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Study the Histopathological Effect of *Aristolochia bracteolata* L. on Common Freshwater Carp *Oreochromis Niloticus*

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Abstract: ***Effects of water-soluble extracts of the fresh mature leaves of Aristolochia bracteolata were studied on common freshwater carp Oreochromis niloticus and observed the Histopathological effect on different tissues, including liver, kidney, and intestine in the laboratory. First, select the two fishes for the experiment. Then, each fish was placed in a glass aquarium containing 10 litres of dechlorinated tap water and named treated and controlled. The 1.5 ml leaf extract exposed fish in the first aquarium; after that, the to and fro movement increased. At the end of the experiment, the mortality of fish occurred. Next, take the tissue sections of organs from both the fishes and treat and stain with haematoxylin and eosin. The stained slides were observed under a compound microscope at 45X and recorded in different observations. Finally, we concluded that the exposure of aqueous leaf extract of Aristolochia bracteolata L. resulted in apparent toxic effects on the kidneys of common carp Oreochromis niloticus.***

Keywords: Tilapia, Histology, Tissue, Embedding, Aristolochia bracteolate.

I. INTRODUCTION

Aristolochia bracteolate is poisonous and harmful to human beings and other animals. It is commonly known as birthwort, pipevine, or Dutchman's pipe. Aristolochia is widely occurring in the most diverse climates; some species like *A. utriformis* and *A. westlandii* are threatened with extinction. *Aristolochia* is a genus of evergreen and deciduous lianas (woody vines) and herbaceous perennials. In 1993, a series of end-stage renal disease cases were reported from Belgium associated with a weight-loss treatment; *Stephania tetrandra* is, an herbal preparation, was suspected of being substituted with *Aristolochia fangchi*[1]. *Aristolochia* is both a potent carcinogen and a kidney toxin. Epidemiological and laboratory studies have identified *Aristolochia* as a dangerous kidney toxin; *Aristolochia* has been shown to be associated with more than 100 cases of kidney failure [2]. Aristolochic acid was linked to aristolochic acid-associated urothelial cancer in a Taiwanese study in 2012 [3]. In 2013, two studies reported that aristolochic acid is a potent carcinogen. Whole-genome and exome analysis of individuals with known exposure to aristolochic acid revealed a higher somatic mutation rate in DNA [4]. Fishes, often called "rich food for poor people," provide essential nourishment, and exceptionally high biological value. The Nile tilapia, *Oreochromis niloticus*, is the most important freshwater fish in the ujani dam, Bhigwan. These fish species have great economic importance, where the Nile tilapia constitutes about 32% of the total country catch. In this concern, tilapia species are the most common fish in the Bhima River. These tilapia species are well adapted to survie in fresh and brackish water.

II. MATERIAL AND METHOD

A. Collection and Preparation of Sample

The Mature leaves of *Aristolochia bracteolata* were collected near the Airport of Baramati, Pune, and identified in the Department of Zoology, Vidya Pratishthan's Art, Science and Commerce College, Baramati (Fig.1). The leaves were thoroughly washed and used for extraction. First, aqueous leaf extract was prepared by dissolving 50 gm of leaves in 100 ml of distilled water using the Soxhlets apparatus. After extraction, the extracted sample is kept at room temperature for 24 hours in the laboratory. After 24 hours, the filtered mixture and extract were used immediately in the experiment.

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Fig 1 Aristolochia bracteolate

B. Selection of Model Organism for Experimentation

The freshwater fish *Oreochromis niloticus* (Tilapia) were selected for the experiment. The *Oreochromis niloticus* was chosen for the study because of its easy availability, hardy nature, rapid growth rate, and tolerance to varied environmental salinity [5]. Fishes were collected from Ujani Dam at Bhigwan, District: Pune. After the collection, it was acclimatised in a glass aquarium (10 litres) for one week. During acclimatisation, the fishes were fed with standard food pellets and were exposed to the natural day and night cycle. The acclimatisation was done at room temperature.

C. Determination of 24hr Sub- Lethal Toxicity Testing

The two fish were selected for the experimentation, and each fish was placed in a glass aquarium containing 10 litres of dechlorinated tap water. The twenty-four hours before the commencement of toxicity testing, stopped feeding the fish. The 1.5 ml extract of *Aristolochia bracteolata L.* was exposed in the first aquarium, and another set served as the control. The aquarium was observed for 24 h for clinical signs like skin pigmentation, swimming pattern, response to stimuli, and mortality. The 24 hr death was recorded in the test tank. One group of the fish did not receive *Aristolochia bracteolata L.* and served as the control group.

