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Induction of Various Diseases in *Clarias batrachus (Linnaeus, 1758)* Following Exposure to Cypermethrin and Fenvalerate, with Reference to Histopathological and Physiological Alterations

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Abstract: Catfishes Clarias batrachus (Linnaeus, 1758), exposed under the Acute toxicity at the lethal concentration dose of LC50 1/4 of 1 µg/ litre for 24 hours; which is 0.25 µg/litre for Cypermethrin. On the other hand, lethal concentration of Fenvalerate for 24 hours was LC 50 (250µg/litre) selected by its 1/10 portion which is LC50 (25 µg/ litre) for the test dose. Generation of different types of diseases observed during the period of research study. These concentrations were maintained throughout the study, with observations made at 24-hour intervals to assess acute toxicity at lethal concentrations.
Keywords: Clarias batrachus (Linnaeus, 1758), Toxicology, Diseases, Cypermethrin, Fenvalerate, Acute toxicity, Histopathological and Physiological observation

I. INTRODUCTION

Causes of the adverse effects of chemicals on living organisms shows the symptoms of toxicity. The reference with the study under the branch of biology, chemistry, pharmacology and medicine is known as Toxicology. The word taken from the Ancient Greek words τοξικός toxikos "poisonous" and λόγος logos.

Mathieu Orfila is considered the modern father of toxicology, having given the subject its first formal treatment in 1813 in his Traité des poisons, also called Toxicologie générale.

Theophrastus Phillipus Auroleus Bombastus von Hohenheim (1493–1541) (also referred to as Paracelsus, from his belief that his studies were above or beyond the work of Celsus – a Roman physician from the first century) is also considered "the father" of toxicology. He is credited with the classic toxicology maxim as, "All things are poisonous and nothing is without poison; only the dose makes a thing not poisonous." This is often condensed to: “The dose makes the poison" or in Latin "Sola dosis facit venenum".

Adverse effects depend on two main factors:

1) Routes of exposure (oral, inhalation, or dermal) and

2) Dose (duration and concentration of exposure). To explore dose, substances are tested in both acute and chronic models.

Acute tests are short-term exposure tests (hours or days) are generally conducted on organisms during a specific time period of the organism’s lifecycle, and are considered partial life cycle tests.

Cypermethrin is (R, S)-alpha-cyano-3-phenoxybenzyl-2,2-dimethyl(1R,1S)-cis, trans-3-(2,2-dichlorovinyl) cyclopropane – carboxylate. A synthetic pyrethroid insecticide used as in large-scale commercial agricultural applications as well as in consumer products for domestic purposes. It is used to kill insects on cotton and lettuce and to kill cockroaches, fleas, and termites in houses and other buildings (Summer, 1996). Fenvalerate [(RS) α-cyano-3-phenoxybenzyl (RS) 2-(4-chlorophenyl)-3-methylbutyrate] most widely used as synthetic pyrethroid insecticides to control insects in food, feed, and cotton products, and for the control of flies and ticks in barns and stables. It is a mixture of four optical isomers in which the 2-S alpha (or SS) configuration is about 23% and support the most insecticidal active isomer (Wang et al., 2010).

Clarias is a genus of catfishes (order Siluriformes) of the family Clariidae, the airbreathing catfishes. The name is derived from the Greek chlaros, which means lively, in reference to the ability of the fish to live for a long time out of water.

Objective measurement of Vital signs of the essential physiological functions of a living organism known as Physiological observation; whereas Histopathological observation is an examination of tissue specimens can identify the presence of a bacterial, viral, and fungal infection.

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II. METHODOLOGY

Adult Catfish *Clarias batrachus* of the same stock and mean weight of 400 grams – 600 grams and the length was ranging from 40-45 cm. Fishes were fed daily with commercial pedigree pellets (Discus Tokyu) and wheat flour mixed meat mincer pellets according to 3% of body weight of fish. The experiment throughout proceeded in winter season in the month of December to January within normal room temperature (± 22 -27ºC). The design of the experiment is a Completely Randomized Design (CRD) performed by analyses through Histopathological and Physiological observation.

