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Effect of Malathion on the Testis of Fresh Water Crab *Barytelphusa Cunicularis* (Westwood-1836) Morphological and Histochemical Studies

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I. INTRODUCTION

The wide spread use of chlorinated pesticides to control pest species creates ecological disturbances which in turn affects the non target organisms. A good amount of information in toxicities of pesticide pollution on aquatic animals are available, (Dalela et al. (1979), Dubale and Shah (1984), Rashatwar and Ilays (1984), Das et al. (2013), Guise et al. (2004), Dutta et al. (1993), Deka and Mahanta (2012), Dode et al. (2012). particularly useful in aquatic environmental studies for several reasons highly social animal. These days pollution of the environment by pesticides is a great problem. Pesticides constitute major agriculture chemical groups which though play an important role in agriculture productivity but have posed potential hazard to non target species. It also contains concentrated forms of proteins, and oil that protect against heart disease. As the crabs are abundantly available locally and used in food by as diet by some people with great nutritive value, there is a potential to project it as **poor man's protein**. Histochemical studies have been useful in evaluating such effect of an organism. Since, the trace amounts of their chemical which do analysis of histological changes in target organ provides a valuable tool in understanding the role of specific cells & organ. However histochemical investigation to determine the effect of pesticides on *B. cunicularis* has received comparatively little attention. Malathion breaks down quickly by the action of water and bacteria present in it. After reacting with other chemicals formed naturally in the air, Malathion broke down quickly by the action of water and bacteria in to more toxic substance called Malaxon, Magar and bias (2013). Once the Malathion is introduced into the environment it may cause serious intimation to aquatic organisms and is notorious to cause severe metabolic disturbance in non target species like crabs, fishes and other aquatic animals like fresh water mussels etc. Pugazhvendan et al. (2009) exposed *ophiocephalus punctatus* for 7 days to Malathion and different concentrations and reported severe histochemical changes in brain, liver, ovary and tissues.

Histochemical studies in cell & tissue of organism provide useful data about effect of different chemical & pesticides on particular organism. It is very simple and common tool for determining the effect of various toxic substances in animal body. In animal kingdom arthropods have largest diversity they are much susceptible towards contamination with water pollutants. They were also taken into consideration for various reasons such as environmental pollution, remedial traits & tourist attraction. Biochemical constitutes like glycogen, protein and lipids are considered as sensitive indicators of pollution effect in crabs. It is of prime importance to understanding biochemical changes in organism under the stress of pollutants **(Kharat et al. 2011)** Since there is practically no information regarding the effect of Malathion on the Reproductive organs of the fresh water crab, the present investigation is being proposed was to determine the histochemical changes in the testis of fresh water crab, *B. cunicularis* after expose with Malathion.

II. METHODOLOGY

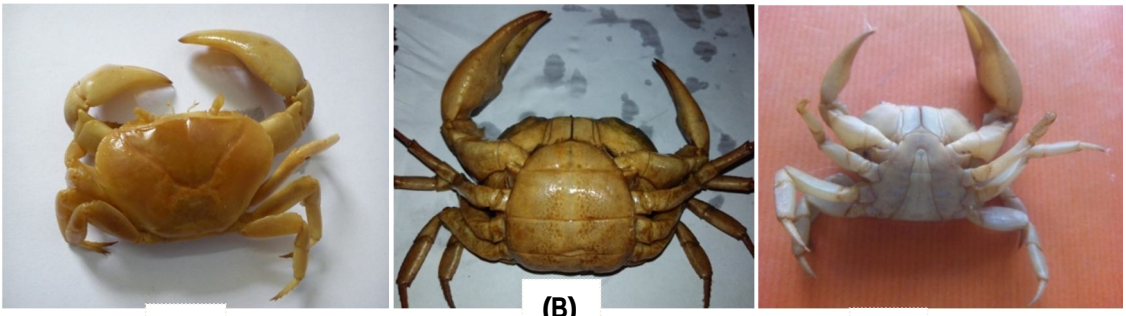
Live specimens *of Barytelphusa cunicularis* was collected from local wet areas, fresh water ponds and garden area of Kalyan P.G. College at Bhilai (Lat: 21^0 13N; long.: 81^0 26E), Chhattisgarh. Samples of the specimens were collected by hand, forceps and trapping nets. After capture prior to the experiment all the specimens were kept in the glass aquarium (80cm×45cm×30cm) under constant aeration and the temperature was maintained approx 27^oC for a week. **(FIG-1-b).** The crabs were sexed on the basis of the shape of the abdomen. Females have oval or rounded abdomen; in contrast, the males have triangular or inverted **"T" shaped** abdomen. The male crabs were kept in separate aquarium. Only healthy crabs were collected and fed with wheat grains. Average weight of crabs varies from (40-50gms), the carapace length and carapace width varies from 3.20-4.50mm and 4.25-5.80mm respectively. The morphometric measurement of the crab was taken through Vernier Caliper and the weight of the crabs was measured through the single pan balance.

