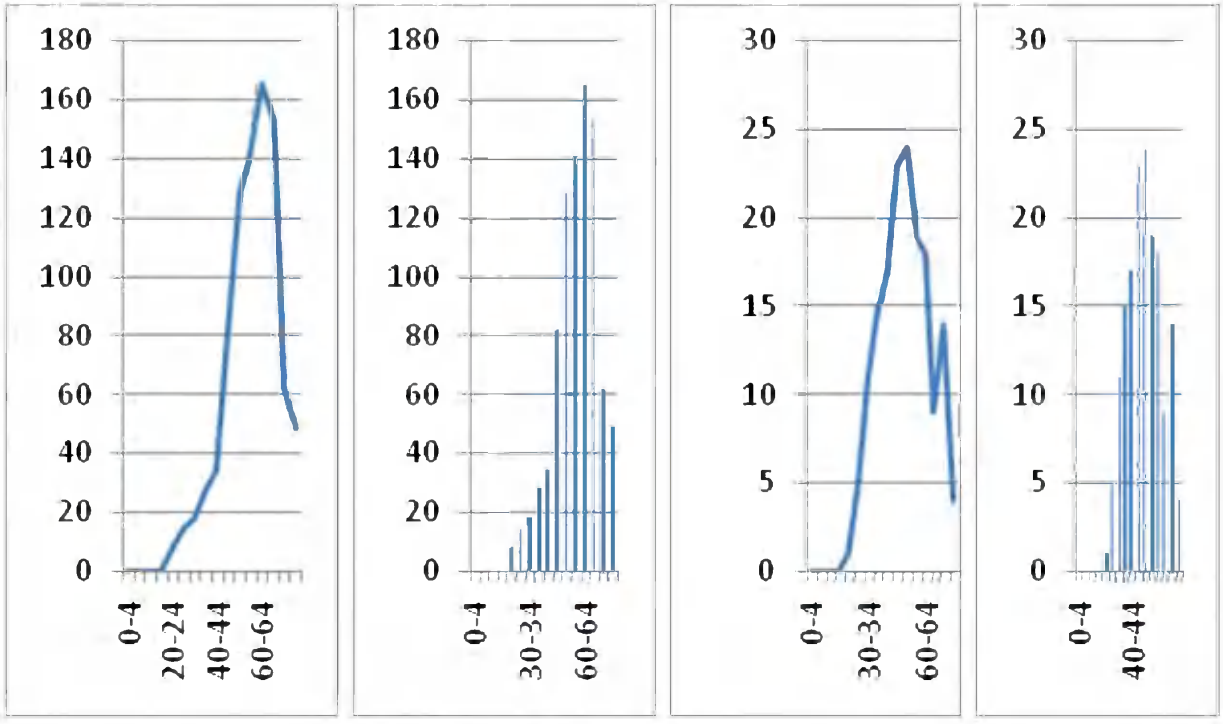

Fig. 14. Age distribution of hypopharynx Cancer

Female

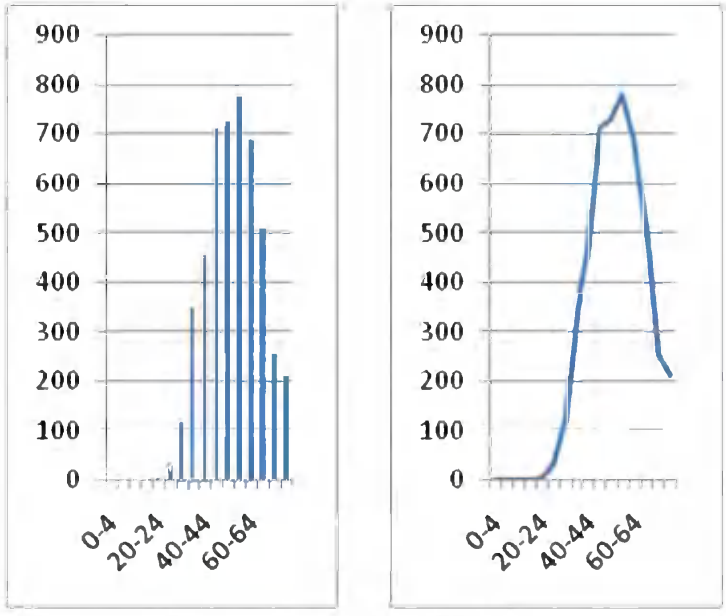

Fig. 15. Age distribution of Cervix Cancer

Laplace distribution is more suitable to model this case.

Female

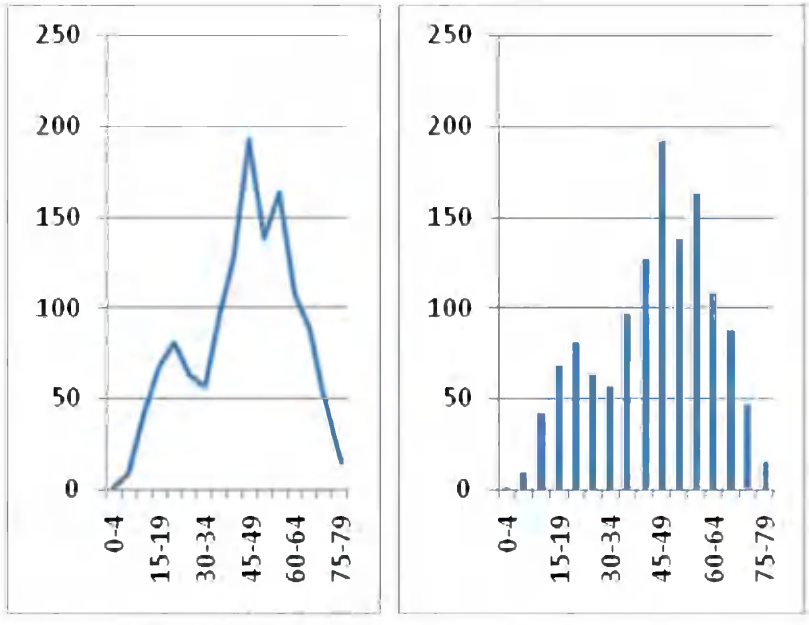

Fig. 16. Age distribution of Ovary Cancer

An asymmetric Laplace distribution is more suitable to model this case.

Female

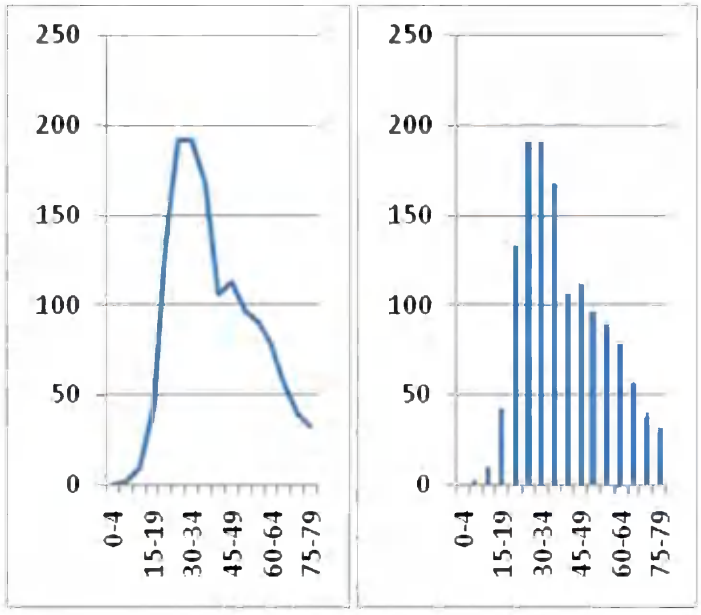

Fig. 17. Age distribution of Thyroid Gland Cancer

An asymmetric Laplace distribution is more suitable to model this case.

Female

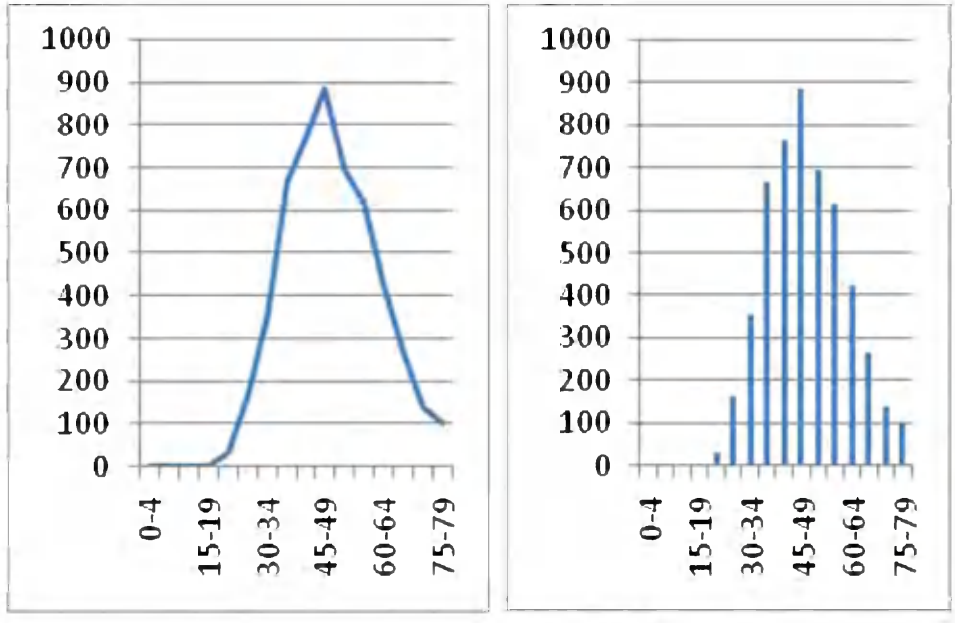

Fig. 18. Age distribution of Breast Cancer

An asymmetric Laplace distribution is more suitable to model this case.

Thus suitable probabilistic models can be used to model cancer data. Time series models may prove more suitable in the study of growth of the disease with respect to age.

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Scientia
Vol. 8, No.1, Jan - Dec 2012, pp 45 - 56

ISSN: 0976-8289

Ultrastructural evidence for role of aposomes in epididymal sperm quality control: Observation in ursolic acid treated rat

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Abstract

Ursolic acid (UA), a triterepenoid compound, is attributed with various pharmacological properties. There are preliminary reports indicating that UA would affect male reproductive health. Hence, the present study was undertaken to evaluate the effect of UA on cauda epididymidal spermatozoa. UA was dissolved in minimum quantity of ethanol, diluted in physiological saline and administered to male Wistar rat through an intraperitoneal route at a daily dose of 10mg/kg body weight for 55 days. Spermatological and histopathological analyses in cauda epididymidal lumen was carried out at the end of the experimental period. The results indicate that UA affects cauda epididymidal sperm as seen in decrease in sperm counts and increased incidence of sperm abnormalities. The observation of cauda epididymidal lumen revealed the presence of spermatozoa with fibrous sheath anomalies. Further, several spermatozoa with such fibrous sheath anomalies remained embedded in a dense matrix, where processing and removal of defective spermatozoa produced due to toxic inflictment was observed. The electron micrographs provided evidence to the effect that spermatozoa get embedded and undergo disintegration by lysosomal/intracellular proteolysis in the vesicles of aposomes pinched off from the epithelium of caput epididymidis into the lumen. The study, thus, reveals yet another mechanism of versatility of epididymis in responding to insults of xenobiotics with newer manifestations to take up newer roles.

Key words: Ursolic acid, cauda epididymis, spermatozoa, aposomes, fibrous sheath

Introduction

A few decades back, the burgeoning world population and its associated risk of damage to the earth and the human health compelled men to share the responsibility in fertility control. WHO constituted a population control programme that included studies on traditional medical plants[1]. Fertility regulation with plants and/or plant products has been reported in ancient literature of indigenous systems of medicine[2]. In a search for plant derived substances, many medicinal plants associated with antifertility properties were discovered [3,4]. Although very few contraceptives have been developed from plant extracts, their potential has not been determined accurately and their modes of action have been beyond our knowledge and, hence, search for a safe and effective plant preparation and/or its active compound for use in male contraception is still on.

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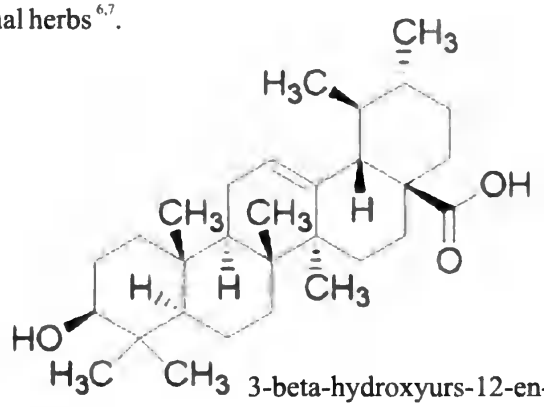
Ursolic acid (UA) is a pentacyclic triterpenoid compound ($C_{30}H_{48}O_3$, Mol. Wt 456.68) isolated from the leaves of *Arctostaphylus uva-ursi* (Fam:Ericaceae), *Ocimum sanctum* (Fam: Lamiaceae), *Vaccinum myrtillus* (Fam: Vacciniaceae), etc., which may occur in their free acid form as glycones of triterpenoid saponin linked to one or more sugar moieties [5]. UA naturally occurs in a large number of vegetarian foods and medicinal herbs [6,7].



3-beta-hydroxyurs-12-en-28-oic-acid

Although for a long time UA was considered to be pharmacologically inactive [8], its alkaline salts as potassium/sodium ursolates were in use as emulsifying agents in pharmaceutical, cosmetic and food preparations [9]. However, upon closer examinations, UA was found to be medicinally active topically and internally [6]. Medicinal plants containing UA have been in use in folk medicine much before it came to be known that the chemical constituents are responsible for the therapeutic effectiveness of these plants. Contemporary scientific research, which led to the isolation and identification of ursolic acid, revealed several pharmacological effects, such as anti-tumor, hepato-protective, anti-inflammatory, anti-ulcer, anti-microbial, anti-hyperlipidemic and anti-viral properties, can be attributed to UA[6]. UA has been suggested to possess antifertility effect in rats of both sexes [10]. But, recent approaches of targeting the post-testicular area namely, the epididymis, to achieve rapid, reversible and safe contraceptive opportunities for men have been speculated [11]. Therefore, a preliminary attempt using light microscopy made by us to find the effect of UA on the male reproductive system suggested it to be a potent disruptor of spermatogenesis, and there were indications of pathological changes in the epididymal epithelium [12]. The latter prompted us to take up a focused study to find the effect of UA on cauda epididymal sperm parameters, morphology and their organelles at the ultrastructural level in albino rats. In our present investigation, we found yet another manifestation in UA-treated rats where in the spermatozoa in sections were missing outer fibrous sheath/peripheral sheath and many of them were found to be embedded in a dense matrix. These matrix-embedded spermatozoa indicated abnormal morphologies. Herein we describe these manifestations and also suggest a role for aposomes in epididymal processing and removal of defective spermatozoa.

Materials and Methods

Animal maintanence

Adult Wistar strain male albino rats, *Rattus norvegicus* (90 day old), weighing 150-200g, raised from a stock procured from the Fredrick Institute for Plant Protection and Toxicology (FIPPAT), now International Institute of Biotechnology and Toxicology (IIBT), Padappai, Chennai, India, were placed in groups of five per cage. The animals were maintained with standard pellet feed (Sai Durga Feeds and Foods, Bangalore, India) and water *ad libitum* under standard environmental conditions of temperature, relative humidity and dark/light cycle. The experiments were approved by the Institutional Animal Ethics Committee (IAEC, Registration No. 418/01/a/CPCSEA/dt. 4.6.2001).

