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Histopatological Impact of Malathion on the Reproductive Organs of Freshwater Crabs Barytelhusa Cunicularis (Westwood, 1836)

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Abstract: ***Crabs are particularly useful in aquatic environment and play a very important role in the ecosystem processes and good indicator for polluted condition. The terrestrial crab plays a significant modal in the study of population and also beneficial for human activities. The present study deals with the histochemical changes of organophosphate, malathion in the freshwater crab Barytelphusa cunicularis. Live specimens of Barytelphusa cunicularis were collected from local wet areas of Maroda-sector, Bhilai, (C.G). The crabs were sexed on the basis of the shape of the abdomen. Average weight of crabs varies from (45-50 gms), the carapace length and carapace width varies from 3.20-4.50mm and 4.25-5.80mm respectively. The crabs were acclimatized in the laboratory condition, and 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. The sections of 5µm were taken for the histopathological examination of testis and ovary; affected due to the impact of Malathion, the testicular follicular cells interstitials cells and nutritive cells were decreased and distracted number of sperm cells it exhibited irregular arrangement of nucleus, in the ovary epithelial layer destruction, degeneration of oocytes, Shrinkage in ooplasm, vacuolization and reduction in cytoplasmic material. The result suggests that rapid utilization to meet the energy demands under the impact of malathion.***

Keywords: Freshwater crab, Ovary, Reproductive organs, Histopathology, pesticides, Malathion

I. INTRODUCTION

Crabs are particularly useful in aquatic environmental studies for several reasons and play important role in ecosystem processes and good indicator for polluted condition. The terrestrial crab plays a significant and useful modal in the study of population and also beneficial for human activities. 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. 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. Histochemical studies have been useful in evaluating such effect of an organism. Since, the trace amounts of their chemical which don't bring mortality over a period are capable of producing considerable organ damage. 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 is very widely used organophosphate insecticide. It used in agriculture and houses for the control of disease vectors it is also a major source of experimental poisoning widely used in the developing countries. Once Malathion is introduced in the environment, usually from spraying on crop or in wide urban or residential areas, droplets of Malathion in the air fall on soil, plants, water or man –made surfaces. 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.

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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.

Histopathological 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. Pollution makes our environment more & more virulent. When the relation among the element of nature like air and water are disturbed, ecological balance is harmed. Our environment is polluted by different types of pollution Including water pollution, air pollution, sound pollution, soil pollution etc. Water pollution is one of those which concern with the undesirable changes in medium which effect hardly to aquatics as well as terrestrial body. Sewage waste, agriculture waste, industrial effluents etc. are various means by which water get polluted.

Aquatic medium is highly contaminated with heavy metal that's all are release from industries, man-made activity by different process. Pollution decreases the floral & fauna diversity on earth. Majority of animals sensitive towards change in the chemical as well as parametric quantity of aquatic medium. 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. The present investigation is being proposed was to determine the histopathological changes in the ovary of fresh water crab, *B. cunicularis* after expose with Malathion.

II. MATERIALS AND METHODS

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. 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.

After acclimatization in the laboratory condition, 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 histopathological analysis the tissues were fixed in Bouin's solution for 12-15 hours. The sections were taken in slides and stained with Haemotoxylin-Eosin through different grades of alcohol. Stained slides of both control and experimental crabs were studied and compared by using microscope and were photographed (10x, 40x, 100x).

III. RESULT AND DISCUSSION

A. Morphology

1) Male Crab

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

1) *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)
2) *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)

3) *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)

2) *Female Crab*

The reproductive system of the female crab is bilaterally symmetrical, in the antero-lateral portion of the cephalothorax (H-shaped Structure) consists of Ovaries, oviduct and female gonophores. (FIG-2)