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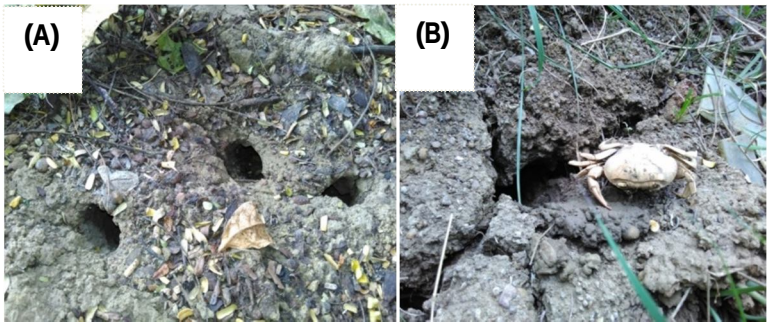
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After acclimatization in the laboratory condition, both male and female crabs were kept in two groups-: the control group set free from malathion and the experimental group was exposed to malathion for LC_{50} at different concentration 0.45ppm, 0.30ppm, 0.26ppm and 0.25ppm for 24hrs, 48hrs, 72hrs and 96hrs respectively. All the crabs were cold anesthetized and the testis and ovary was dissected from both the control and experimental crabs and fixed in Bouin's solution for 12-15 hours. The tissues were dehydrated in increasing concentration of ethanol, cleaned in xylol and soaked in paraffin in order to make the sections of 5µm thick cut with digital rotatory microtome. For histochemical analysis the tissues were fixed in 70% alcohol for 24hrs. The sections were taken in slides and stained with different techniques for histochemical localization of carbohydrate, protein and lipids. For carbohydrate estimation, Periodic acid Schiff (PAS) **(Humason 1972).** For the protein estimation, the Mercury Bromophenol blue method **(Pearse, 1968).** For lipid estimation Sudan black B staining was followed (**Pearse, 1968).** Stained slides of both control and experimental crabs were studied and compared by using microscope and were photographed (10x, 40x, 100x).

A. Observation And Result



Freshwater Crab- *Barytelphusa cunicularis* (FIG-1a)



Crab Burrows (FIG

B. Observation And Result

1) Morphology

a) Male crab: The reproductive system of the male *Barytelphusa cunicularis* consists of the paired testis, vasdeferences, seminal vesicles and genital aperture. **(FIG-1a-c)**

b) Testis: Each testis is elongated, lobulated creamy white in colour. It extends interiorly on the cephalothorax on the top of the hepatopancreas below the carapace and continues laterally to the stomach. The width and the diameter of the testis is not uniform along its length. The distal end of each testis and the anterior end of the vasa differentia are joined together to form a commissure or cross bridge which give "H" like shape. The length of each testis lies between 3 to 6mm. (**FIG-2ab)**

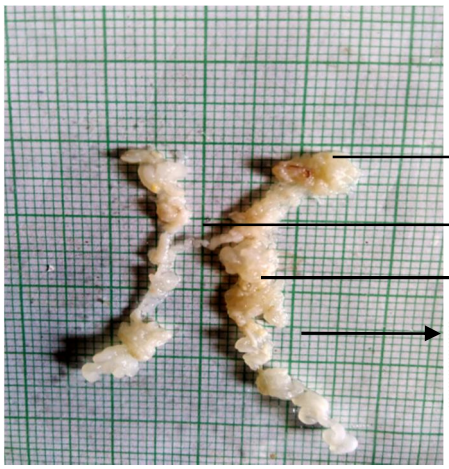
c) Vasa Deferentia: A pair of vas differentia arises from the posterior end of the two testes. Each vas deference is creamy white colored thin, extensively coiled tube, ending into the ejaculatory duct. Due to coiling and the folding of the tube, it forms a wider and lobulated, elongated structure and tenged in mass of muscular tissue. Diameter of each tubule varies from 1 to 1.5mm. The distal end of the each vas deference arise a thin tubule called ejaculatory duct they are transparent whitish in color and about 8 -13 mm long tubule. It leads to gonophores of respective sides and release spermatophore in to penis during copulation. (FIG-2ab)