Preparation of test compound and treatment

The UA (Sigma Chemical Co., MO, USA) was dissolved in minimum quantity of ethanol, diluted quantitatively in physiological saline and administered to 10 rats in the experimental group through intraperitoneal (i.p.,) route at a daily dose of 10 mg/kg body weight for 55 days, according to Akbarsha et al. [12], the duration of one complete spermatogenic cycle [13]. Rats in the control group, in equal numbers, received the vehicle alone.

Sperm dynamics [14]

Dilution of semen

After the termination of the experiment, five rats in each group were dissected under MS222 anesthesia to expose the cauda epididymis on one side. The connective tissue capsule around the epididymis was incised, teased out and the epididymal duct was uncoiled. The duct was punctured and the semen that oozed out was transferred to an embryo cup and quickly sucked into a capillary tube up to 0.05l mark and transferred and diluted 200 times using phosphate buffered saline (PBS).

Sperm counts

The diluted semen was thoroughly mixed and a drop of the semen was transferred to an improved Neubauer counting chamber and a cover glass was laid over. The counting chamber was observed in a research microscope at x400 magnification and the sperm in the central square were counted. The sperm counts were calculated using the formula {(Number of sperm in 25 squares / 25) x 10 x dilution factor x 2000 which gives the number of sperm in one ml of cauda epididymidal oozate. Data from each group was used to calculate the mean and standard deviation (Mean ± SD).

Sperm morphology

Sperm smears, stained with Papanicoloau`s stain, were used to record the sperm abnormalities. The sperm abnormalities were observed at different magnification [100x, 400x and 100x].

Histopathological studies

Light and electron microscopic studies adopting resin embedding

At the end of the experimental period, the reproductive system of five rats in each group was perfused with Karnovsky`s fluid under mild MS222 anesthesia and dissected to remove the epididymis. The epididymis (cauda epididymidal segment) was fixed in 2.5% glutaraldehyde in cacodylate buffer, post-fixed in 1% osmium tetroxide and embedded in thin viscosity resin (Spurr mix, Sigma Chemical Co., MO, USA). Semi-thin sections (1μm thickness), cut in an ultra-microtome (Reichert Jung, Austria), were stained with toluidine blue-O (TBO) and observed in a Carl Zeiss (Jena, Germany) Axio 2 Plus research microscope. Images were captured in a CCD camera and processed using Carl Zeiss Axiovision software. Areas in TBO-stained sections were chosen for transmission electron microscopic analysis. Ultrathin sections cut in a Leica (Germany) ultra-microtome were stained with uranyl acetate and lead citrate and observed in a Phillips (Holland) 201C transmission electron microscope (TEM). The spermatozoa in the cauda epididymal lumen and also the luminal content were critically observed.

Statistical Analysis

The data were analyzed statistically, using Students' "t" test and the significance of difference was set at $P \leq 0.01$ (significant) and $P \leq 0.001$ (highly significant) levels [15].

Results

Sperm Dynamics

In UA-treated rats sperm counts decreased to a significant level. On the other hand the percentage of abnormal sperm in UA-treated rats increased manifold (Table 1).

Table 1. Data on sperm parameters of male rats treated with UA (Values are Mean ± SD)

Group	Sperm counts ($x10^6$/ml)	Sperm with abnormal morphologies (%)
Control	29.87±3.95	06.21±2.42
UA-treated	18.38±1.52*	45.24±0.73*

*$p < 0.01$

Changes in the epididymis

The UA treatment resulted in spermatozoa possessing different kinds of abnormalities as observed among epididymal spermatozoa (Fig. 1). In a few spermatozoa, extrusion of the outer fibrous / mitochondrial sheath was observed (Fig. 2). Majority of the spermatozoa showed disruption of fibrous sheath (Fig. 3). Many of the spermatozoa were embedded in a dense matrix (Fig. 4).

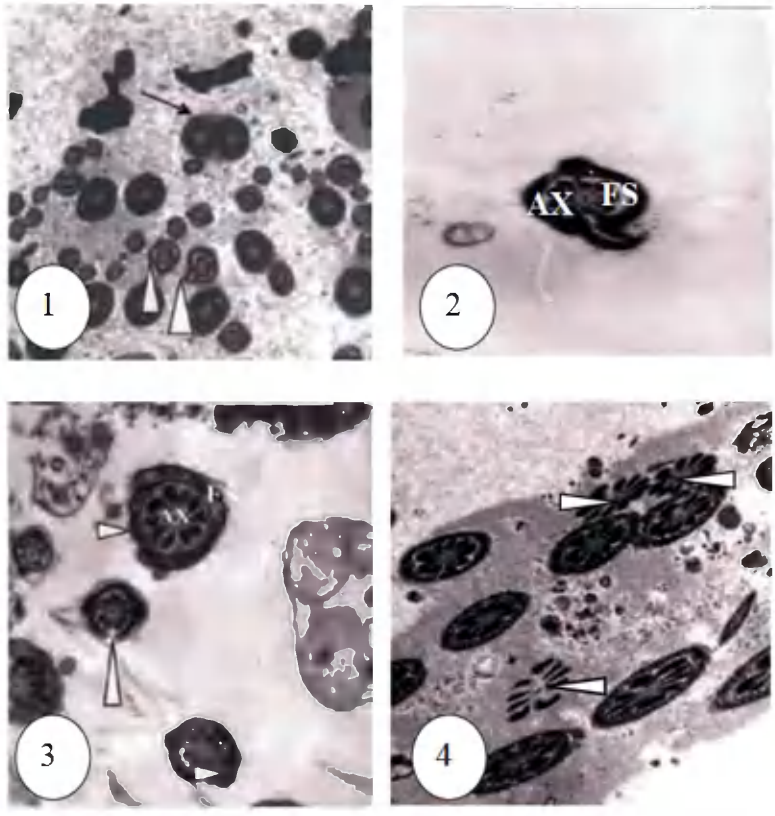


Fig.1.TEM of transverse section (TS) of the cauda epididymal lumen of UA-treated rats showing the presence of abnormal spermatozoa with two axonemes in a common cytoplasmic mass (arrows) and with disrupted fibrous sheath (arrowheads) that surrounds the axoneme (15,000x).

Fig. 2. TEM of TS of sperm tail of UA-treated rat showing extrusion of the fibrous sheath (FS) that surrounds the axoneme (AX) (30,000x).

Fig. 3. TEM of sperm tail showing disruption (arrows heads) of fibrous sheath (FS) that surrounds the axoneme (AX) (30,000x).

Fig. 4. TEM of cauda epididymidis of UA-treated rat showing matrix-embedded spermatozoa undergoing disintegration. Arrowhead points to sperm with fibrous sheath missing. Note the presence of small vesicles (arrows) (15,000x).

On screening through the manifestations of the matrix-embedded spermatozoa, we found the presence of a fairly a high percentage of sperm with intact outer dense fibres, but with missing / abnormal mitochondrial sheath / fibrous sheath (Fig. 4). In a search for source of the matrix in which spermatozoa get embedded, a meticulous exploration through the tissue sections was made. We found the epididymal epithelium of caput epididymidis to produce cytoplasmic blebs, known in the literature as aposomes [16], varied in their morphic features (Figs. 5, 6, 7, 8, 9, 10) and release electron lucent roughly spherical membrane bound vesicles/bodies (Figs. 7, 8, 9, 10).

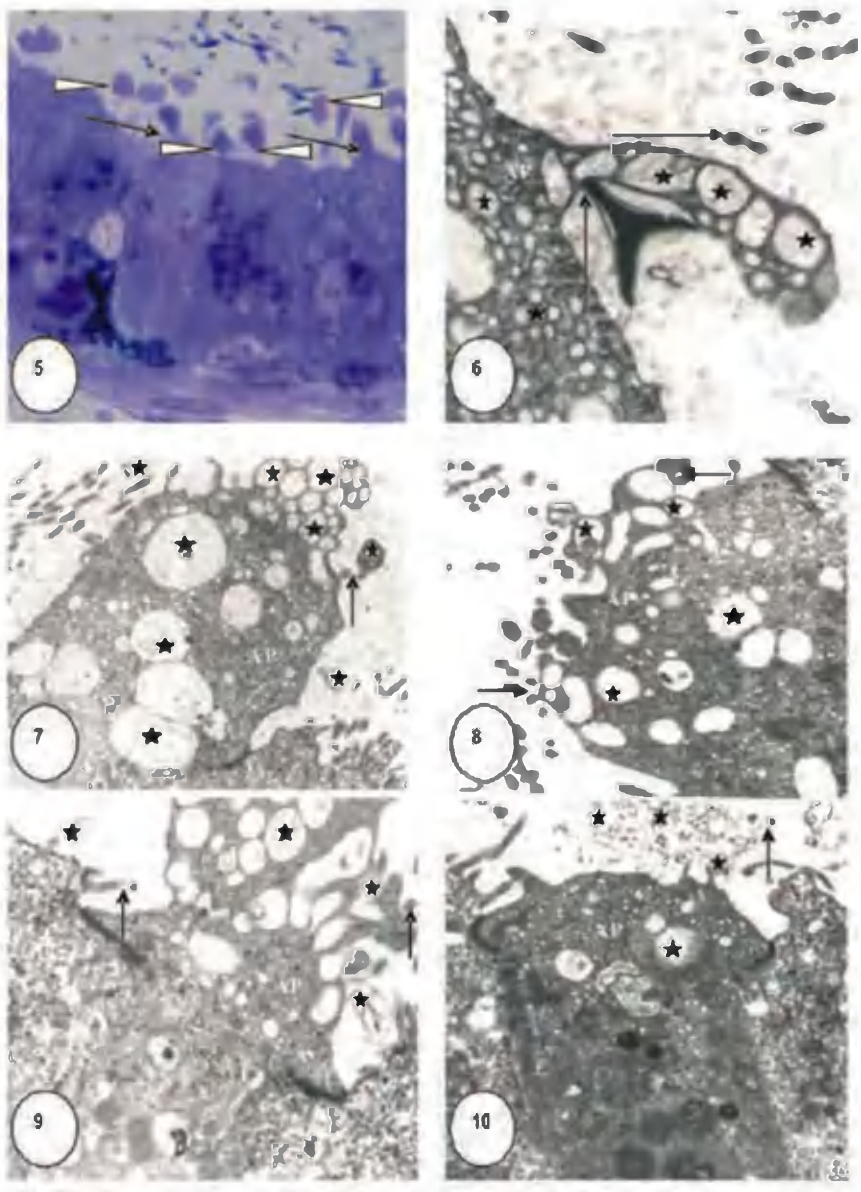


Fig. 5. TBO-stained semithin section of caput epididymidis of UA-treated rats showing protrusion of apical cytoplasmic blebs, aposomes (arrows), and pinching of aposomes (arrowhead) into intraluminal compartment (100x).

Fig. 6-10. TEM of caput epididymidal epithelium of UA-treated rats showing the apical cytoplasmic blebs , aposomes(AP).They are seen to release the electron lucent roughly spherical membranous vesicles (asteriks). The vesicles were seen to scatter in lumen and a few were associated with spermatozoa (arrowheads) (30,000x, 7,000x, 20,000x, 20,000x, 10,000x).

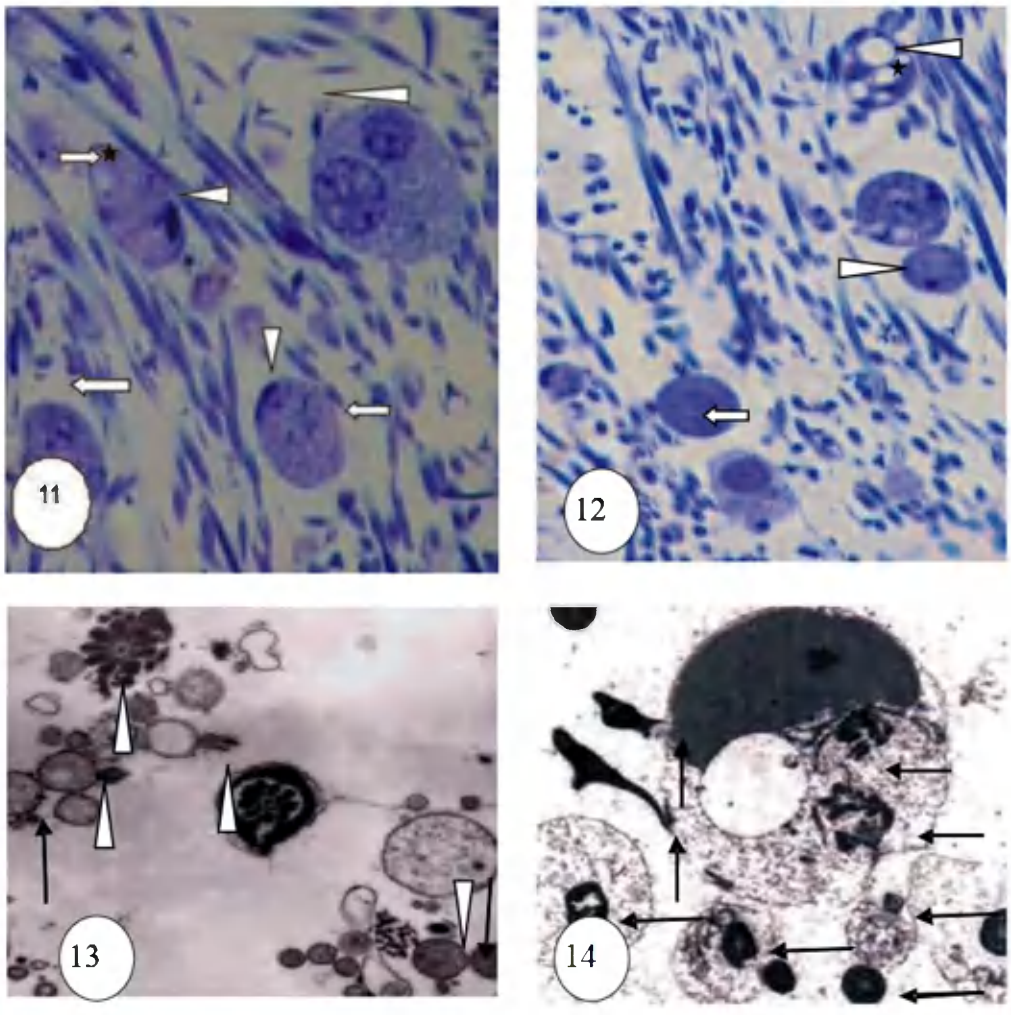


Fig. 11. TBO stained semithin section of cauda epididymal lumen of UA-treated rat showing the presence of spermatozoa (arrow heads) in a less dense matrix (arrow) with vesicles (1000x).

Fig. 12. TBO-stained semithin section of caput segment of epididymis of UA-treated rat showing the fusion of vesicles of aposomes (arrowheads) towards formation of a dense matrix (arrow) (1000x).

Fig. 13. TEM showing the fusion of vesicles (asterisks) of aposomes and association of the vesicles with defective sperm (arrow heads) (30,000x).

Fig. 14. TEM showing disintegration of matrix - embedded spermatozoa (arrow) in vesicles (10,000x).

The densities of vesicles in the aposomes were reflecting different grades, compact, moderate and scattered. The vesicles arrived at the lumen and were found loosely scattered (Figs. 7, 8, 10, 11), spermatozoa were found to lie scattered amongst these vesicles (Fig. 6, 8, 11, 12). The vesicles were seen to associate among themselves by fusion and formed into conglomerates (Fig. 12, 13) losing their identity. The spermatozoa were found entangled in it and undergo disintegration (Fig. 14).