Catfishes *(Clarias batrachus)* exposed under the Acute toxicity at the lethal concentration dose of LC50 ¼ of 1 µg/ litre for 24 hours; that is 0.25 µg/litre for Cypermethrin. On the other hand, Fenvalerate lethal concentration for 24 hours was LC 50 (250µ g/litre) selected by its 1/10 portion that is LC50 (25 µg/ litre) for test dose of the present study.

During the first level, after acclimatization period of 48 hours (2 days) in Normal water, fishes were randomly divided in two groups each group were put in 2 separate tanks of 200 litres of water. Again, Fishes were acclimatized with pesticides Fenvalerate and Cypermethrin mixed toxic agent in two different tanks for 96 hours (4 days) at the second and third level respectively. Observation taken during the whole experiment and proceed as to obtaining the results. Smear slide was also prepared within two methods, Thin and Thick Smear slide.

III.RESULT AND DISCUSSION

Following diseases were found as results during this Experiment observation:

A. Infectious Hematopoietic Necrosis Virus (IHN)

1) Virus disease of salmonids that results in a severe anemia

2) Clinical signs are usually severe mortality, exophthalmia, darkened color, extremely pale gills, petechial hemorrhages, and abdominal distention from ascites

3) Blood may be straw colored with few cells present- hematocrits may be as low as 5 - 10%

4) Along with the clinical signs that accompany the disease the use of blood smears and kidney imprints aid in the presumptive diagnosis of the disease

5) Characteristics of the blood smears are: few cells are present because of severe anemia, bilobed erythrocytes are usually present in small numbers, necrobiotic bodies (degenerate cellular debris) are often present foamy macrophages often with engulfed cellular debris are sometimes present

6) Characteristics of blood imprints are: foamy macrophages with and without engulfed cellular debris are often present necrobiotic bodies and other cellular debris such as cytoplasmic material are often present.

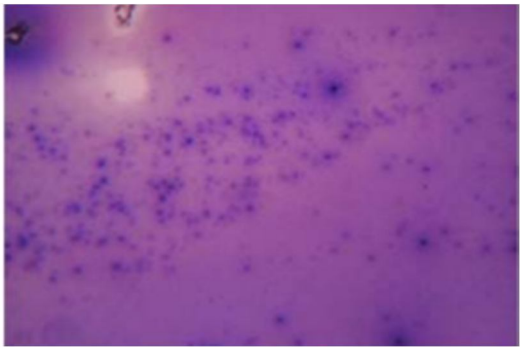


Plate 1: Blood film of IHN virus while experiment done

B. Cell Injury and Necrosis - Causes and Patterns

1) Reversible Cellular Changes

According to Heidel and Smith (2007), a variety of changes in cell morphology and function in response to injury are adaptive and are compatible with cell survival. Injuries often induce changes in cellular structure which are not lethal and are reversible. These changes include acute cellular swelling, hydropic change, and fatty change. These changes may be distinct, or can blend or progress from one to another, or occur simultaneously within a tissue. Reversible injuries result in structural and functional changes, but adaptation by the affected cells can maintain cell viability. Without adaptation, the changes caused by the injury can progress and may lead to cell death (necrosis).

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Figure 1: Skin eruptions observation while experiment done

C. Disturbances of Circulation: Hemorrhage

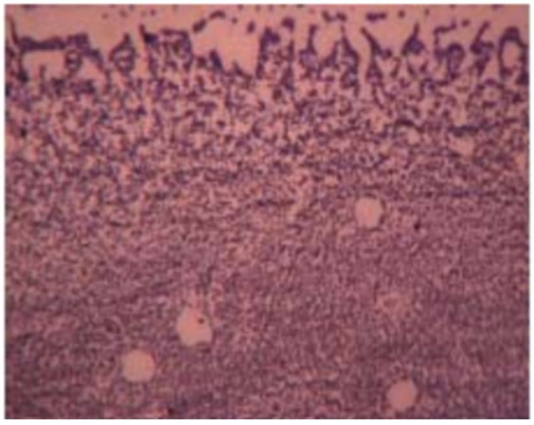


Plate 2: Blood Slide, showing hemorrhage while experiment done

Hemorrhage is the escape of blood from the vascular system. It is caused by injury to vascular endothelium; this can be due to infection, inflammation, necrosis, neoplasia, or trauma. During the experiment fishes also suffered by Hemorrhage and eye bulging also been seen.