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d) Gonopods: Paired gonopods (Gonopods-1 and Gonopods-2) are hollow tubular organ with the apical opening in the terminal part. The gonopods are short but sharp thread like end. The size of both the gonopods varies. Gonophores are paired appendages present in the abdominal that are modified to copulatory organ. In *Barytelphusa cunicularis* they are tubular organ in the terminal part ending into along tube with a broad base which gradually tapers distally in to slightly blunt end. The gonopod-2 is shorter then gonodopod 1 it also had broad base with thin thread like end. **(FIG-2ab)**



Male Crab Dissected Testis (FIG-2a)



(C) Male reproductive organs (FIG-2b)

C. Observation And Result

HISTOLOGY

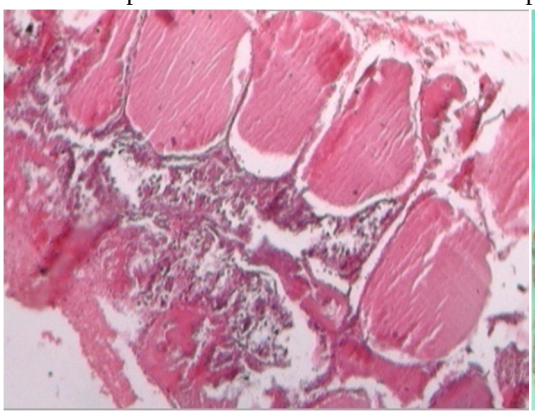
1) Testis

Entire testis is enveloping by fibrous layer made of collagen fiber, each testis consists of large number of testicular lobes or somniferous tubules for histopathological differentiation heamotoxylin-Eosin were used. Histological the testicular follicles are surrounded by a single layer of germinal epithelium that encloses each testicular lobule. These lobules give rise to spermatogonial cells which are stain with blue color the lumen of testicular cells stain with pink color the nutritive cells helps in the process of spermatogenesis, non germinal cell called as sertoli cells acting as accessory cell, interstitial cells, nurse cells or nutritive cells were found. The proximal vas deferens consists of luman filled by colloid or loose spermatozoids and had a tall cylindrical cellular epithelium. The medial vas deferens had a lumen filled by spermatophores with cylindrical cellular epithelium. The distal vas deferens was made up of basal cellular epithelium, filled with colloid and spermatophores, it is larger in dimension than median vas deferens.

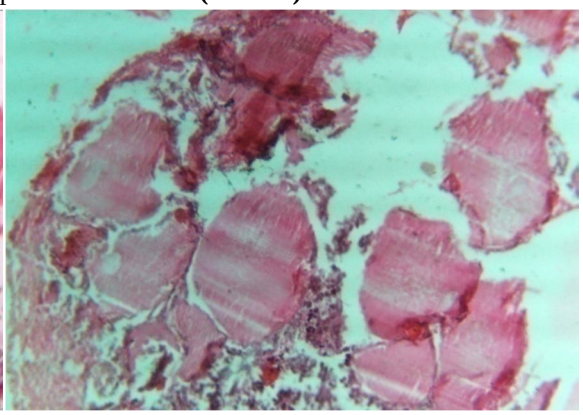
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The cells in each testicular lobe seem to be in a single stage of spermatogenesis however cells in deferent lobe may be in deferent stages of spermatogenesis. **(FIG-4a)**The cross section of each vas deference is circular and elongated thick muscular sheath folded by the layer of granular epithelium, it secrets the kind of fluid that serves in the transport of spermatophore, the epithelium and spermatophore are stain deep violet with Heamotoxylin- Eosin. Spermatophores consist of various spherical shaped spermatozoids enveloped by the thin membrane.. The lumen of vas deference contains number of spermatophore and numerous spermatozoa which are carried to the pennies. During copulation the function of vas deferens is to transfers spermatozoa in the form of spermatophores to the external opening of the male reproductive system, towards the posterior end of the endocrine gland there is endocrine structure termed as androgenic gland, responsible for development of secondary sexual character in male crabs **(Charniaux-Cotton, 1960; Charniaux-Cotton and Payen, 1985).** The epithelial cells of the ejaculatory duct are flattenethe vasd end surrounded by thick muscular layer of smooth muscle cells the smooth circular muscle cells are arranged around the circular layer. The proximal end of