Discussion

The decreased sperm counts in the epididymis may be attributed to the impairment of spermatogenesis, sloughing of premature germ cells, generation of symplasts and multinucleate giant cells, apoptosis of round spermatids, and so on [12]. A decrease in sperm counts due to anti-estrogenic effect of UA leading to a reduced spermatogenesis has also been suggested [10]. The decrease in sperm counts and increase in the incidence of sperm abnormalities points to the spermatotoxic effect of UA.

The epididymal epithelium, especially at the caput region, besides the classical exocrine secretory process, contributes apocrine secretion [16]. This secretion pathway involves formation of apical cytoplasmic blebs that will detach into the intraluminal compartment. The apical blebs, the aposomes, disintegrate, liberating their content which is contained in small membranous vesicles [16, 17, 18]. The epididymal principal cells occasionally produce material in a granular form and release them by lysis of apical membrane at the instance of toxic inflictments [19]. These are adaptations involving structural changes, mostly associated with specific functional attributes. Recently, apocrine secretion activity in the five segments of mouse epididymal epithelium and the formation of vesicular structures has been reported [16]. These authors observed that principal cells emitted apocrine protrusions along the entire epididymal tract, but most notably in the caput epididymidis, as in the present study. In agreement with the observations of these authors [16], the proximal epididymis (i.e., segments I–III of the caput) was very active in terms of apocrine secretion. However, contrary to what was reported initially by these authors, it was later found that the corpus and cauda epididymides were very poorly active [20]. We found discrepancies in the structure of blebbing cells and vesicles. Free vesicles resulting from blebs emitted by principal cells were also observed in the epididymal lumen. The small vesicles were observed to contain uniformly distributed electron-dense granular material whereas the larger vesicles looked rather empty. Even though a cytoplasmic continuity between these vesicles and epithelial cell cytosol was noticed during the apocrine secretion, the content of both these entities varied between each other. A few of the vesicles had granular material aggregated in the vicinity of their membrane. Thus, their ultrastructure was almost the same as that observed for human prostasomes or for prostasome-like vesicles in rat epididymis, stallion semen, or bovine seminal vesicles [21,22,23,24,25].

An observation of dense bodies of size 900 nm in the epididymal lumen of mouse, restricted to the corpus and cauda epididymides and vas deferens, with no representation in the caput has been reported [26]. They are different from the ones observed in this study as they lack clear membrane around the vesicle, and the size also varied largely. About the origin of dense bodies, the authors did not arrive at any lucid conclusion. A similar type of vesicles of size between 25 and 75-nm diameter has been observed in rams [27]. These

authors also observed that some of the vesicles contained electron-dense material and a large majority showed a typical membrane bi-layer. It has been established that a large proportion of vesicles derived from epididymis are found in seminal plasma in humans.

During the last decade, efforts have been made to understand how aposomes interact with sperm cells and what functions are associated with these structures. Aposomes are nothing but the protrusion of a portion of the apical cytoplasm of principal cells into the lumen which is otherwise termed as apical blebs. Aposomes were reported to possess fusogenic membranous properties, which has been clearly substantiated in the present study, allowing the exchange of materials with spermatozoa [20]. Besides proteins, lipids also were shown to be transported by aposomes [28]. The apocrine secretion of some proteins in the epididymis involves the apical blebs which contain mainly free ribosomes, 20 nm vesicles (circles) and a few ER cisternae. As apical blebs of the epididymis were immunoreactive for ubiquitin, it was proposed that these proteins are synthesized on free ribosomes in the apical blebs and that the apocrine secretion represents the manner whereby they enter the lumen to effectively protect sperm from free radical injury and ubiquitinate proteins for degradation.

Ubiquitin, which is a usual component of most eukaryotic cells, has been shown to play a crucial role in intracellular proteolysis [29, 30] and post-translational modifications of proteins. In the proteolytic pathway, ubiquitination is a cascade of process requiring ubiquitin conjugating enzymes which allow the binding of ubiquitin to proteins targeted for degradation [30]. Major components of the ubiquitin-dependent proteolytic pathway are expressed in the epididymis [31, 32]. In other words, ubqituin appears to be secreted in apocrine fashion by the principal cells of the epididymal epithelium and binds preferentially to the surface of defective spermatozoa. This has been reported in bulls, men, rhesus monkeys, and mice [33, 34]. The percentage of defective spermatozoa decreases as the spermatozoa descend through the epidiymal duct because of the disposal of such spermatozoa along with the ubiquitinated cytoplasmic droplets by the epididymal epithelium [35]. Thus, amongst its many functions, ubiquitin plays a role in extra-lysosomal protein degradation also. In this context, ubiquitin in the lumen of the duct may be conjugated to various proteins and target them for degradation, such as those arising from degenerating sperm present in the lumen [36] and from the numerous cytoplasmic droplets following their breakdown after being released from sperm as they pass through the epididymis [37].

We have reported the presence of varying number of clusters of spermatozoa embedded in abundant dense matrices in the lumen of caput epididymidis onwards. One or more spermatozoa were found entangled in the dense matrices and these spermatozoa were in different degrees of disintegration. Quite a few reports support the presence of a dense material towards the disintegration of spermatozoa in the epididymal

milieu [38, 39, 40, 41, 42, 43]. Therefore, the dense material could be a device to mass-ubiquitinate the defective spermatozoa and seclude the viable spermatozoa from the ubiquitination system, to facilitate the removal of defective / dead spermatozoa. Also, the apical blebs of principal cells of the epididymis and vas deferens have been reported to contain numerous vesicles with a diameter of 20 nm and these vesicles are of similar size and appearance [16]. It has been suggested that the 20nm vesicles may be derived from the Golgi apparatus of principal cells and represent small primary lysosomes containing glycosidases. So, here the lysosomes may have a role in degradation/dissolution of defective spermatozoa. Thus, this part of the study, for the first time, links aposomes of epididymal epithelial principal cell origin to the dense matrix which entangles the defective/dead spermatozoa, towards a sperm quality control mechanism, which is adequately substantiated, and the second part of release of ubiquitin by aposome is only hypothetical, based on rationalistic inferences, but remains to be established through adequately designed analyses.

We suggest that aposomes in this context are concerned with contributing to the dense matrix and the enzymatic mechanism for degradation/dissolution of the defective spermatozoa, thereby excluding the normal sperm from enzymatic degradation, which is another aspect of versatility of epididymis.

Acknowledgments

The ultracut and TEM facility of Wellcome Trust Laboratory, Christian Medical College and Hospital, Vellore, India, is gratefully acknowledged. The authors are grateful to the Department of Science and Technology (DST), Govt. of India, New Delhi, for the financial assistance provided.

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Scientia
Vol. 8, No.1, Jan - Dec 2012, pp 57 - 64

ISSN: 0976-8289

Influence of environmental parameters on the Odonata assemblages in palakkad, south india

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Abstract

Odonata have long been studied as an insect order that plays an important role in the balance of aquatic invertebrate communities, and [1]their general visibility, diversity around lentic waters that makes them an ideal ecological indicator. The current study was carried out to investigate the influence of environmental variables on the assemblages of Odonata.[2]Abiotic factors are known to be important in determining species composition of Odonata assemblage. Three wetland sites namely paddy fields, ponds and streams of seven selected areas of Palakkad district were studied as sampling sites. Water samples for physico-chemical studies were collected from these sites and were examined.Temperature, pH, Conductivity, Dissolved oxygen, TDS and Turbidity were determined and differences were evaluated using analysis of variance (ANOVA). A total of 556 individuals, belong to forty four species under 11families were collected from seven sites. Higher diversity of Odonata was found at Kallikad. ANOVA testing showed that water quality parameters are all significantly different among the three wetland sites of seven selected areas and suggested strong correlation between species diversity and water quality parameters. None of the parameters had a strong correlation to odonata abundance. Among the environmental variables taken into account for the study, pH, conductivity, atmospheric temperature are found to be influencing the distribution of Odonata.

Key words: Assemblage, Odonata. Wetland, turbidity, dissolved oxygen.

Introduction

Since the abundance and diversity of species that colonize and establish populations in the wetlands is the ultimate indicator of healthy wetlands,determining sensitive species of taxonomic groups that can be used to indicate the health of eco systems is very important[3].Insects often makes good indicators because they are present in some capacity in almost in every type of habitat and many are habitat specialists.[1]The order Odonata represents one set of insects that is widely studied for its potential in indicating the environmental quality. This is largely because many of the criteria of good indicator species, such as being taxonomically well known, relatively easy to identify .and having distinct habitat requirements are fulfilled by Odonata. Besides this they are also the early colonizers of new lentic habitats. They are reasonably diverse and have a well established taxonomic frame work[4]. As several species are stenotopic and require specialized habitat conditions, a given assemblage reflects the effects of environmental variables.

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Assessment of water quality is carried out through the use of wide range of physical, chemical or biological variables. pH, Temperature ,Conductivity, Turbidity and Dissolved oxygen are considered as the common water quality parameters. The term dissolved oxygen refers to the amount of oxygen that is dissolved in water at a given temperature and a given atmospheric pressure. Dissolved oxygen is considered as one of the most important limnological variable, both for the characterization of aquatic ecosystems and for the maintenance of aquatic life[5]. Many organisms, especially the indicators of good environmental quality require high concentrations of dissolved oxygen for their survival. Water can hold more dissolved oxgen at low temperatures than at high temperatures. The primary source of dissolved oxygen is the photosynthetic activities and reaeration[2]. The major demands on dissolved oxygen come from plant respiration and decomposition of organic matter by bacteria. Water pH is important because it can affect aquatic insect survival and emergence. pH naturally varies both daily and seasonally[3]. Water becomes more basic during the day and acidic during the night.[2]Since conductivity is a numerical expression of the capacity of water to conduct electric current, thus depending on the ionic concentrations and temperature, it indicates the amount of existing salts in the water column

Palakkad district lies between north latitude 10° 46` and 10° 59` and east longitude 76° 28` and 76° 39`. lies near the Palakkad pass, a pass or natural depression through the Western Ghat ranges. The climate is hot and humid for most part of the year.[2]The Western Ghats is one among the 34 global hotspots of biodiversity and it lies in the western part of peninsular India in a series of hills stretching over a distance of 1,600 km from north to south and covering an area of about 1,60,000 sq.km. It harbours very rich flora and fauna. The Western Ghats form an important watershed for the entire peninsular India.

Materials and Methods

Seven wet land sits were selected. Sampling was conducted during 2010-2011 from June to December. Water samples for physico-chemical studies were collected from different sites. Water samples were collected in triplicate. Measurement of water temperature was performed with a Mercury thermometer. pH and conductivity and TDS were determined using a portable pH/EC/TDS/ meter while dissolved oxygen were determined by Winkler method .The Turbidity was determined by Nephelometric method. Method used for Odonata identification was random survey, which was carried out in selected habitats best suited for collecting Odonates. The specimens were observed and identified up to the best possible taxonomic level in the field using appropriated keys;

Data analysis

SPSS applications were used to calculate the analysis of variance (ANOVA) Alpha diversity indices of

Simpson index were calculated. Relative abundance was done to evaluate Odonata composition

Result

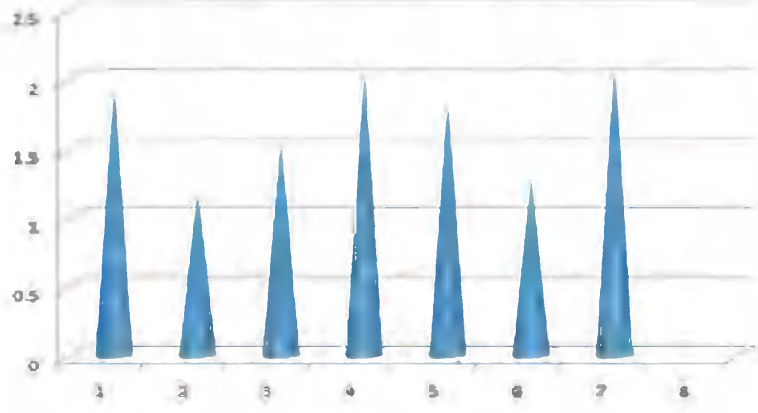
Odonata abundance and diversity

A total of 556 odonates from 44species were collected,including one rare species Phylloneura westermani(2.2%,),4 IUCN red listed(12.2%)),3endemic species(6.7%).The number ofspecies collectedwere24,8,10,23,14,8,10atkKallikad,Pirayiri,Puthussery,Mannur,Chirakad,Tathamangamand Dhoni-the seven sites where sampling is done.The cumulative abundance from the seven sites differ significantly.

Ceriogrion cerinorubellum sp. of the sub order zygoptera was present in all sites. The individuals belonging to *Orthetrum Sabina* Sp. and *Pseudogrion indicu*m Sp. were found to be present in five out of seven sampling sites.B*rachythemis contaminata* resisent to pollution is found only in one site. A total of 16 species in this study were found to be present in single sites. Of these, *Libellago lineata, Euphae fraseri, Copera vitata* are found in the hill streams of site7.It is a part of often undisturbed riparian ecosystem. Lemny (1982) explained their need for rather unpolluted water with high dissolved oxygen and low siltation. *Ictinogomphus rapax* is found to be present in a single site, site-1 which is characterized by the presence of the riparian vegetation in relatively undisturbed ecosystem. The highest aquatic Odonate diversity was found to be in Site-1(24species) followed by Site4 (23 species), Site-5 (14 species), site3&7(10 species) Site-2&6 (8 species). The value of Simpson diversity Index was found to be highest for Site-1 (0.05) and least for Site-7 (0.20) (Table 1) respectively. The maximum Odonata diversity is noticed in Kallikad. Dhoni showed minimum diversity and highest abundance is in Mannur. Least abundance is in Pirayiri (Fig. 1.)

Table. 1. Species diversity index

Name of the site	Species diversity
Kallikad	0.05
Pirayiri	0.06
Puthussery	0.15
Mannur	0.10
Chirakkad	0.17
Thathamangalam	0.15
Dhoni	0.20



1. Kallikad 2. Pirayiri 3. Puthussery 4. Mannur
5. Chirakkad 6. Tathamangalam 7. Dhoni

Fig. 1. Species abundance of Odonates in seven selected sites

Water quality analysis

Table 2 .Water quality parameters of paddy fields at seven wetland sites.