Eye bulging

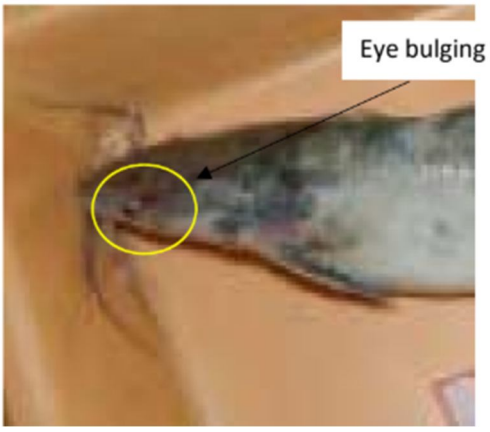


Figure 2: Found while experiment done



Figure 3: Heidel and Smith, 2007

In Leucocytotoxic test after interaction of Fenvalerate and Cypermethrin, blood film ruptures and clump in seconds before preparation of smear slide film, which indicates that severity of toxicity the given concentration of experimental pesticides on fish's blood (Kumar, December 2024).

D. Pigments

Substances in cells and tissues that have innate color are pigments. They are a diverse group of substances, and may or may not have health significance. Most are varying degrees of tan to brown in histological preparations. Melanin is a pigment responsible for color of skin, eye, and other tissues. In fish, accumulations of melanin are common at sites of tissue injury, often being visible grossly. Melanin is also present in melanomacrophage centers where it acts as a scavenger for free radicals. These centers increase in size and number in kidney, spleen, liver, and other organs after various types of injury. Melanin is a dark brown granular pigment. Such types of these pigmentation seen in effected catfish examined under the study. Fishes clearly seen suffered by stiffy barbels and dark pigmentation with swelling specially on the head portion.

E. Melanoma



Figure 4: Melanoma of barbels of catfish

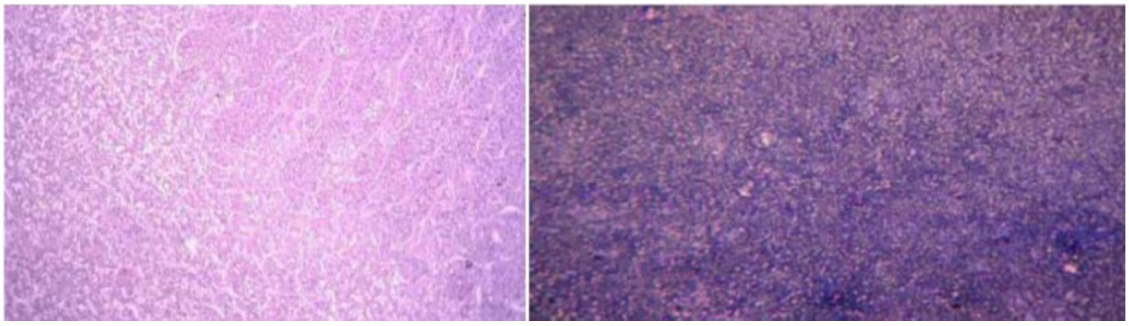


Plate 3 Plate 4

Plate 3 and 4: A well- differentiated hepatocellular carcinoma with widened cords and basophilic hepatocytes. Some normal hepatocytes are on left of corner.