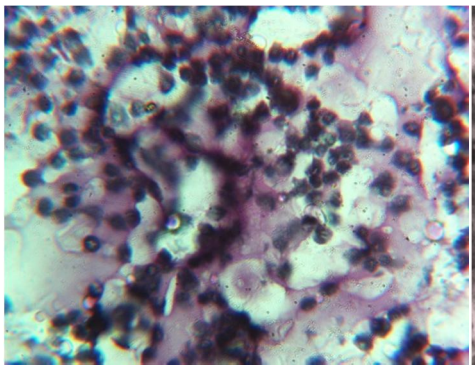
The testis show different changes in their structure, the testicular follicular cells interstitials cells and nutritive cells were decreased and distracted number of sperm cells also affected due to the impact of malathion. **(FIG-4b)**



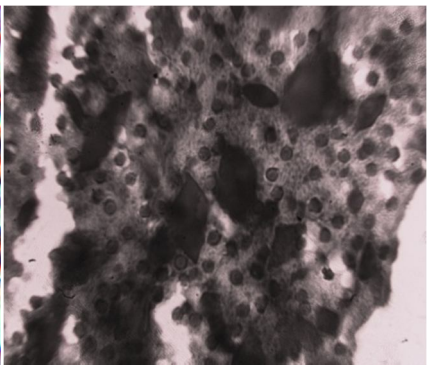
T.S of Testis (Control) (FIG-4a)



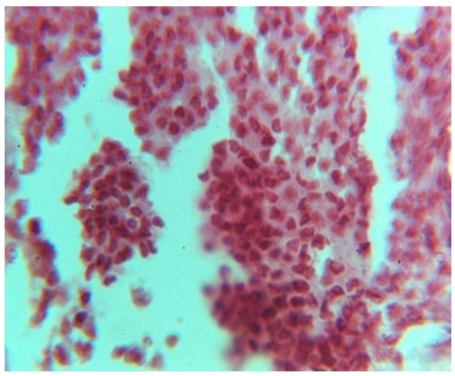
T.S of Testis (Effected) (FIG-4b)



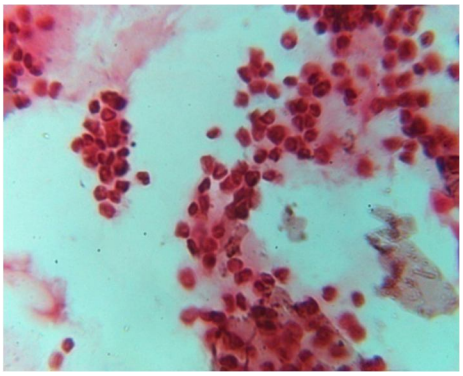
(A)



(B)

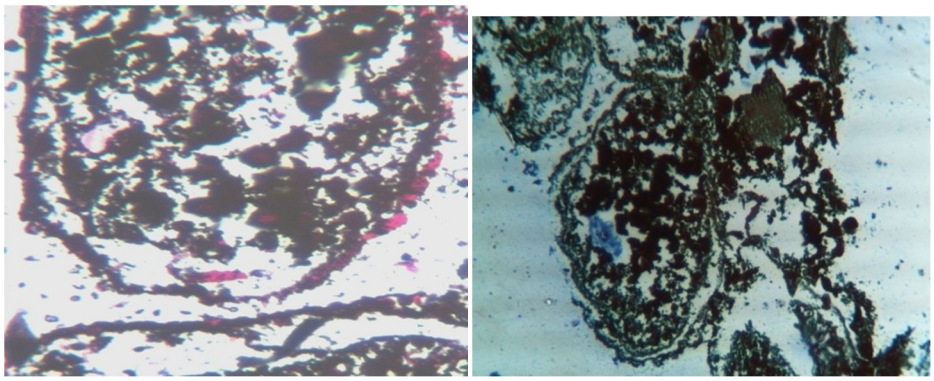


(C)