1) *Ovary:* The color of the ovary varies from milky white to deep orange in color according to their maturity. The shape of each Ovary is Filliform, cylindrical to oval and transparent in appearance depending upon the maturity of ova and visibility of the oocyte. The ovary is bilobed and extends from behind the antero-lateral carapace along the anterior edge of carapace medially to the stomach. The width and diameter of right and left ovary varies and is not uniform along the length. (FIG-6 and Fig-7)
2) *Oviduct:* The distal ends of both the ovary are joined the anterior ends of both the oviduct together the ovary and oviduct from a commissure or isthmus so as to give 'H' like structure. Behind the stomach, the oviduct then dips ventrally under the pericardial sac. The oviduct then extend posterior into extensively dense structure called bursa, each bursa is deep brown in color and the distial end of the oviduct ultimately opens into thin female gonopores.
3) *Female Gonophores:* The size and color of receptacles varies and may be small, hard and creamy in color with a jelly plug on the external pore.

IV. HISTOLOGY

A. *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.

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)

B. *Histology*

Testis

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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-9 and FIG-10). 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.

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C. Ovary

The ovary of the control crab consists of outer epithelium and inner germinal epithelial layer. The oocytes are covered with the follicles cells. And the follicles are filled with different types of maturing oocytes and responsible for supplying nutrients. The ooplasm consists of thick yolk granules.

In mature oocytes there is no visualization of connective tissues, the ovarian capsule is very thin, breaks and easily releases mature oocytes.

Behind the stomach, the oviduct then dips ventrally under the pericardial sac. The oviduct then extend posterior into extensively dense structure called bursa, each bursa is deep brown in color and the distal end of the oviduct ultimately opens into thin female gonophores. (FIG-11)

When exposed to Malathion ovaries of *Barytelphusa cunicularis* exhibited irregular arrangement of nucleus, epithelial layer destruction, degeneration of oocytes, Shrinkage in ooplasm, vacuolization and reduction in cytoplasmaic material. (FIG-12)

V. DISCUSSION

Treatment of malathion also changes the biological parameter in some reproductive organ (Bhatnagar et al. 1996), a fall in the glycogen level maybe due to the interference of glycogenolysis. 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).

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. Indira et al. (1989) noticed effect in the developing oocytes inter viewing the enzyme system in metabolism under stress conditions. Machle et al. (1990) studied that cuprous oxide exposure induced significant alterations in the ovary of the crab *Barytelphusa* querini, arrangement of oocyte and disappearance of nucleus were observed in fresh water crab *Barytelphusa cunicularis*. Similaraly the level of lipid and protein also affected by the impact of Malathion on the experimental crabs.

Crab Burrows (FIG-1a)

Fig.1b Crab Dorsal view

Fig.2 Crab Ventral View (Female)

Fig.3 Crab ventral View (Male)