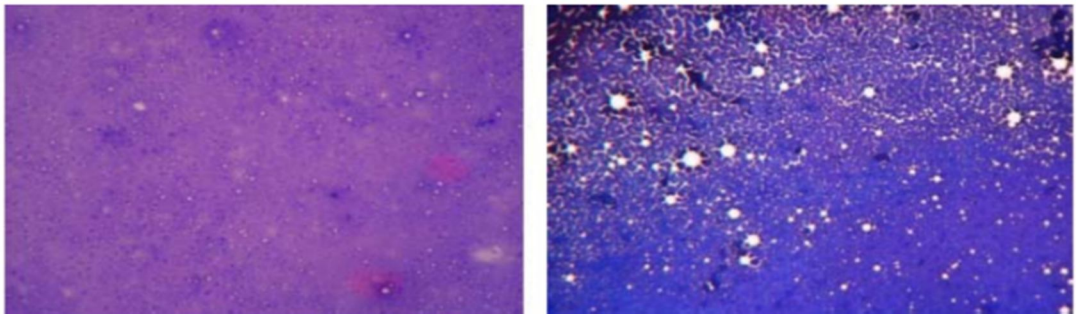


Plate 5 Plate 6

Plate 5 and 6: Blood film of Melanoma observed during Experiment.

Comparing of Plate 3, 4 ,5 and 6 it is clearly justifying the causes of Melanoma due to the toxicity of water which also shows the bad effect of blood slides prepared during the experiment.

F. Infectious Disease

It is found in Research that fishes suffered by different type of infectious diseases due to toxicity of water which clearly seen in their histological slides as well as their outer body surface. These types of infectious diseases are shown below in figures in comparison with Reference and work done by Beth Mac Conell, Vicki Blazer, Sonia Mumford, Charlie Smith and John Morrison, 2007.

1) Coldwater Disease (CWD) Flavobacterium psychrophilia: Skin and Muscle erosion

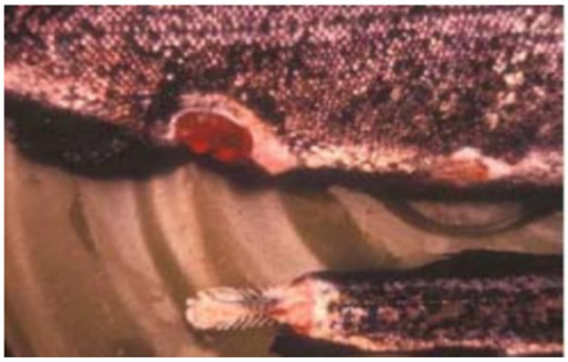


Figure 5: Beth et al., 2007



Figure 6: According to Experiment done

2) Parasites: Myxopsporean-Proliferative kidney disease (PKD) – Tetracapsula bryosalmonae

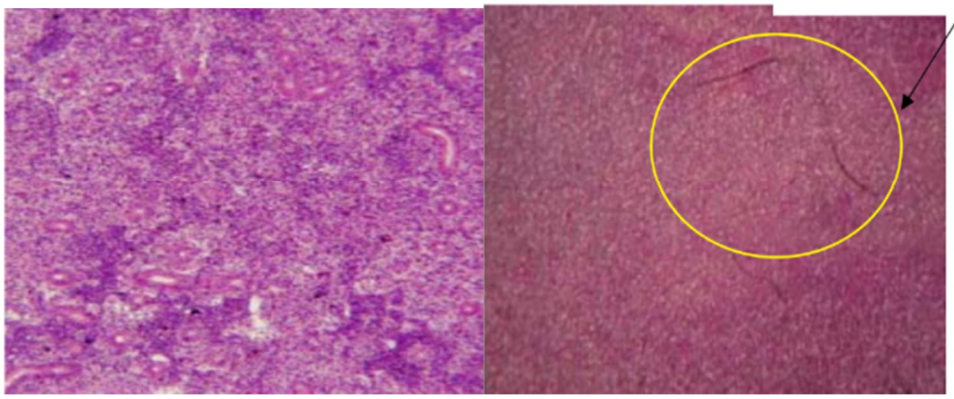


Plate7: Beth et al.,2007

Plate 8: According to Experiment done

3) Ceratomyxosis – Ceratomyxa shasta

The whole body whorled get stiffy and appear as twisted with swelling.