Sites	pH	Temperature (^{0}C)	Conductivity (mS/Cm)	Dissolved Oxygen (Mg/L)	Turbidity (NTU)	TDS (Mg/L)
Site 1	8.23	28.9_{a}	288.00_{a}	3.20_{a}	37.00_{a}	204.00
Site 2	7.26_{a}	27.3_{b}	287.00	4.80_{b}	37.00_{a}	202.00
Site 3	7.46	27.8_{bc}	586.00	5.40_{bc}	37.00	417.00
Site 4	6.71_{b}	29.1_{ad}	107.80_{a}	4.33_{abcd}	64.00	76.70
Site 5	7.93	28.3_{ace}	504.00	5.00_{bcde}	17.00	357.00
Site 6	7.26_{ab}	28.7_{ade}	431.00	4.00_{abde}	40.00	307.00
Site 7	6.26	27.7_{bce}	78.20	6.00_{bce}	60.00	55.40

Note: Means followed by same subscripts are not significantly varying at P<0.05

Table 3. Water quality parameters ofStreams at seven wetland sites

Sites	pH	Temperature (^{0}c)	Conductivity (mS/cm)	Dissolved Oxygen (Mg/L)	Turbidity (NTU)	TDS (Mg/L)
Site 1	7.20	27.60_{a}	288.00	4.20_{a}	33.00_{a}	253.00_{a}
Site 2	7.54_{a}	26.70_{ab}	516.00_{a}	5.00_{a}	8.00	2700.33_{a}
Site 3	7.32	28.10_{ac}	64.43	4.80_{a}	34.00_{a}	46.00_{a}
Site 4	6.95	28.90_{cd}	99.10	6.40	86.00	70.30_{a}
Site 5	7.64_{a}	28.30_{acde}	515.00_{a}	5.00_{a}	18.00	365.00_{a}
Site 6	7.82	29.00_{cde}	521.00	5.00_{a}	29.00	370.00_{a}
Site 7	6.10	27.60_{abce}	329.50	4.00_{a}	60.00	91.90_{a}

Note: Means followed by same subscripts are not significantly varying at P<0.05

Table 4. Water quality parameters of Ponds at seven wetland sites

sites	pH	Temperature (^{0}c)	Conductivity (mS/Cm)	Dissolved oxygen (Mg/L)	Turbidity (NTU)	TDS (Mg/L)
Sites 1	7.80_a	28.73_a	285.00	3.20_{ab}	36.00	260.00
Sites 2	7.17_{ab}	27.00	307.00	4.20_{ab}	34.33_a	280.00
Sites 3	6.93_{ab}	28.10_{ab}	667.00	4.50_{abc}	117.00	473.00
Site 4	6.73_b	29.03_{abc}	116.80	5.00_{bcd}	34.00	82.90
Site 5	7.29_a	28.10_{abd}	357.00	3.00_{ab}	18.00	253.00
Site 6	9.40	28.60_{acde}	365.00	5.00_{bcde}	28.00	370.00
Site 7	5.13	27.90_{bde}	77.70	5.00_{bcde}	38.00	55.10

Note: Means followed by same subscripts are not significantly varying at P<0.05.

Site 1 has high pH and site 7 has low pH .Site 1is significantly varying from site7. site1has 187 and site7 has 33individuals. Similarly site 1 and site4 are significantly varying in pH. site 4 has 129 individuals.site 3 and site 5 are significantly varying. 58 individuals in 3 and site5 got 63 .

Temperature of thePaddy fields of the seven sites are not significantly varying. Temperature is not a factor influencing either abundance or diversity. But high temperature usually facilitates assemblages.

Site 1 has low DO and site 7 has high DO. Generally low DO facilitate Odonate assemblages.

Fig. 3 Comparison of Turbidity conductivity in the paddy fields of seven selected sites.

Site1,2, have moderate conductivity. Site 7 has very low conductivity. Moderate conductivity is good for Odonate distribution. Site1 has low turbidity. But turbidity alone is not a factor for Odonate distribution. Turbidity and TDS are connected.

Site 1 and site 4 are significantly varying in pH. Site 6 has got very high pH and hence low diversity. Site7 has very low pH, not supporting the assemblage. Temperature is not significantly varying in all sites.

. In site 7 conductivity is low, but low pH and high DO level restrict the Odonate assemblage. Site 1 and site7 got low conductivity hence high species content. Low turbidity and low TDS are favourable factors for Odonata.

Streams of different sites show pH ranging from 6.10 to 7.82.Site 7 alone is on the acidic side . pH of all other sites are favouring the Odonata.site 4 and site6 shows higher temperarures.Temperatures of all other sites are not significantly varying..Conductivity of all sites are significantly varying.DO of all sites are in the same range.except site1 and 3, the turbidity is not significantly varying in rest of the sites .TDS of all the sites are not significantly varying.

Discussion

Among the different habitat taken for the study, the ponds of the site 1 are found to be the favourite place because of its high diversity and abundance[3]. Alkaline pH, low Conductivity, low DO, low Turbidity, high TDS offers optimum conditions for the distribution of the Odonata. Ponds of the site 4 show low Conductivity, but high DO and low pH restricts Odonata assemblage.

High pH, low DO, high Temperature, low Conductivity, TDS at the range of 117to270are found to be favourable for odonate assemblages. pH correlates broadly with the distribution of certain taxa but is seldom the proximate factor determing the distribution. Numerous species occur across a wide range of pHvalues.[5]pHmayinteract with substances in water to change their effects on dragonfly larvae. Zygoptera may be more toletrant of high pH than Anisoptera [3]. Very low Conductivity and high Conductivity had negative impact on Odonate distrubution. Variations in diversity obtained could be as a result of moderately high conductivity values and DO levels that were significantly different between the sampling stations within the wetland[5]. Increased Conductivity has an influence on osmoregulation of the aquatic invertebrates leading to sensitive freshwater organisms either to disappear or adapt. The low diversity in Station S2 andSite6 were probably due to few macro habitats observed in the area coupled with [2]an open access for livestock invasion that leads to herbivory of aquatic vegetation and nutrient input via urine and fecal deposition and trampling of sediments which have direct impact on aquatic ecosystems. The highest diversity and richness observed in stationS1 could not only be due to several microhabitats but also the absence of frequent and major human activities that could cause hydrological perturbations to such areas[6]. This concurs with Matthaei *et al.*, who showed that the distribution of benthic macroinvertebrates in aquatic systems is dynamic and is strongly influenced by the hydrological disturbance regime. It was therefore realized in this study that diversity is a function of seasonal differences, and human disturbance which influences the availability of organic matter as also supported by Mason[7].

Changes in water quality between the stations can be largely attributed to land-use practices. DO for instance was lowest in stations S6 probably due to higher temperature as a result of reduced vegetation

cover and high human activities like washing, agriculture and even higher turbidity[10]. High temperature reduces the solubility of oxygen while turbidity reduces ligpenetration thus low primary productivity which in turn affects the availability of DO. There was increased agriculture and cattle grazing along the wetland during the dry seasons that consequently reduced vegetation cover which is a likely cause of the variation .Conductivity was highest during the dry months of November and December the time when the wetland experienced negligible flow and the solutes were not effectively diluted which concurs with Busulwa and Bailey .High temperature, which facilitates the release of ions, recorded during the same period might have also played a role in high conductivity values obtained..Low pH values were recorded during the period from October to December which can be attributed to the dry spell experienced during this period. [8]Prolonged drought has been found to induce re-acidification of aquatic systems, which in turn lowers the pH. Water temperature showed both spatial and temporal variation. Station S1 which had heavier vegetation cover recorded lower temperature values as compared to non-covered stations.[8]Vegetation cover limits direct solar radiation reaching the water thus contributing to minimal fluctuations of temperature. [4]High solar radiation due to low macrophyte and little water volume can explain high water temperature during the dry periods. Variations recorded in the nutrient concentrations among the stations, could possibly be due to the difference in the magnitude of activities like crop cultivation, animal grazing, and domestic washing that all have an effect on the concentration of nutrients. Robert and Rankin[10] obtained similar results at a site where anthropogenic impact seemed to be more, Nutrients availability in an aquatic environment is primarily influenced by several factors which include application of fertilizers .Griffith[5] showed a negative correlation of macroinvertebrate diversity with increased nutrient levels. This could imply that high nutrient levels which are a sign of disturbance have an impact on macroinvertebrates ecology and diversity.

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University Press Sage House, 512 East State Street New York

Scientia
Vol. 8, No.1, Jan - Dec 2012, pp 65 - 71

ISSN: 0976-8289

Evaluation of the *in vitro* antioxidant and antimicrobial activities of *Heracleum candolleanum* (Wight & Arn.) Gamble

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Abstract

Heracleum candolleanum (Wight et Arn.) Gamble (Apiaceae) is widely used in traditional systems of medicine for a variety of diseases. It is an endemic plant usually found at an elevation above 1100-1800m at the high ranges of Idukki and Kottayam districts, Kerala State. According to IUCN, it is a vulnerable species. The Kani tribes administer decoction of the whole plant internally for nervous disorders and inflammatory conditions. In the present study, the antioxidant activity of the methanol extract of seeds, roots and leaves were investigated by dot blot assay. DPPH radical scavenging activity of the methanol extract of seeds was also screened for *in vitro* antioxidant activity. Antibacterial activity of the methanol extract of seeds was evaluated by Agar well diffusion method. The results obtained in the present study suggested that the seed extract of *H. candolleanum* might be a potential source of natural antioxidant and anti-microbial agents.

Key words: Traditional medicine, phytochemical evaluation, anti-oxidant activity, anti-microbial activity.

Introduction

Study on the effect of exogenous and endogenous antioxidants against cellular free radicals has attracted the attention of scientists worldwide. This is because of the major role of free radicals in the pathogenesis of many diseases including atherosclerosis, ischemic heart disease, cancer, Alzheimer's disease, Parkinson's disease, and even in the aging process[1]. Medicinal plants, especially those used in folk medicine, have much therapeutic value owing to the presence of an array of antioxidant components. Metabolites from medicinal plants exert their antioxidant and anti-inflammatory activity by scavenging oxidants and also by decreasing the formation of reactive oxygen species (ROS) by activated phagocytes[2]. Thus, novel anti-inflammatory metabolites of plant origin can have much application to suppress the inflammatory basis of disease process. In addition to this, challenges from pathogenic microorganisms also demands search for metabolites with broad antimicrobial activity. Natural products from medicinal plants, either as pure compounds or as standardized extracts can thus provide unlimited opportunities as new drug leads because of their extreme structural diversity[3].

Heracleum candolleanum (Wight et Arn.) Gamble, of Apiaceae is endemic to the Western Ghats and is locally known as 'Vathamparathi'. In Siddha medicine, it is described as 'Chittelam'. The plant is found in the Western Ghats of peninsular India at altitudes above 1000 m and is used by tribes for various ailments

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ranging from nervous disorders to inflammatory conditions[4]. The tribal people of Silent Valley and Idukki in Kerala use fruits of *Chittelam* in digestive complaints [5]. The decoction made from the root of this plant is used by the tribes as an anti-arthritic agent and also as a nerve tonic[6]. The crude methanol extract of the root *of H. nepalense* is known to have activity against gram-positive and gram-negative bacteria[7]. There are also reports on the isolation of mono-terpenoids; 2-exo, 3-endo-camphanediol and 2-pinene-4, 10-diol from the seeds of *H. candolleanum*[8]. In addition to this a number of furanocoumarins were also reported from the fruits and roots of *H. candolleanum*[9]. Coumarins have recently been reported to have interesting pharmacological and biochemical properties, including antioxidant, anti-inflammatory and anti-allergic activities, inhibition of platelet aggregation, wound-healing, antiviral, antiobesity and antimutagenic effects[10-13]. In this study, phytochemical profiling and preliminary screening for antioxidant activity of the methanol extracts of leaf, root and seeds of *H. candolleanum* were conducted. As an array of active principles was isolated from the seeds of *H. candolleanum they were selected for* further studies.

Materials and Methods

H. candolleanum was collected from the Vagamon Hills, Kerala, India. A voucher specimen was kept in the herbarium of Navajyothi Sree Karunakara Guru Research Center for Ayurveda and Siddha, Uzhavoor as reference. Approximately 50gm dried powder of root, leaf and seeds were taken extracted using methanol in a soxhlet apparatus. The filtrate was dried using rotavapor and the extract was used for the preliminary phytochemical analysis, antioxidant and antimicrobial studies. The methanol extracts were redissolved in a minimum amount of methanol and made up to the required volume in 1% gum acacia[14].

Phytochemical screening of the plant extract

An aliquot of the dry extract was used for the phytochemical evaluation for compounds which include cumarins, flavonoids, alkaloids, saponins, and steroids following standard procedures with minor modifications.

Qualitative determination of antioxidant activity

Dot-Blot by DPPH staining method: Antioxidant capacity of *H. candolleanum* was detected semi-quantitatively by a rapid DPPH staining TLC method. Each diluted crude extract of plant parts (seeds, roots and leaves) were applied as dots on a TLC layer (Merck Silica gel F_{254}) in the order of increasing concentration (10-50 mg/ml), along the row. The TLC plate was then stained with 0.4mM DPPH solution. Staining of the silica plate was based on the procedure of Soler-Rivas et al.[15]. A 0.4mM DPPH solution in methanol was used as control[16-18]. Ascorbic acid was spotted as the standard drug.

Quantitative antioxidant assay (in vitro DPPH Radical Scavenging Assay)

Antioxidant activity of the extract was determined spectrophotometrically by DPPH free radical scavenging assay by the method of Blois[19]. In this assay, 1 ml of various concentrations (10-1000 µg/ml) of the methanol extract of seed was mixed with 2 ml of 0.1mM DPPH solution in methanol. The mixture was allowed to react at room temperature in the dark for 45 minutes. Blank solutions were prepared with DPPH solution. Ascorbic acid was used as positive control. The decrease in absorbance was measured at 517nm.

Antimicrobial activity

The antibacterial activity of the methanol extract of the seed was determined by agar well diffusion method. Nutrient agar plates were inoculated with overnight grown cultures of *Escherichia coli*, *Pseudomonas aeruginosa* and *Serratia marcescens*. Wells of approximately 10mm was bored with well cutter and different concentrations of seed extracts (50µl and 100µl of 1mg/ml solution) was added, the zone of inhibition was measured after overnight incubation and this was compared with that of streptomycin.

Results and Discussion

Preliminary Phytochemical screening

Phytochemical screening of the extracts of *H. candolleanum* revealed the presence of alkaloids, coumarins, sterols, quinines, phenols, resins, reducing sugars, carbohydrates and volatile oils (Table 1).

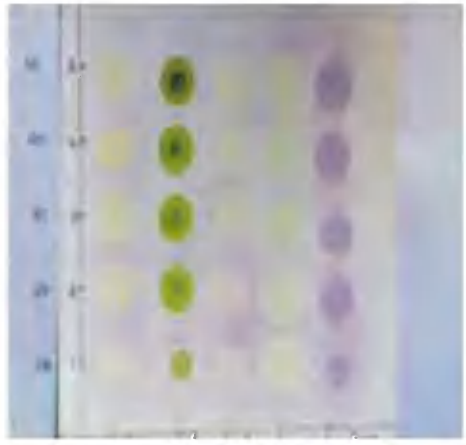
Dot-Blot DPPH Staining

Antioxidant capacity of *H. candolleanum* was detected quantitatively by a rapid DPPH staining method. This method is based on the inhibition of the accumulation of oxidized products. The diluted crude extracts of root, leaf, seed and ascorbic acid were applied as dots on a TLC plate and was then stained with DPPH solution. The crude extracts of *H. candolleanum* (10-50mg/ml) were applied from bottom to top in sample columns; ascorbic acid (10-50 mg/ml) and DPPH were applied from bottom to top in separate columns. DPPH (0.4mM in methanol) was used as positive control. The purple area on the plate indicates the absence of antioxidants and the yellow spot against a purple background indicates the presence of antioxidants[20]. The spots which have more intense colour possess higher antioxidant activity (Fig. 1). In leaf, the yellow colour can be masked by chlorophyll.

Table 1. Phytochemical profile of *H. candolleanum*

Extracts tested?	Root Extract	Leaf Extract	Seed Extract
Components?			
Flavanoids	-	-	-
Coumarins	+	+	+
Alkaloids	+	+	-
Saponins	-	-	-
Sterol/Terpenoids	+	-	-
Quinines	-	+	-
Anthroquinones	-	-	-
Tannins	-	-	-
Phenols	+	-	+
Resins	+	-	+
Glycosides	+	-	-
Reducing sugars	+	-	-
Carbohydrates	+	-	+
Volatile Oils	+	+	+
Proteins	-	-	-

Note: '+' indicates presence and '-' indicates absence



Root Leaf Seed V-C DPPH

Fig. 1. TLC Plate stained by DPPH showing antioxidant Activity of Root, Leaf and Seed with standards.