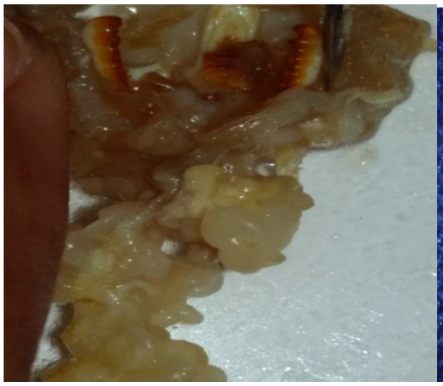


Fig.4

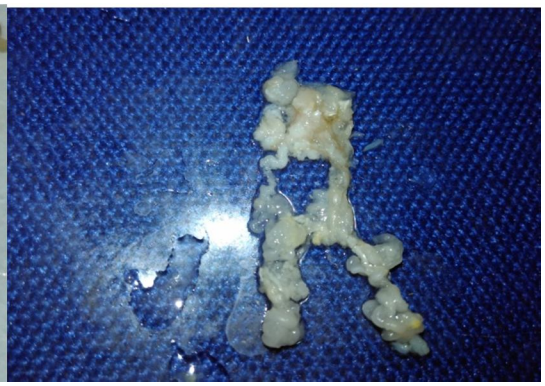


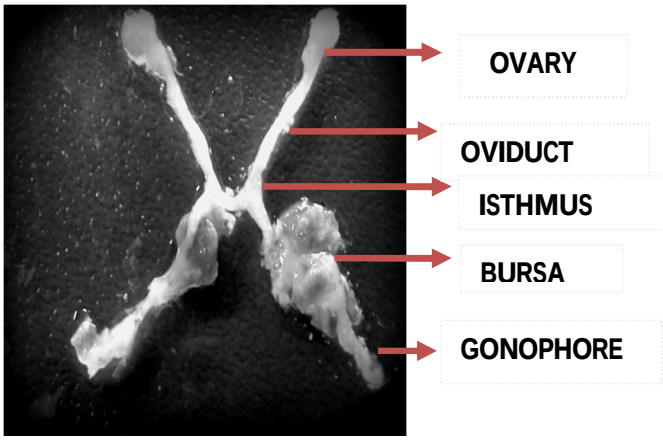
Fig.5

Fig.4 and Fig.5 (Male Crab dissected Testis)

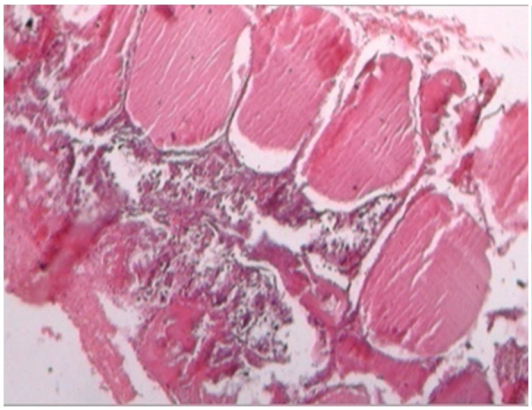
Fig.6

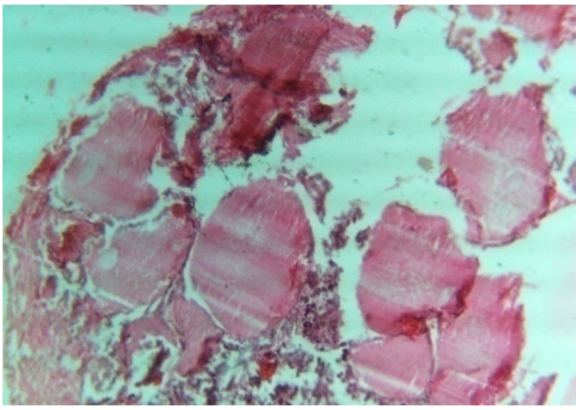
Fig.7

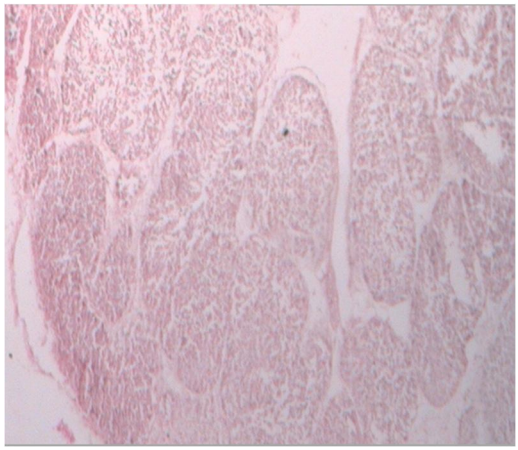
Fig. 6 and Fig.7 (Female Crab Dissected Ovary)

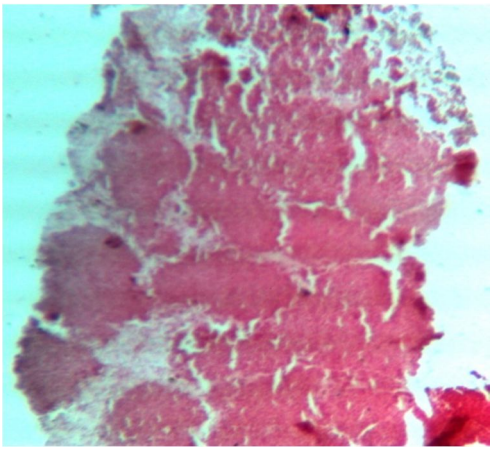


Female reproductive organs (FIG-8)