Figure 7 : Ceratomyxosis (According to present study)

4) Aphanomyces invadans

Development of water mould disease caused by Aphanomyces invadans, known as Epizootic ulcerative syndrome, Mycotic granulomatosis (MG) or Red spot disease (RSD) in which fishes suffered by Ulcerative lesions on the body texture.



Figure 8: Beth et al.,2007



Figure 9: According to Experiment done

At first, fish develop red spots on the skin. These lesions expand to form ulcers and extensive erosions filled with necrotic tissue and mycelium. This is followed by the development of granulomas on the internal organs and death.

A great biochemical variation found between the results of Normal, Fenvalerate and Cypermethrin during the blood biochemical examination in Clarias batrachus shown in Table-1(Kumar and Dhakad, April 2017).

Table 1: *Biochemical Results*

Test Level	Range	Normal	Fenvalerate	Cypermethrin
Bilirubin (Total Serum)	0.08-1.02 mg/l	0.11 (+)	0.05 (-)	0.06 (-)
ESR	20- 34 mm	22 (+)	10 (-)	22 (+)
SGOT (AST)	155.0-162.72 u/L	161.5 (+)	72.4 (-)	261.0 (-)
SGPT (ALT)	40.17-54.01 u/L	45.3 (+)	31.3 (-)	48.7 (+)
Post Prandial Plasma Glucose (After Meal)	109.8-184.5 mg/dl	151.6 (+)	71.4 (-)	271.9 (-)

Legends: (+): Significant towards Non-toxicity
(-): Significant towards toxicity

Bilirubin (Total Serum), ESR, SGOT (AST), SGPT(ALT), Post Prandial Plasma Glucose (After Meal) all were below their Lower Standard Limit and are more significant towards the toxicity of Fenvalerate whereas SGOT (AST) and Post Prandial Plasma Glucose (After Meal) both showed their harmful effects and were far away from their Upper Standard Limit range in Fish affected with Cypermethrin (Kumar and Dhakad, December, 2017).

Demerits of agents are also observed in behavioral and physiological such as aggression, slum secretion, without shoal, active less, stiffy barbels, discoloration of skin color, depressive skin. These symptoms highlighted the extent of suffering experienced by the fish during exposure to toxicity (Kumar, December 2024).

IV. CONCLUSIONS

1) When smear slide prepared after the sampling of blood at different stages of normal, toxic and herbal phases, it has been seen that fishes infected by many types of diseases during the whole experiment are as follow:Infectious Hematopoietic Necrosis (IHN)Virus (Smith and Mac Connell *et al.*,2007)
2) Cell Injury and Necrosis (Heidel and Smith, 2007)
3) Hemorrhage (Heidel and Smith, 2007)
4) Pigmentation as:

5) Monocytic leukemia
6) Melanoma
7) Infectious Diseases (Mac Connell *et al.*,2007) like as:
8) Coldwater Disease (CWD) caused by *Flavobacterium psychrophilia.*
9) Parasitic Diseases due to Myxopsporean as Proliferative Kidney Disease (PKD) infected by the parasite *Tetracapsula bryosalmonae.*
10) Ceratomyxosis due to presence of Ceratomyxa shasta
11) Development of water mould disease caused by Aphanomyces invadans, known as Epizootic ulcerative syndrome, Mycotic granulomatosis (MG) or Red Spot Disease (RSD) in which fishes suffered by Ulcerative lesions on the body texture.