(D)

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(E) (F)

Fig: 6- TESTIS

Control (A) Glycogen (C) Lipid (E) Protein Experimental (B) Glycogen (D) Lipid (F) Protein

D. Observation And Result

1) Histochemical

After exposure of malathion on different concentration such as 0.45*ppm*, 0.30*ppm*, 0.26*ppm* and 0.25*ppm*. The LC-50 values for 24hrs, 48hrs, 72hrs and 96 hrs respectively of malathion, The concentration of protein, lipid and carbohydrate get decreased as compared to control crabs. The glycogen contents in the testes of *B. cunicularis* were examined through PAS method, the contents of the glycogen is examined in the experimental condition which gradually decreases, the deletion of glycogen may be due to its rapid unitization to meet the energy demands under the impact of heavy metal pollution stress by **Farooqui et al. 1983.** Treatment with malathion also changes the biological parameters in some reproductive organs **Bhatnagar et al. 1996.** Fall in the glycogen level may be due to the interference of glycogenolysis. The general metabolism and constant supply of glucose is necessary for proper functioning of testes. Hence the glycogen is a good energy source for metabolism. The protein content is elevated due to hepatic detoxification which results in the inhibitory effect on the activities of enzyme involved in androgen biotransformation **(Venkataramana et al., 2006)**. The depletion of glycogen may be due to its rapid utilization to meet the energy demands under the impact of heavy metal pollution stress by **Farooqui et al. (1983)**. Similarly by the impact of Malathion on the experimental crabs, after exposure of malathion suggest that decrease in levels of protein, lipid and carbohydrate in different tissues, may be increased due to glycogenolysis, glycolysis, proteolysis and lipolysis under stress to meet increase energy demands for survival.

III. REVIEW OF RESEARCH AND DEVELOPMENT IN THE SUBJECT

The histochemical study on the testis of the freshwater crab *Barytelphusa cunicularis* changes due to malathion caused specific changes and abnormality in the tissue level .the changes induced in the level of proteins lipid and carbohydrate due to the rapid utilization to meet the energy demands under the impact of malathion which gradually decreases. Treatment of malathion also changes the biological parameter in some reproductive organ (Bhatnagar et al. 1996). Kharat et al., 2011 reported that the histology change in the tissue of fresh water prawn. *Macrobrachium kistensis* exposed to TBTCL. The reproductive system of *Barytelphusa cunicularis* a general layout was similar to those found in other decapods i.e. paired testis and vas deferens (Krol et al 1992; Cumberlidge, 1999; Gracia and Silva 2006; Castilho et al 2008) Garcia and Silva, 2006 repoarted that the testis of some crabs consisted of anterior, intermediate and posterior regions based on the histological observation. Charniaux-Cotton, 1960; Garcia and Silva 2006, Castilho et al., 2008, studied in many crustaceans the presence of androgenic gland or structure similar to the functions to those carried out by the androgenic glands. Sherkhane et al 2010, reported the androgenic gland is known to produce some hormones that regulate the aspects of male reproduction. In *Barytelphusa cunicularis* and some decapods the first two pleopods are modified to serve as gonopods in the insemination of female. Histology or histochemical studies of gonopods was not reported in the present study, however in some crustacean species the role of gonopods in the transfer of spermatophore during copulation. Cumberlidge, 1999; Berg and Sandifer, 1984, describe the dired role of the gonopods in transferring sperm to the female. The structure of spermatozoa and spermatophores in crustacean provide useful information on phylogenetic relationship and evolutionary divergence, especially in the decapods (Tudge, 1997; Kronenberger et al. 2004). .