***In vitro* DPPH free radical scavenging assay**

The DPPH radical has been used widely in the *in vitro* model system to investigate the scavenging activities of natural compounds such as phenolic compounds, cumarins or crude extracts of plants. DPPH radical is scavenged by antioxidants through the donation of proton, forming reduced DPPH and the color changes based on the number of electrons accepted. After reduction, sample's colour changes from purple to yellow which can be quantified by its decrease of absorbance at 517 nm. The degree of discoloration indicates the free radical scavenging potential of the sample/antioxidant by its hydrogen donating ability.

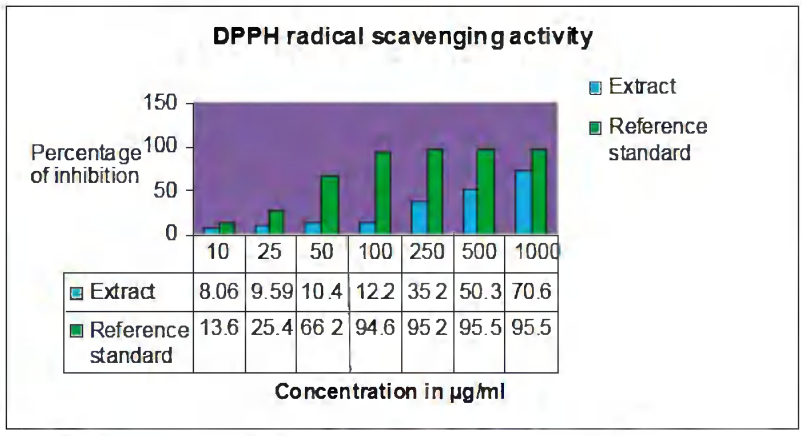


Fig. 2. *In vitro* DPPH free radical scavenging activity of the methanol extract of *H. candolleanum* seed.

Free radical scavenging activity of seed extract and the standard drug ascorbic acid are shown in Fig. 2. The IC_{50} value was calculated and is given as Table 1. From the result it is clear that the antioxidant capacity of seed extract of *H. candolleanum* is comparable with that of ascorbic acid.

Antimicrobial activity

Antimicrobial activity of seed extract was determined by agar well diffusion method using Streptomycin as positive control. The test compound was dissolved in DMSO to obtain the stock concentration of 1000µg/ml. The antimicrobial spectrum evaluated against *Escherichia coli*, *Pseudomonas aeruginosa* and *Serratia marcescens are* shown in Table.2. Several other species of *Heracleum* showed antimicrobial activities. *In vitro* evaluation of antimicrobial activity of hydro-distilled oil of *Heracleum thomsonii* showed that the oil exhibited moderate to high antimicrobial activity[21].The zone of inhibition of the seed extract was comparable with that of standard drugs.

Table 2. Antibacterial activity of the methanol extract of seeds of *H. candolleanum*

Bacteria	Zone of inhibition (cm)			
	DMSO	Streptomycin	Methanol extract of seed	
			50µl	100µl
Escherichia coli	-	4	1.2	1.4
Pseudomonas aeruginosa	-	2.3	1.5	1.8
Serratia marcescens	-	3.5	1.1	1.4

Note: DMSO – Dimethyl sulphoxide (negative control), Streptomycin – positive control (values are the average of results from three repeated experiments).

The free radical scavenging ability of *H. candolleanum* expressed its antioxidant capacity. Plants can be utilized as a source of natural antioxidant compounds as their crude extracts exhibit antioxidant activity[22]. The sensitivity against *Escherichia coli*, *Pseudomonas aeruginosa* and *Serratia marcescens* proved its antimicrobial activity. On the basis of these results, it could be deduced that the seed extract of *H. candolleanum* contained principles that were capable of scavenging free radicals and can also be an effective antimicrobial drug. The plant could, therefore, be regarded as a natural antioxidant and antimicrobial agent.

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Scientia
Vol. 8, No.1, Jan - Dec 2012, pp 72 - 80

ISSN: 0976-8289

A Computational Study on the Enolization of Acetone – Reaction Mechanism and Substituent Effects

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Abstract

The enolization of acetone is studied in the gas phase which involves an intra-molecular proton transfer. The calculations were carried out first at HF/6-31G*, reoptimized at B3LYP/6-311++G** and then extended to MP2/6-31G* level of theory. MP2 method has been found to give better results than RHF and B3LYP methods. All the methods are in agreement with the fact that the aceto-form is stable than enol form. An interplay of various factors such as the inductive effect, resonance, hydrogen bonding, spatial arrangement and steric effects of the substituents are found to affect the energetics of keto-enol equilibria. The mechanism of enolization remains the same throughout irrespective of the nature of substituents. In general, –I groups stabilize the transition state which is supported by Mulliken charge distribution. The energy barriers of gaseous state enolisation of acetone and substituted aceto-forms were found to be higher, all greater than 60kcal/mol.

Key words: Enolisation; Hartree-Fock theory; tautomerism; substituent effects

Introduction

Two or more labile isomeric forms of a compound existing in equilibrium are called Tautomers[1]. Keto-enol tautomerism is one of the most commonly studied forms of prototropic tautomerism[2]. This interconversion occurs in different tautomeric systems containing one or more carbonyl groups linked to sp3- carbons bearing one or more hydrogen atoms. It was observed that while phosphoenolpyruvate has high phosphate transfer potential because stable keto form is formed on dephosphorylation, rare enol tautomers of the bases guanine and thymine can lead to mutation because of their altered base-pairing properties[3]. Rate of enolisation is slow in neutral medium but accelerated by acid or base catalysis. The enol form has a major role in many of the organic reactions. The intra-molecular proton transfer especially in keto-enol prototropy may occur for isolated molecules in the vapor phase at a very low pressure or for molecules at high dilutions in aprotic solvents.[4] Tautomeric preferences in the gas phase often differ from preferences observed in aqueous solution or in the solid state[2,5]. This great variability of tautomeric preferences is usually due to very complex internal and external effects that influence the tautomerization process.

A few computational studies about the enolisation of acetone have been reported so far. Studies on keto-enol tautomerism, both in the gas phase and in the presence of water by the ab initio metadynamics method

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gave interesting results on the mechanism of tautomerism[6]. When acetone is solvated by neutral water, an intermolecular mechanism was observed and the simulations reveal that

C-deprotonation is the kinetic bottleneck of the keto–enol transformation, in agreement with experimental observations. Here the short H-bonded chains of water molecules provide the route for proton transfer from the carbon to the oxygen atom of acetone[6]. The enolization enthalpies at 0 and 298 K for the aliphatic carbonyl compounds were found to be 9-24 kcal/mol[8] by ab initio CBS-4 (complete basis set fourth-order) model calculations. An insight into the mechanistic aspects of enolization and the substituent effects would surely enrich many areas of chemistry. The present study which involves a detailed investigation into the gas phase mechanism and substituent effects of acetone is an instigating effort in this direction. The energy barriers for keto-enol tautomerization of acetone at RHF, B3LYP and MP2 levels are computed and the effect of electron withdrawing and donating substituent at á-C on enolisation at MP2 level are studied here. All the systems were studied at 298.15K and 1atm pressure.

Materials and methods

Computational methodology

All the calculations were performed with 'Gaussian 03' version. Acetone, its enol form and transition state were first optimised using 6-31G(d) basis set at Restricted Hartree–Fock level. The geometries were further confirmed by performing vibrational frequency calculations at the same level. RHF method is based on the Variation Theorem where the approximate energies calculated are always greater than the exact energy. Basis set used provide crude energy value devoid of correlation energy. Therefore better hybrid HF-DFT method, B3LYP, which considers exchange functional suggested by the Becke and the correlation functional by Lee, Yang and Parr, was used. The optimisation of RHF geometry of acetone using 6-311++G** basis set at B3LYP level yielded a structure with one imaginary frequency on vibrational frequency analysis. The geometry of acetone obtained has been observed to be different from the RHF optimised geometry. An examination on the experimentally reported structure of acetone shows that RHF geometry is the correct one, though it gave poor energy values. Therefore, MP2 /6-31G* was performed at RHF optimised geometry followed by vibrational frequency analysis. Therefore, substituent effects were analyzed by computing monosubstituted systems at MP2/6-31G* level. The computed geometrical parameters of optimised structures and energetics were tabulated for each of the substituted systems and that of acetone. Different molecular systems considered for the present work are shown below. For better understanding, the following labels are used.

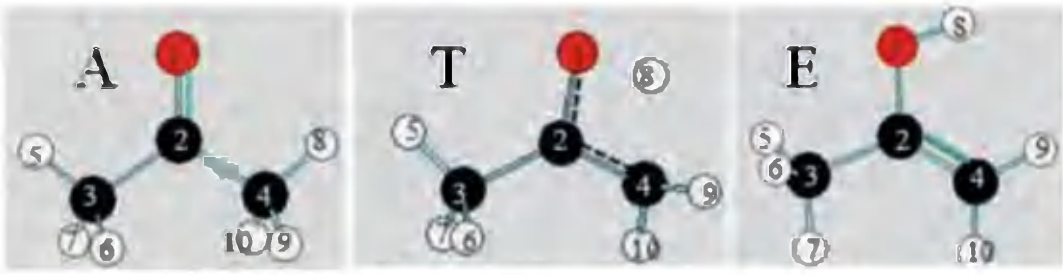

Fig. 1. Geometries of Acetone, its transition state and enol.

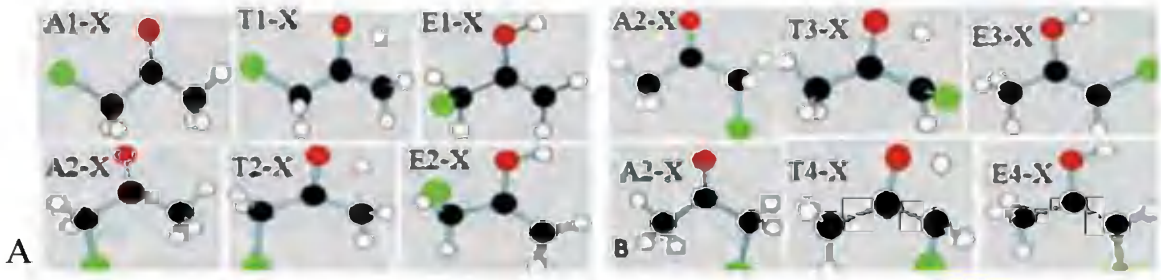

Fig. 2. Different conformation of acetone, corresponding transition structures and enols considered for the study. (X = Cl, F, OH, OMe, Me, COOH, NO_2, CN & NH_2).

A) Enolization from un-substituted carbon

B) Enolization from substituted carbon

The parent systems acetone, its enol and corresponding transition states are labelled as A, E and T for convenience. The different labels employed for the substituted systems are also shown in Fig. 2. 'X' represents the substituent. Activation energy (**ÄE***) for enolisation, **ÄE***=E(transition state) - E(aceto form) and Relative energy, **RE** = E(enol form) - E(aceto form). The unit of energy calculated by the program is Hartree/particle. 1Hartree/particle = 627.50919 kcal/mol.

Results and Discussion

The geometrical parameters of optimised A, E and T are given in Table -1. The atom numberings are shown in fig.3. During the enolisation process, (A) transformed into a transition structure, in which one of the planar H8 is nearly transferred to the carbonyl oxygen, O1. In the transition structure, C2-O1 bond has been elongated and has lost its double bond character. The C4-H8 bond is elongated (starts breaking up) where as O1-H8 bonds has started forming in the optimised T geometry. The product of enolisation, E has a shortened C2-C4 bond and a newly formed O-H bond.

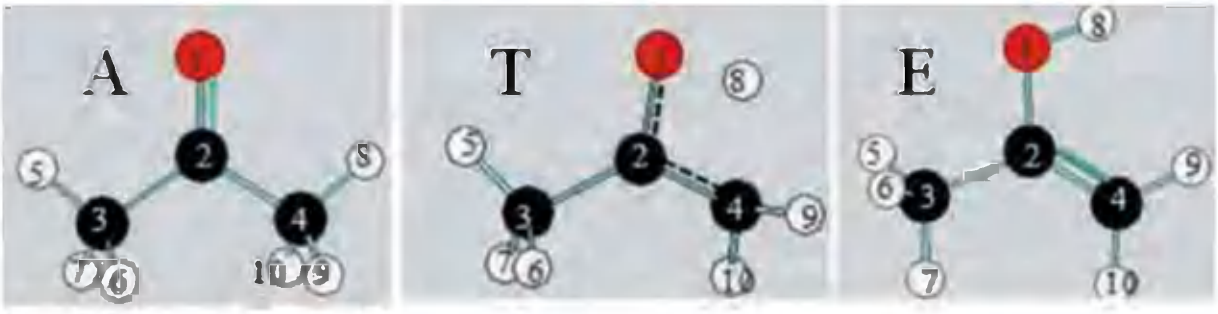

Fig. 3. Numbering of A, E and T.

In T, C2-O1 bond length is 1.257 and 1.300 A^0 at RHF and MP2 levels respectively, which shows that the bond between carbonyl carbon and carbonyl oxygen gets elongated. In A, this bond length is 1.192 and 1.227 respectively, which shows that C2-O1 bond gets shortened. More or less the same C2-C3 distance by all the three methods shows that this bond remains unchanged in the transition state. H8 is in an unbound state in optimised geometry of T and is closer to O1 than to C4 at all the three levels of theory. The energetics of acetone-enol tautomerism is summerised in Table 2.

Table 1. Optimized geometrical parameters of acetone, its transition structure and enol at HF/6-31G*, B3LYP/6-311++G**;and MP2/6-31G* level of theory. * B3LYP level of theory with the given basis set fails in the case of acetone. # The geometrical parameters in the column is insignificant.

Structure	Symmetry	Method	Bond length (A^0)					Bond angle (^{0})		
			C2-O1	C2-C4	C2-C3	O1-H8	C4-H8	C3-C2-O1	C4-C2-O1	C2-O1-H8
A	C2v	RHF	1.192	1.513	1.513	#	1.081	121.668	121.668	#
		B3LYP	*	*	*	*	*	*	*	*
		MP2	1.227	1.513	1.513	#	1.090	121.710	121.711	#
T	C1	RHF	1.257	1.433	1.491	1.218	1.510	121.269	107.441	80.540
		B3LYP	1.286	1.424	1.490	1.279	1.496	120.959	108.653	77.897
		MP2	1.300	1.415	1.490	1.277	1.511	120.291	109.24	77.255
E	Cs	RHF	1.353	1.321	1.497	0.947	#	110.627	124.037	110.347
		B3LYP	1.354	1.321	1.497	0.948	#	110.618	124.019	110.348
		MP2	1.376	1.340	1.494	0.974	#	110.304	124.064	108.088

Table 2. Electronic energies and relative energies (kcal/mol) of A, T and E at various levels of theory. # Activation energy (ΔE*) for keto-enol tautomerism. * Relative energy **B3LYP/6-311++G** gives one imaginary frequency for A

Method	Basis set	Structure	Electronic energy (Kcal/mol)	E*/RE($K_{cal/mol}$)
RHF	6-31G(d)	Acetone	-120458.0672	0
		T.s.	-120369.141	88.93#
		Enol	-120439.3091	18.76*
B3LYP	6-311++G**	Acetone	**	
		T.s.	-121178.2727	
		Enol	-121233.961	
MP2	6-31G*	Acetone	-120702.6634	0
		T.s.	-120634.6502	68.01#
		Enol	-120683.8494	18.81*

As expected, in all the methods A is found to be more stable than E. The activation energy for aceto-enol tautomerism is 88.93 kcal/mol by RHF method and 68.01 kcal/mol by MP2 method in gaseous state with 6-31G* basis set. This value obtained is very close to the energy barrier reported using MP2/6-31G* for the gas phase intramolecular enolisation process, which is 69.17kcal/mol (for water-catalysed process, it is 43.08 kcal/mol only)[6,7]. The energy barrier reported using MP2/631G* for the gas phase intramolecular enolisation is 69.17kcal/molB3LYP method with higher basis set shows one imaginary frequency for the experimental geometry of acetone as explained earlier and hence is not considered here. In all the three methods, E is always less stable than A. RHF energy barrier for T is greater than that of MP2 by 20.91 kcal/mol. It has to be noticed that RHF method gives a very high energy barrier than MP2. This may be due to the fact that the RHF level does not incorporate exchange or correlation energy while evaluating the integrals. MP2 method explains the energetics better than the other two. Thus, it could be concluded that MP2 method gives reliable results both for the energetics and the geometry for enolisation.