During immunological study of Skin Prick Test (SPT) and Leucocytotoxic Test (LCT) following conclusion are come out:

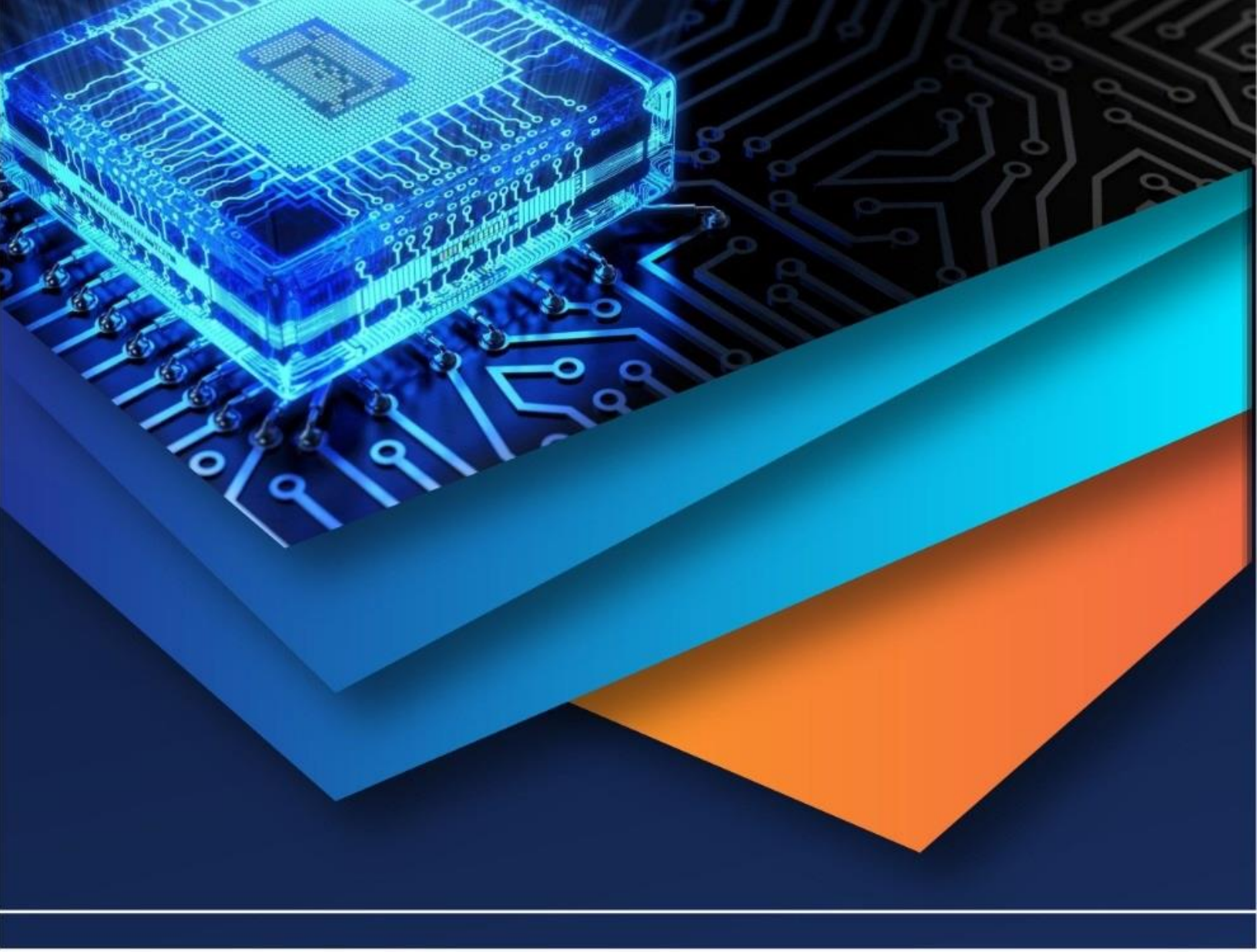
- In Skin Prick Test (SPT), it is observed that the whole body harshly affected by poison of Fenvalerate and Cypermethrin due to which body got stiffy and twisted after its application on the skin surface of fish.
- The head and tail portion of fish badly affected after interaction of blood with Fenvalerate and Cypermethrin.

V. ACKNOWLEDGMENT

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