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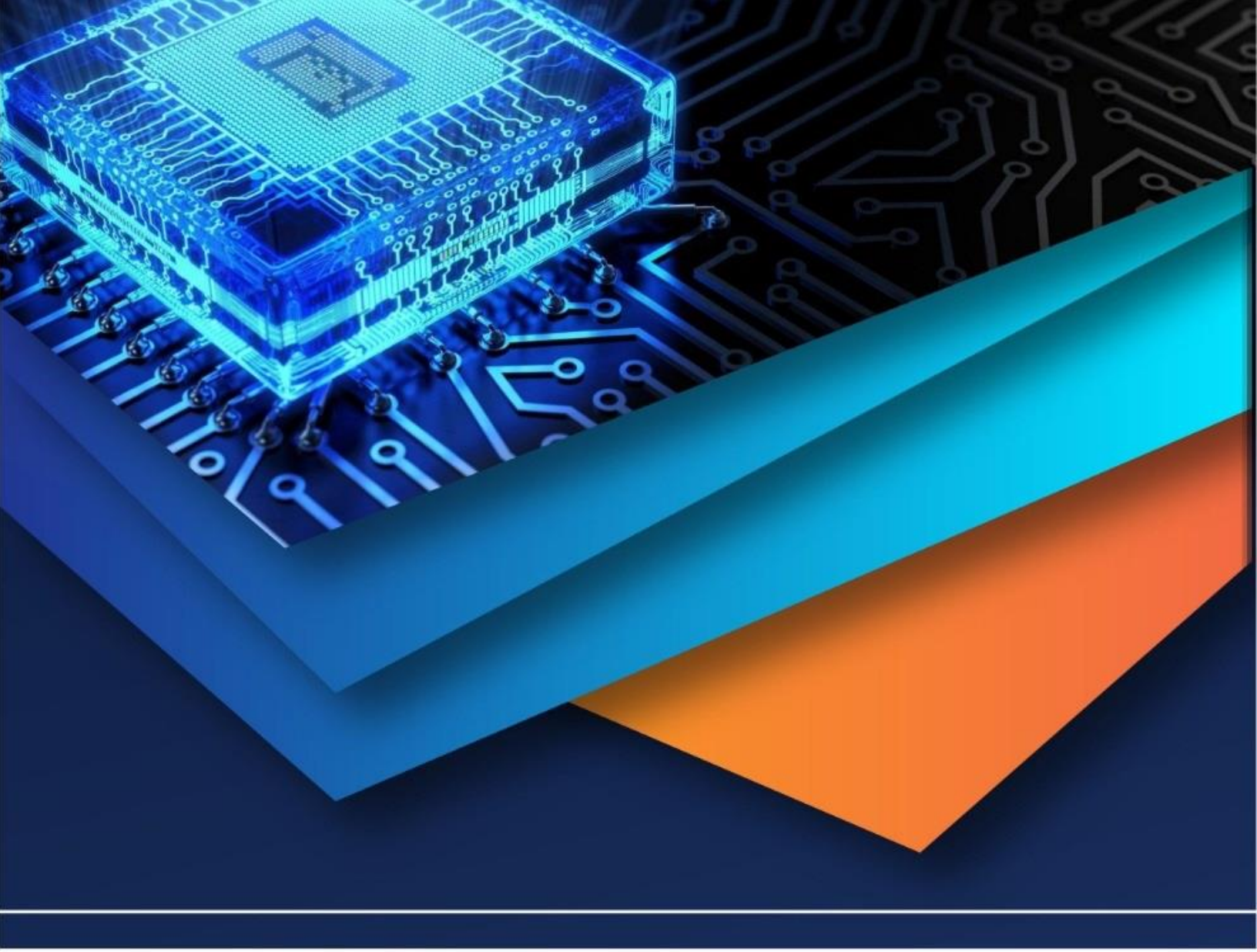
Thus the present study helps to investigate the effect of malathion on the histochemical aspects of proteins, Lipids and carbohydrates in the testis of fresh water crab *Barytelphusa cunicularis*. Lopez Greco et al; 2007 reported the morphology of male reproductive system and spermatophores formation in the freshwater 'red claw' crayfish *Cherax quadricarinatus.* Castilho et al., 2008, studied the Morphology and Histology of the male reproductive system of the Mangrove crab. In the periacrosomal and peripheral regions, the presence of glycogen in the spermatozoa of *Gecarcinucus steniops* and in other decapods like *Carcinus macnes* the glycogen reported to be absent (Anderson and Personne, 1970). Stored glycogen in spermatozoa could serve as endogenous source of energy under aerobic and anaerobic conditions.

Treatment with malathion also changes the histochemical parameters of reproductive tract, a fall in the glycogen level was observed as the glycogen is an energy source of general metabolism and constant supply of glucose is essential. Changes the biological parameters in some reproductive organs when treated with Malathion. (Bhatnagar et al. 1996). Machle et al. (1990) studied that cupreus oxide exposure induced significant alterations in the ovary of the crab *Barytelphusa* querini**.**

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