Substituent Effects

a. Geometry

Enolisation from un-substituted Carbon (Fig. 2.a.)

The O1-H8 distance in E1-X is same (0.974A^0) in all systems which is also equal to that of E. The C3-X bond gets slightly elongated during the enolisation of A1-X. O1-H8 bond length in T is 1.277 A^0. In T2-X, where, X is an electron withdrawing group (CN, F, Cl, COOH and NO_2) and OH or OMe, O1-H8 bond length is higher, which weaken the O1-H8 interaction in the transition state. +I groups, NH_2 and Me have

no considerable effect on the geometry of T2-X. In T1-F, the C3-F bond is shorter than that in A1-F and E1-F. This shows that the F atom is closer to the enolic O which may result in a more polar transition state. Similar result is shown by A2-F / E2-F system also.

Enolisation from substituted Carbon (Fig. 2.b.)

In T3-X and E3-X, the substituent is closer to the enolic group. For T3-CN, T3-COOH and T3-NO_2 the C2-O1 bond length is 1.284, 1.284 and 1.289 A^0 respectively. These values are lower than that of T and other T3-X species. O1-H8 distance in E3-X is in the range of0.973-.979 A^0 except for E3-NO_2, E3-COOH and E3-NH_2 (0.995, 0.982, 0.987 A^0 respectively) which shows that steric effect of these groups have a considerable effect on enol geometry. C2-C4 distance of T4-CN, T4-NO_2 and T4-COOH (1.438, 1.440 and 1.437 A^0 respectively) are found to be higher than other systems due to steric strain of these groups. A similar effect is seen for their enol forms. O1-H8 distance in T is 1.277 A^0. In T4-X, where X is electron donating groups such as OH, OMe, NH_2 and Me, the O1-H8 distance is considerably higher than that of T, whereas, when X is –I groups including F, this bond is shorter than that of T. So transition structure with respect to O1-H8 bond is stabilised by –I groups inT4-X. F-substituted systems show similar results to that of parent system except for the C2- CF bond distance.

b. Energy barriers and Relative energies

Table 3. Activation energies of substituent systems (in kcal/mol).

System	E*	System	E*	System	E*	System	E*
T	68.01	T	68.01	T3-NO_2	64.84	T4-C0OH	64.45
T1-Cl	68.61	T2-Me	73.90	T3-C0OH	66.06	T4-NO_2	64.84
T1-NO_2	68.99	T2-Cl	75.77	T	68.01	T4-CN	64.86
T1-C0OH	69.13	T2-OMe	76.02	T3-CN	69.32	T	68.01
T1-CN	69.35	T2-OH	76.03	T3-Me	77.19	T4-Cl	70.57
T1-OH		T2-NH_2	76.09	T3-Cl	79.75	T4-NH_2	70.69
T1-NH_2		T2-C0OH	76.38	T3-NH_2	80.52	T4-OH	72.20
T1-OMe		T2-F	76.43	T3-OH	84.44	T4-Me	73.01
T1-F		T2-NO_2	76.45	T3-OMe	84.96	T4-OMe	73.17
T1-Me		T2-CN	76.54	T3-F	89.03	T4-F	75.65

Table 4. Relative Energy of substituted systems (in kcal/mol).

System	RE	System	RE	System	RE	System	RE
E1-NO_2	16.72	E2-Me	17.37	E3-NO_2	4.65	E4-CN	10.13
E1-Cl	17.64	E2-NO_2	17.47	E3-COOH	5.99	E4-COOH	12.86
E1-CN	18.02	E2-Cl	17.70	E3-CN	7.86	E4-NO_2	14.44
E1-COOH	18.33	E2-CN	17.72	E3-NH_2	9.47	E4-NH_2	16.14
E	18.81	E2-COOH	18.37	E3-Cl	12.34	E4-Cl	16.48
E1-OH		E2-NH_2	18.39	E3-OH	12.44	E4-Me	17.08
E1-NH_2		E	18.81	E3-OMe	13.01	E4-OH	17.25
E1-OMe		E2-F	19.86	E3-F	15.92	E4-OMe	17.54
E1-F		E2-OMe	20.23	E3-Me	16.20	E	18.81
E1-Me		E2-OH	20.50	E	18.81	E4-F	19.41

Activation energies ÄE* for A1-NO_2, A1-COOH & A1-CN are very close to each other, ranging from 68.99 to 69.35 Kcal/mol. These values are higher than that of A (68.01 Kcal/mol). This slight increase may be due to the steric effects offered by these groups since they are at the same plane of C2, C3 and O1. An imaginary frequency is shown by A1-X where X is OH, OMe, F, NH_2 and Me. E1-F and E1-Me also show imaginary frequencies. Except for T2-Me and T2-Cl all other T2-X shows very closer ÄE* values. The range of ÄE* for T2-X series is 73-76 kcal/mol whereas that of T is 68.01 kcal/mol. The energy barrier of T is lowest in both T2-X series and T1-X series. In general, T2-X systems have comparatively higher activation energies than T1-X systems when X is an electron withdrawing group.

Enolisation from substituted Carbon (Fig. 2.a.)

T3-X with –I substituents have comparatively lower energy barrier than that with +I substituents. Electron withdrawing groups such as NO_2, COOH and CN in the C4-carbon will decrease the activation energy of the system. T3-NO_2 and T3-COOOH have lower ÄE* than T and their enols are the most stable enols among all systems studied in this work. This is due to the resonance effect and H-bonding. T4-X with –I substituents have least activation energy that those with +I groups. In E4-X series, the lowest relative energy of enol form is 10.14Kcal/mol for E4-CN which shows an exceptional stability due to the delocalisation of electron density between C3 and CN. ÄE* of T3-F is 89.04 kcal/mol whereas, in T4-F, it is 75.65 Kcal/mol. The exceptionally high energy barrier of T3-F and T4-F is attributed to electrostatic repulsive forces operating between the highly electronegative F and enolic O which is more strong in T3-F since F is more close to enolic O. The enol forms are also destabilised by F.

Mulliken charge distributuion

The magnitude of negative charge density on C3, C4 decreases during enolisation but that on O1 increases from -0.538 to -0.743. H8 is in an unbound state and has more positive charge density. In E, the positive charge density on carbonyl carbon is reduced than in A. It is thus clear that a low energy transition state is possible when the substituent can disperse the negative charge on C4 or on C3. –I groups withdraw electron density from these carbons and hence stabilise the transition state geometry to a greater extent than the +I substituents. Electron-donating substituents will enhance the electron density at á-carbon which will destabilise the transition state.

Table 5. Shows the Mulliken charge distributuion of A, T and E.

	Atom									
	O1	C2	C3	C4	H5	H6	H7	H8	H9	H10
A	-0.54	0.531	-0.575	-0.575	0.211	0.184	0.184	0.211	0.184	0.184
E	-0.74	0.44	-0.515	-0.53	0.188	0.188	0.177	0.435	0.162	0.181
T	-0.69	0.586	-0.572	-0.776	0.214	0.212	0.201	0.425	0.227	0.174

An imaginary frequency is shown by A1-X where X is OH, OMe, F, NH_2 and Me as well as E1-F and E1-Me. This is due to the fact that electron donating substituents will enhance the negative charge density on C3. The electrostatic repulsion between the +I group and electron rich carbonyl group also contribute to the formation of high energy transition state since, substituent is closer to C=O in A1-X. The same type of electrostatic repulsion operates in A1-F and E1-F. The range of ÄE* for T2-X series is 73-76 Kcal/mol whereas that of T is 68.01 kcal/mol. So, inductive effect of substituent does not have a major role in determining the energy barrier in T2-X series, while the steric effects and electrostatic repulsive forces presumably have a predominant role here. The electron-withdrawing group attached to C4 lowers not only the activation energy of transition state but also the relative energy of enol form by dispersing the excess negative charge density on C4.

Conclusions

Four different conformations of á-monosubstituted enol forms and their respective transition state and aceto forms are constructed with both electron withdrawing and donating substituents. Geometry and energetics of enolisation of acetone and á-substituted acetones were calculated at MP2/6-31G* level of theory. All systems were studied in the gaseous state at 298.15 K and 1 atm pressure. Energy barrier for enolisation of acetone is 68.01 kcal/mol and the relative energy of enol formed is 18.81 kcal/mol. The inductive effect, resonance, hydrogen bonding and the spatial arrangement and electrostatic interactions of the substituents are found to affect the energetics of keto-enol equilibria. In all the substituted keto forms, the C=O length remains almost the same. Changes are observed only at transition state and enol geometries. Electron-withdrawing group lower the energy barrier for enolisation except in A2-X series. The enol-forms with highest relative energies are those with electron-donating substituents irrespective of the conformation.

Acknowledgements

JEG acknowledges CMSD, Hyderabad for providing the computing time to perform the Gaussian calculations.

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Scientia
Vol. 8, No.1, Jan - Dec 2012, pp 81 - 86

ISSN: 0976-8289

On gracefulness of join of graphs

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Abstract

Graceful labelings of graphs have always fascinated researchers as they have not only theoretical applications, but also so many practical life applications. This paper deals with the gracefulness of the join of graphs. It is clearly demonstrated that the graphs $P_n+P_2 (n\geq 1)$ and $S_n+K_2 (n\geq 1)$ are graceful.

Keywords: Graceful labeling, Join of graphs, Graph $P_n+P_2 (n\geq 1)$, Graph $S_n+K_2 (n\geq 1)$, applications.

Introduction

The research topic of labeling of graphs has been receiving increased attention worldwide[1]. Many studies on graceful labeling of graphs have been conducted by talented and studious mathematicians all over the world and they have made breakthrough not only in proving complex theorems, but also in proposing new applications and thus identifying unusual opportunities to make use of them in many practical applications of day today life.

A graceful labeling of a graph G with m edges is a function f: V(G)→{0, 1, 2,.....m} such that distinct vertices receive distinct numbers and {| f (u) – f(v) | : uv ? E(G)}={1, 2,.....m}.A graph is graceful if it has a graceful labeling[2]. In order for a graph to be graceful, it must be without loops or multiple edges[3].

Let G_1 and G_2 be two graphs with disjoint point sets V_1 and V_2 and sets E_1 and E_2 The join of G_1 and G_2 defined by Zykov is denoted by G_1+G_2 and consists of $G_1 \cup G_2$ and all edges joining V_1 with V_2 (union $G = G_1 \cup G_2$ has $V=V_1 \cup V_2$ and $E=E_1 \cup E_2$). This is illustrated in (Fig.1) as an example. If $G_1 = K_2 = P_2$ and $G_2 = K_{1,2} = P_3$, G_1+G_2 is as shown below.

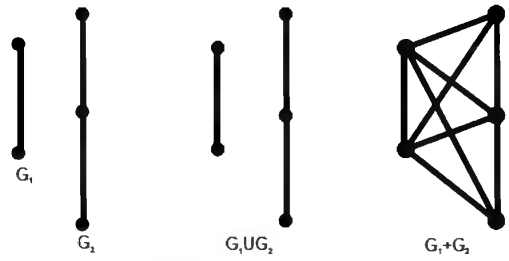

Fig.1. Join of grap

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Materials and methods

Two specific families of graphs are obtained from the particular join of graphs. Further, it is proved and demonstrated through examples that for $n \geq 1$, the graph P_n+P_2 and the graph S_n+K_2 are graceful.

Results and Discussion

Researchers have investigated graceful labeling on various specific families of graphs [4,5]. We show that few classes of join of graphs are graceful.

Theorem 1

For $n \geq 1$, the graph P_n+P_2 is graceful.

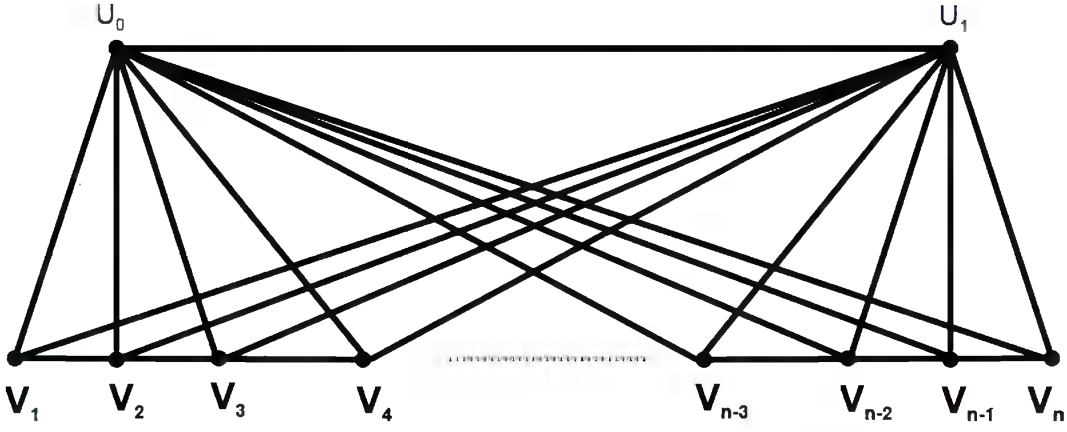

Fig.2. Graph $P_n + P_2$

Proof:

Observe that, $|V(P_n+P_2)| = n+2$, $|E(P_n+P_2)| = 3n$.

Let $m = |E(P_n+P_2)|$. Define $\phi : V(P_n+P_2) \rightarrow \{0, 1, 2, \ldots\ldots m\}$ by $\phi(u_0)=0, \phi(u_1)=m$

$$\phi(v_i) = 3\frac{(i+1)}{2} - 2, \text{ for } 1 \leq i \leq n \text{ and } i \text{ odd}.$$

$$\phi(v_i) = m+1-(3i/2), \text{ for } 1 \leq i \leq n \text{ and } i \text{ even}.$$

It is clear that the vertex labels $\phi(v_i)$'s are distinct, for $1 \leq i \leq n$. Let A be the set of edges of P_n+P_2 which are adjacent to u_0,

i.e., $A = \{u_0 v_i \in E(P_n+P_2)$ and $u_0 u_1 \in E(P_n+P_2)$, for $1 \leq i \leq n\}$

Let B be the set of all edges of P_n+P_2 which are adjacent to u_1 but not in A, *i.e.*, $B = \{u_1 v_i \in E(P_n+P_2)$, for $1 \leq i \leq n\}$

Let C be the set of all edges of P_n+P_2 which are in the path P_n, *i.e.*,C= $\{v_i\ v_{i+1} ? E (P_n+P_2)$, for $1\leq i \leq n-1\}$

Let A', B', C' denote sets of edge labels of the edges in the sets A, B, C respectively. Observe that

A'= {m, m-2, m-5, m-8,.....7, 4, 1}

B'= {m-1, m-4, m-7, ...8, 5, 2}

C'= {m-3, m-6, m-9,.....9, 6, 3}

It follows that edge labels in the sets A', B', C' are distinct and

A'∪B'∪C' = {1, 2,... m-1, m}.Hence P_n+P_2 is graceful.