T.S of Testis (Control) (FIG-9)


T.S of Testis (Effected) (FIG-10)


T.S of Ovary (Control) (FIG-11)


T.S. of Ovary (Effected) (FIG-12)

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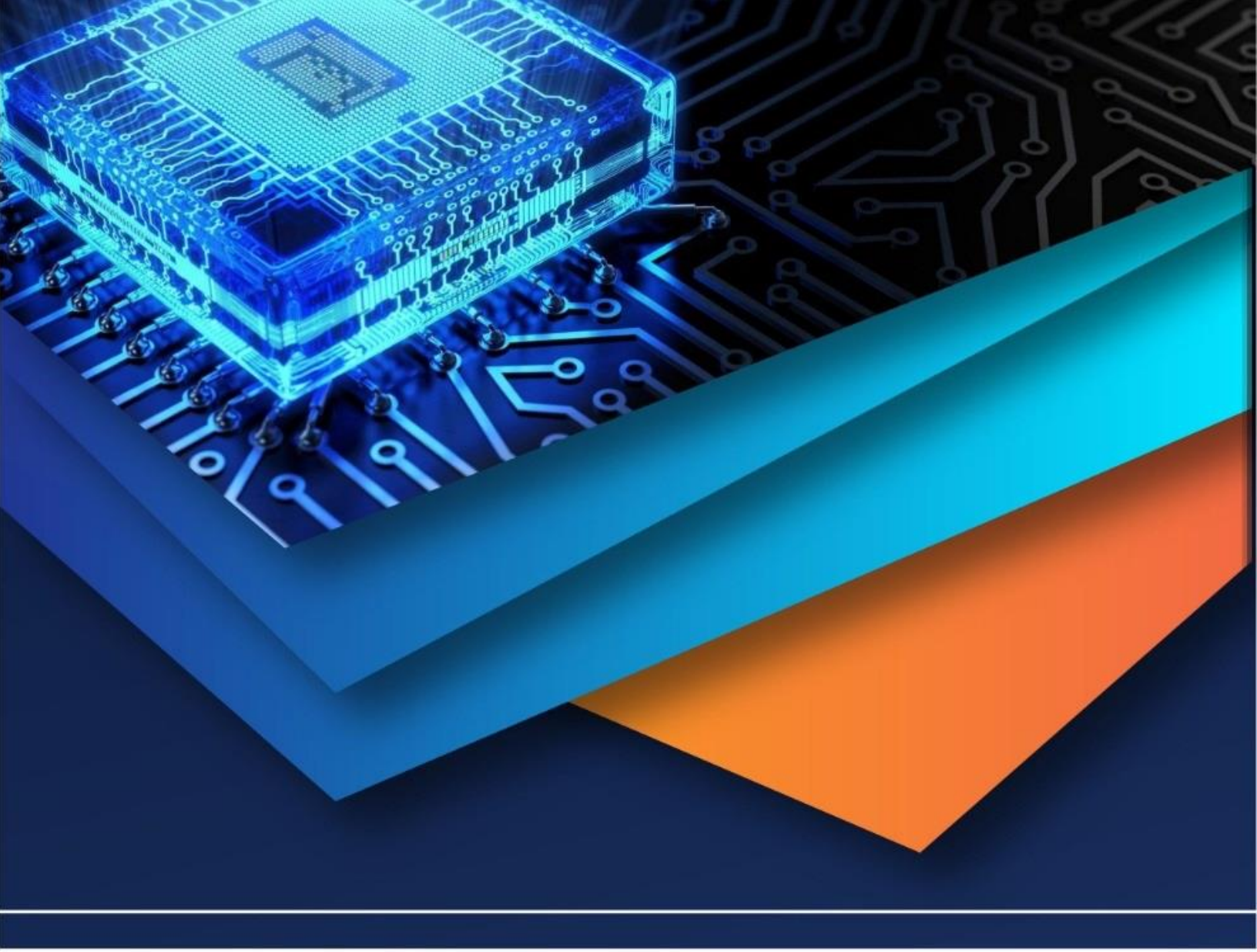

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