An illustrative example : $P_{10}+P_2$ is graceful.

Observe $|V (P_{10}+P_2)| = 12$, $|E (P_{10}+P_2)| = 30 = m$, $n = 10$.

Define ϕ: $V(P_{10} + P_2) \rightarrow \{0.1.2.....30\}$ as $\phi (u_0) = 0.\phi (u_1) = 30$

$\phi (v_i)$, when i is odd

$\phi (v_1) = 1$, $\phi (v_3) = 4$, $\phi (v_5) = 7$, $\phi (v_7) = 10$, $\phi (v_9) = 13$

$\phi (v_i)$: when i is even

$\phi (v_2) = 28$, $\phi (v_4) = 25$, $\phi (v_6) = 22$, $\phi (v_8) = 19$, $\phi (v_{10}) = 16$

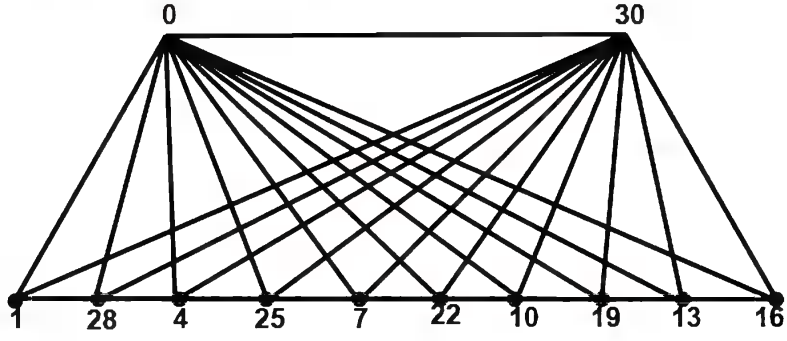

Fig.3. Graph $P_{10} + P_2$

Vertex labels are distinct.

$A=\{u_0v_1, u_0v_2, u_0v_3, u_0v_4, u_0v_5, u_0v_6, u_0v_7, u_0v_8, u_0v_9, u_0v_{10}, u_0u_1\}$

$B=\{u_1v_1, u_1v_2, u_1v_3, u_1v_4, u_1v_5, u_1v_6, u_1v_7, u_1v_8, u_1v_9, u_1v_{10}\}$

$C=\{v_1v_2, v_2v_3, v_3v_4, v_4v_5, v_5v_6, v_6v_7, v_7v_8, v_8v_9, v_9v_{10}\}$

A' = {30, 28, 25, 22, 19, 16, 13, 10, 7, 4, 1}

B' = {29, 26, 23, 20, 17, 14, 11, 8, 5, 2}

C' = {27, 24, 21, 18, 15, 12, 9, 6, 3, 0}

Edge labels A', B', C'are disjoint.A'∪B'∪C'={1, 2, ...29,30}.

$P_{10}+P_2$ is graceful.

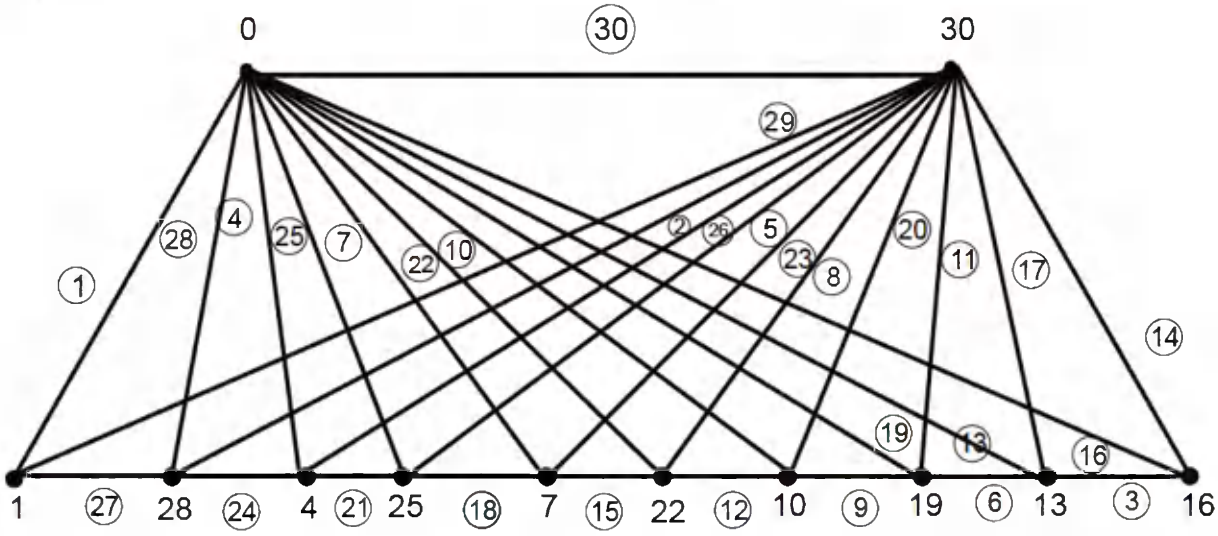

Fig.4. Graceful labeling of $P_{10}+P_2$

Theorem 2

For n≥1, the graph S_n+K_2 is graceful.

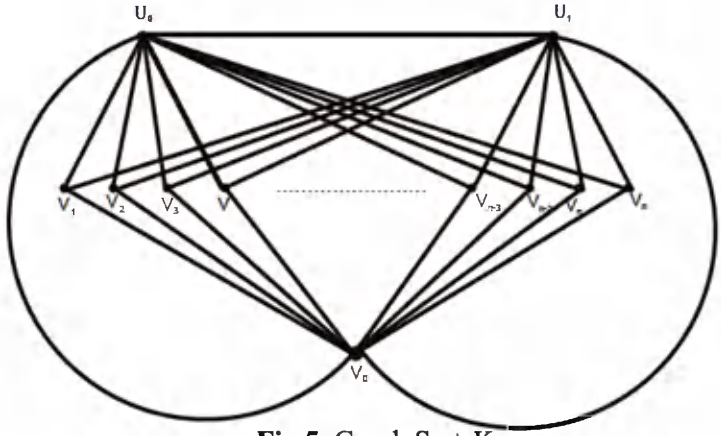

Fig.5. Graph S_n+K_2

Proof:

Observe that $|V(S_n+K_2)|= n+3$. $|E(S_n+K_2)|=3n+3$.

Let $m=|E(S_n+K_2)|$.Define $\phi: V(S_n+K_2) \rightarrow \{0, 1, 2...m-1,m\}$ by

$\phi(u_0) = m$, $\phi(u_1) = m-n-1$, $\phi(v_0)=0$ and $\phi(v_i)=1$ for $1\leq i \leq n$.

Vertex labels $\phi(u_0)$, $\phi(u_1)$, $\phi(v_0)$, $\phi(v_i)$'s are distinct. for $1\leq i \leq n$.

Let A be set of all edges of S_n+K_2 which are adjacent to v_0, *i.e*

$A= \{v_0u_0, v_0u_1, v_0v_1, v_0v_2, \ldots\ldots\ldots v_0v_n\}$.

Let B be the set of all edges of S_n+K_2 which are adjacent to u_0, but not in A. *i.e* $B= \{u_0u_1, u_0v_1, u_0v_2, u_0v_3, \ldots\ldots\ldots u_0v_n\}$.

Let C be the set of all edges of S_n+K_2 which are adjacent to u_1, but not in A and not in B, *i.e* C= $\{u_1v_1, u_1v_2, u_1v_3, \ldots\ldots\ldots\ldots\ldots u_1v_n\}$.Let A', B', C' be the sets of edge labels of the edges in the sets A, B, C respectively.

A' = {m, m-n-1, 1, 2, 3,.....n-1, n}

B' = {m-1, m-2, m-3,.....m-n, n+1}

C' = {m-2n-1, m-2n, m-2n+1, m-2n+2,....., m-n-3, m-n-2}

The edge labels in the sets A', B', C' are distinct and A'∪B'∪C' = {1, 2, 3,.....m-1, m}.Hence S_n+K_2 is graceful.

An illustrative example : S_6+K_2 is graceful

Observe that $|V(S_6+K_2)|= 9$, $|E(S_6+K_2)|=21$.Let m= 21, n= 6.

Define $\phi: V(S_6+K_2) \rightarrow \{0, 1, 2, \ldots\ldots\ldots\ldots 21\}$ by

$\phi(u_0)=21, \phi(u_1)=14, \phi(v_0) = 0, \phi(v_1) = 1, \phi(v_2) = 2,$

$\phi(v_3) = 3, \phi(v_4) = 4, \phi(v_5) = 5, \phi(v_6) = 6.$

Vertex labels $\phi(u_0), \phi(u_1), \phi(v_0), \phi(v_1), \phi(v_2), \phi(v_3), \phi(v_4), \phi(v_5),$

$\phi(v_6)$ are distinct.

Let A be t he set of all edges of S_6+K_2 which are adjacent to v_0, *i.e* A = $\{v_0u_0, v_0u_1, v_0v_1, v_0v_2, v_0v_3, v_0v_4, v_0v_5, v_0v_6\}$

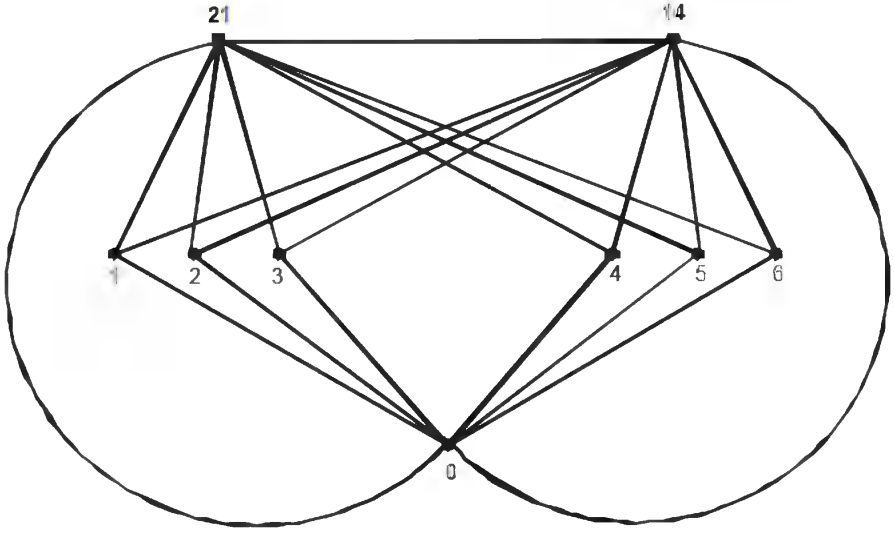

Fig.6. Graph $S_6 + K_2$

Let B = $\{u_0u_1, u_0v_1, u_0v_2, u_0v_3, u_0v_4, u_0v_5, u_0v_6\}$

Let C= $\{u_1v_1, u_1v_2, u_1v_3, u_1v_4, u_1v_5, u_1v_6\}$

Let A', B', C' be sets of edges labels in the sets A, B, C.

A' = {21, 14, 1, 2, 3, 4, 5, 6}.B' = {20, 19, 18, 17, 16, 15, }.C'={8, 9, 10,11,12,13}and A'∪B'∪C' ={1,2,3,.....20,21}.Thus S_6+K_2 is graceful.

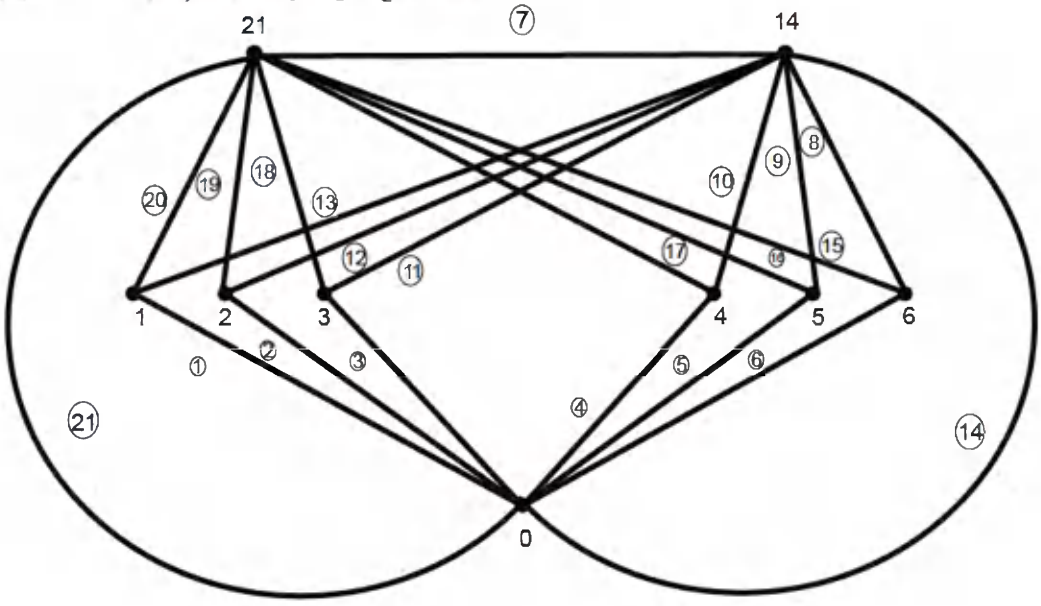

Fig.7. Graceful labeling of $S_6 + K_2$

Conclusion

It is clarified that join of graphs $P_n + P_2$ and $S_n + K_2$ is graceful. Graphs $P_{10} + P_2$ and $S_6 + K_2$ are graceful. Also graceful labeling is a useful branch of labeling as it has many important applications in the modern world like Optical MPLS (Multi Protocol Label Switching) networks, coding in computer programmes and so on. Graceful graphs are related to many mathematical topics including Golomb rulers, permutation and other graph labeling problems. They have practical applications such as radio astronomy, crystallography etc. So we conclude with the note that the study on graceful labeling remains relevant for the times.

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Scientia
Vol. 8, No.1, Jan - Dec 2012, pp 87 - 94
ISSN: 0976-8289

Rediscovery of seven 'possibly extinct' plants from the Shola forests of kerala state'

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Abstract

Seven endemic plants categorized as 'Possibly extinct' were rediscovered from the Shola (Tropical Montane) forests of Kerala State as a result of detailed floristic expeditions for more than 1½ decades. The study also yielded about 669 taxa of flowering plants out of which, 246 are endemics, 48 belonging to various threat categories and 49 being new distributional records to Kerala. The study areas included major shola forest regions of Idukki High ranges in Kerala such as Mannavan Shola (the largest shola forest of the state) Pambadam Shola, Pullaradi Shola, Idivara Shola, Sholas of Eravikulam National Park, Vellari Mala Shola of Wayanad etc. Detailed taxonomic description, distributional status and notes on each species are given. Need for continued exploration in the sholas and conservational importance of the area are discussed.

Key words: Possibly extinct, Rediscovery, Conservation, Tropical Montane, Shola forest, Kerala

Introduction

'Shola (Tropical Montane) forests are evergreen forests situated in the high altitude regions in the Western Ghats, above 1500 m. They are the continuation of the Tropical Wet Evergreen Forests' in the higher altitudes. They are found mainly in the sheltered ravines, troughs, hollows and other depressions, where there is abundance of moisture, and are occasionally seen 'flowing' to the valleys along with the streams. These forests have high ecological significance in protecting the head waters of rivers by holding up of water received by precipitation like a sponge and thus preventing rapid run off. The trees are stunted, profusely branched, without a straight bole and with an umbrella shaped canopy. The crooked branches are densely covered with epiphytic mosses, ferns, lichens and orchids. The species are basically of a tropical stock. But, temperate species dominate in the forest ecotones.

In the state of Kerala, typical shola forests can be located in Eravikulam (*Idukki*), Devarmala (Pathanamthitta), Agasthyamala (*Thiruvananthapuram*), Sispara Ghat (*Palakkad*), New Amarambalam reserve forest (*Malappuram*), Vellarimala sholas (*Kozhikode*) and Brahmagiri hills (*Wayanad*). Some of these regions are actually extensions of the forest types of Tamilnadu state, from the Nilgiris, Pulneys or Anamalai hills. However, it is to be noted that majority of shola forests are distributed in the '*High ranges*' of Idukki district and even the largest shola forest of the state, namely Mannavan Shola (presently under

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Anamudi Shola National Park), is located in this region (Kumar, 2004). Apart from the few areas that are today protected as sanctuaries, national parks and reserve forests, the remaining areas have been deforested or replaced with plantations of various sorts. In total, the area under shola-grassland vegetation in Kerala is estimated to be approximately 70 km^2. (CESS, 1984)

Very few studies have been conducted in the shola forests of Kerala, perhaps due to their remote occurrance and unfavourable climates. A comparable floristic account of the shola forests of Kerala is due to the pioneering studies of Sebastine and Vivekananthan (1967) and Shetty & Vivekananthan (1968, 1970. 1971, 1972, 1973, 1975 and 1991). Rice (1984), Jose *et al.* (1994), Karunakaran (1997) etc have added further documentation on the plant wealth of the region. But since most of these studies were concentrated mainly on the ecological aspects, botany of the region remained scanty. Later, Swarupanandan *et al.* (1998) conducted some studies on the floristic and ecological aspects of a few sholas of the High Ranges of Idukki district, which was later, broadened to other areas of the state by Kumar (2004) as a continuation of the research project.

Materials and Methods

The study areas included major shola forest regions of Idukki High ranges in Kerala such as Mannavan Shola (the largest shola forest of the state) Pambadam Shola, Pullaradi Shola, Idivara Shola, Sholas of Eravikulam National Park, Vellari Mala Shola of Wayanad etc. From 1994 onwards, regular field trips were organized to the various study areas for collecting plant specimens. The study thus yielded about 669 taxa of flowering plants out of which, 246 were endemics, 48 belonged to various threat categories and 49 were new distributional records to Kerala. All the specimens were deposited in *Kerala Forest Research Institute* (KFRI) Herbarium, Peechi. The specimens were critically studied using the available literature and herbaria. Apart from those collected, other specimens from the study area available at MH, CALI and KFRI herbaria were also referred.

Result and Discussion

Enumeration of the taxa

The species are given in the alphabetical order. The local names if any and the family names are given after the citations.

Actinodaphne bourneae Gamble, Kew Bull. 1925: 128. 1925 & Fl. Pres. Madras 1231. 1925; Fyson, Fl. South Indian Hill Stat. 505. 1932; Matthew, Ill. Fl. Palni Hills t. 605. 1996 & Fl. Palni Hills 1065. 1999. (Family: *Lauraceae*)

Medium sized trees. Leaves to 15 x 6 cm, elliptic-lanceolate, base acute, apex acuminate; branchlets, petiole, nerves and pedicels fulvous tomentose. Flowers 3-6, in sessile lateral clusters; perianth yellowish, 5 mm wide, tube 4 mm, lobes 6, ovate. Berry globose.

Fl. & Fr.: January-February.

Distr.: Endemic to Southern Western Ghats. Occasional in high altitude sholas above 2000 m. Eravikulam, Anamudi slope, *KKK 18546.*

Notes: New record to the flora of Kerala. It was collected by Gamble from the sholas of Palni hills/ Kodaikanal. Later it could not be collected during this century and was considered extinct (Nayar, 1997).

Arisaema attenuatum Barnes & Fischer, Bull. Misc. Inform. Kew 1936: 275. 1936; Chatterjee, Bull. Bot. Soc. Bengal 8: 121. 1959. Manilal, Fl. Silent Valley 331. 1988; Sasidh., Fl. Parambikulam WLS 365. 2002. **Pambu Cholam.** (Family: *Araceae*)

Tuberous herbs, dioecious; corms to 3 cm across, oblate. Leaf solitary, petiole slender, to 75 cm long, mottled; leaflets 6-12, to 20 x 5 cm, elliptic-lanceolate to oblanceolate, base tapering, apex acuminate. Tube of spathe cylindric, to 9 x 1.5 cm; limb elongate-lanceolate, to 6 cm long, long acuminate. Spadix to 9 cm, tip acute and curved at right angles and shortly exserted. Male inflorescence 3 cm long, flowers crowded near base; female inflorescence 1.5 cm long, ovaries subglobose.

Fl. & Fr.: February-March

Distr.: Endemic to Southern Western Ghats. Occasional; found in shola borders or scrublands at the altitudes of 1700-2000 m. Mannavan Shola, Chengalar, *KKK 31016;* Eravikulam MPCA, *KKK 17526.*

Note: The species was *treated as 'possibly extinct'* by Shetty & Vivekananthan (1991). *But its collection* from the Chengalar dam region at Mannavan Shola and from the Umayamala valleys (MPCA plots) at Eravikulam National Park confirms its existence. The species was later collected from Parambikulam WLS also (Sasidharan, 2002).

Arisaema psittacus Barnes in Hook., Icon. Pl. ser. 5, 5: t. 3405. 1940; Chatterjee, Bull. Bot. Soc. Bengal 8: 131. 1959; Nair *et al.*, Rheedea 7(2): 101-106. 1997. **Pambu Cholam.** (Family: *Araceae*)

Tuberous herbs, dioecious; corm to 3.5 cm across, subglobose; petiole to 65 cm long, green with purplish mottlings. Leaves digitate compound, leaflets 8-10, to 20 x 5 cm, elliptic-lanceolate, base cuneate, apex caudate-acuminate, dark green above, light and glossy below. Spathe to 18 x 4 cm, tip caudate acuminate, recurved, tube striped with green and white; limb hemispherical to 15 x 4 cm. Spadix 8 cm long; appendix to 5 cm. Male flowers purplish; female flowers deep green; ovaries ovoid.

Fl. & Fr.: May-September.

Distr.: Endemic to Southern Western Ghats. Very rare and threatened species found at 1800-2000 m in shola borders. Mannavan Shola, Idlimottai, *KKK 18180;* Pambadum Shola, Vandaravu, *KKK 18192.*

Note: *The species* could not be relocated for a long time after the type collection by Barnes in 1937 from Chunduvurrai estate regions and Mannavan Shola (Barnes, 1940). It was so considered extinct in its natural habitat. However it could be rediscovered from the same localities by Nair *et al* (1997). I could collect the species from both these locations as well as from Pambadum Shola National Park.

Impatiens anaimudica Fischer, Bull. Misc. Inform. Kew 1935: 92-93. 1935 & Gamble, Fl. Pres. Madras 1871. 1936; Vivek. *et al.* in Hajra *et al.,* Fl. Ind. 4: 117-118. 1997; Biju, Rheedea 11(2): 109-113. 2001. *I. konalarensis* Chandrab. et al., Journ. Bombay Nat. Hist. Soc. 81: 676. 1984. (Family: *Balsaminaceae*)

Herbs, to 20 cm tall; stems terete, reddish green. Leaves rosulate at tip, to 3 x 2 cm, ovate-acuminate, margins with serrations ending in cilia, slightly pilose on veins. Umbels 3-4 flowered. Flowers scarlet red; lip cymbiform, small; spur reduced to a small rounded projection at base; lateral sepals ovate-acuminate; standard orbicular; not spurred; wings with 2 subequal lobes, obtuse; stamens 5; ovary ovate-elliptic. Capsule to 1 cm long, obliquely elliptic, aristate.

Fl. & Fr.: October-December

Distr.: Endemic to Southern Western Ghats. Very rare and endangered; found in grassland ecotones usually above 2000 m altitudes. Eravikulam, Umayamala, *KKK 18015.*

Note: This species was considered as *'possibly extinct'* (Nayar & Sastry, 1988) since it could not be traced out after the type collection by Barnes in 1933 from Anamudi (Barnes & Fischer, 1936; Barnes, 1938), until it was relocated by Biju (2001) from the same locality. Only a single collection could made from Umayamala (Eravikulam NP) after a thorough search, which indicates the rarity of the species.

Impatiens platyadena Fischer, Bull. Misc. Inform. Kew 1934: 393. 1934; Gamble, Fl. Pres. Madras 1871. 1936; Vivek. *et al.* in Hajra *et al.,* Fl. Ind. 4: 193. 1997. (Family: *Balsaminaceae*)

Undershrubs. Leaves alternate, to 15 x 5 cm, elliptic-lanceolate, apex acuminate, crenate-serrate, petiole with 6-8 flattened green tipped white glands on margins. Racemes with drooping peduncles, to 4 cm long. Flowers 1.5 cm long; lateral sepals suborbicular, cream coloured; lip funnel shaped; spur tubular; standard 7 mm long, ovate; wings 15 mm long, distal lobes scarlet. Capsule 6 mm long, ovoid, acute.

Fl. & Fr.: July-October

Distr.: *Endemic to Idukki District of Kerala State. Very rare and endangered; found along stream sides at 1800-2000 m altitudes.* Eravikulam, *Nilagiri Teri, KKK 31089.*

Notes: *This species was considered 'possibly extinct' by Ahmedullah & Nayar (1987) and Hajra et al. (1997). The present collection forms a rediscovery after the type collection by Barnes from the Nyamakkad gap of Idukki District during 1933. The species can be easily identified by the green tipped white glandular hairs on the petiole.*

Pimpinella pulneyensis Gamble, Kew Bull. 228. 1919 & Fl. Pres. Madras 560. 1919; Fyson, Fl. South Indian Hill Stat. 253. t. 201. 1932; Mukh. & Const., Umbell. India 141. 1993; Matthew, Ill. Fl. Palni Hills t. 354. 1996 & Fl. Palni Hills 568. 1999. (Family: *Apiaceae*)

Tall herbs; branchlets hispid pubescent. Leaves simple at base, to 5 x 5.5 cm wide, broadly cordate, apex acute, margin denticulate, pubescent below; cauline leaves 3-foliolate, often dissected. Umbels 4 cm wide; rays 10-12; umbellules 10-12. Flowers 3 mm wide; sepals absent; petals 1.2 mm wide, emarginate; stylopodium subconical; ovary ovoid. Fruits not seen.

Fl.: August-September.

Distr.: Endemic to Southern Western Ghats. Occasional; along grassland ecotones above 2000 m altitudes. Mannavan Shola, Idlimottai, *KKK* 16603.

Note: This species is so far reported from it's type locality (Palni hills) and was considered '*possibly extinct*' (Nayar, 1997; Matthew, 1999). Present collection thus forms both a rediscovery and extension of distribution of the species to Kerala.

Symplocos monantha Wight, Ic. t. 1236. 1848; Clarke in Hook. f., Fl. Brit. India 3: 581. 1882; Gamble, Fl. Pres. Madras 784. 1923; Hore, Fl. India Fasc. 20: 160. 1990. (Family: *Symplocaceae*)

Shrubs; branchlets tomentose. Leaves to 5 x 2 cm, ovate, apex acuminate, serrate, coriaceous; nerves obscure, glabrous. Flowers sessile or shortly pedicelled, axillary; sepals 1.5 mm long, triangular, acute, ciliate; corolla lobes 4 x 3 mm, united at base, obtuse, glabrous; stamens 30-40 in 5-6 bundles, attached at the base of the corolla; ovary hairy at apex. Drupe 12 x 5 mm, ovoid, truncate at apex, white.

Fl. & Fr.: November-January

Distr.: Endemic to Southern Western Ghats. Rare; in shola ecotones above 2000 m altitudes. Mannavan Shola, Idlimottai, *KKK 31111.*

Note: This species was considered as '*possibly extinct*' (Nayar, 1997) since it could not be relocated after the type collection by Robert Wight, which was made from the Shevagherry Hills of Tamil Nadu, until it was relocated from Periyar Tiger Reserve after 150 years (Sasidharan, 1998). Present collection extends the distribution of the species to Mannavan Shola (Idukki Dt.)

Conclusions

Identification of endemic species of a region or a country is considered a priority exercise in any programme of conservation of biodiversity. Since endemic species usually have specific ecological niches, (mountain peaks, islands) and edaphic gradients, the habitats of endemic species are far more vulnerable than other species. It is a known fact that out of the 1272 endemic species present in Kerala, 460 belongs to rare, vulnerable and endangered categories, which occur in isolated populations. Such species would become extinct in the near future unless appropriate conservation measures are taken (Nayar, 1997).

A total of 669 angiosperm taxa collected during this study with 246 (37%) endemics certainly indicate the high diversity and endemism prevailing in the high altitude shola regions. Perhaps the unique altitudinal and climatic factors, which favour diverse microclimatic situations, might be leading to the high endemic concentration and diversity in these regions. The discovery of above mentioned seven endemic species, earlier documented as '*Possibly extinct*' shows the importance to conserve such mega centres of diversity, which acts as a refugium of several rare and endangered taxa.

About 48 shola plants which belong to various threat categories could also be collected (Kumar, 2004). It is a fact that a lot of species, which were not reported in other earlier works (Joseph, 1977; Henry *et al,* 1979; Jain & Sastry, 1984; Ahmedullah & Nayar, 1987; Nayar & Sastry, 1987, 1988, 1990), were found locally in very rare and threatened conditions. About 49 flowering plants were also reported for the first time from the state of Kerala as a result of this study (Kumar, 2004; Kumar & Sasidharan, 2010).

From the study it is becoming clear that sholas are one of the most diverse ecosystems in the Western Ghats, which is considered as one of the 'hottest' of the hotspots of biodiversity. They are floristically unique in all respects and have to be protected and explored thoroughly to elucidate the floral diversity and status of the endemics, before they get extinct due to degradation, depletion or conversion of natural vegetation.

Acknowledgements

I thank the support received by the Directors of *Kerala Forest Research Institute* (KFRI), during the study. Guidance and helps received from Dr. N. Sasidharan, Dr. K. Swarupanandan, Dr. A.R.R. Menon, Dr. K.

Balasubramaniam, scientists of KFRI and Dr. M. Sivadasan, Dr. P.V. Madhusoodanan, Dr. A.K. Pradeep & Dr. M. Sabu of Botany Department, University of Calicut are gratefully remembered. Finanical support from the *Kerala Forest Department* (Wildlife), World Wide Fund (WWF) and *Council of Scientific and Industrial Research* (CSIR), New Delhi in the form of a *Senior Research Fellowship* (SRF) are sincerely acknowledged.

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