D. Histological Analysis

After the experimentation, the fishes proceeded for histological analysis. For histological examinations, organs like liver, kidney, intestine, and stomach were collected from the fish (Control and experimental). Collected organs were gently rinsed with physiological saline solution (0.9% NaCl) and immersed in a fixative composed of glacial acetic acid, formaldehyde, and ethanol (1:3:7). The fixed tissues were passed through the alcohol series 50 to 100% for dehydration and finally cleared in alcohol. Tissues were then embedded in paraffin wax (58°C). After embedding, the blocks were subjected to sectioning at 5µ in thickness in a rotary microtome. The tissue section slides were stained with Haematoxylin and Eosin stains. These slides were treated with xylene and mounted in DPX, observed under a research microscope attached to a digital camera, and photographed.

III. RESULT AND DISCUSSION

A. Major Chemical Constitutes of Aristolochia bracteolate

The *Aristolochia bracteolate* has been investigated for chemical constituents that have been characterised (Table 1). The 15 different secondary metabolites are commonly found in *Aristolochia*. It includes aristolochic acids and esters, aristolactams, aporphines, protoberberines, isoquinolines, benzylisoquinolines, amides, flavonoids, lignans, biphenyl ethers, coumarins, tetralones, terpanoids, benzenoids, steroids, and others respectively. However, most chemical constituents are pharmaceutically important, showing some clinical significance [6].

Table No 1: Major Chemical Constituents of *Aristolochia bracteolata*

Genus	Compound	Reference
Aristolochia bracteolata	Aristolochic acids and esters, Aristolactams, Aporphines, Protoberberines, Isoquinolines, benzylisoquinolines, Amides, Flavonoids, Lignans, Biphenyl ethers, Coumarins, Tetralones, Terpanoids, Benzenoids, Steroids and others.	*Ping-Chung Kuo et al., 2012*

B. Fish Mortality

The effect of water-soluble extracts of the fresh mature leaves of *Aristolochia bracteolata* was studied on common freshwater carp *Oreochromis niloticus* and observed mortality in set 1 (treated with extract) at 24 hr. Untreated control fish showed no mortality. After that, both the fishes were dissected and preceded for histological study.

Histology study of fish tissues:

There were different histological changes recorded in tissues of freshwater carp *Oreochromis niloticus*.

1) Liver

Untreated: In control fish, the histological structure of tissue appeared in normal shape and size. However, the liver tissue showed irregular lobules separated by the hepatocytes and bile ducts. Observed the polygonal cells with a central spherical nucleus and a densely stained nucleolus in the liver tissue.

Treated**:** The plant extract treated fish liver had slightly vacuolated cells showing evidence of fatty degeneration. In addition, necrosis was observed in some portions of the liver tissue, probably resulting from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification. Cells may also have led to necrosis.

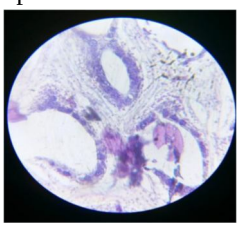


Fig.2 Control Liver

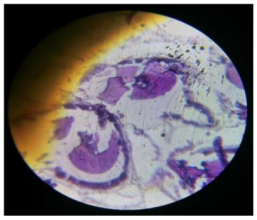


Fig.3 Treated Live

2) Kidney

Untreated: In this tissue, the nephron showed glomerulus and bowman capsule, and renal tubules consist of a single layer of epithelial cells. The distal tubules showed a more rounded shape and sharper apical surface cells. In addition, the cuboidal cells observed in collecting tubules it was smaller than the cells of proximal tubules.

Treated**:** In the tissue of the kidney observed several histological changes. First, the renal tubules became highly expanded, and their epithelial lining separated from tubular cells. Some renal tubules lose their cellular integrity. In renal tubules observed, dilation, oedema and hypertrophied nuclei. Glomeruli showed vacuolisation and disorganised blood capillaries. The mesenchymal tissue showed necrosis and pyknotic nuclei.

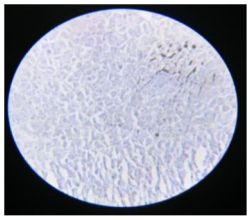


Fig.4 Control Kidney

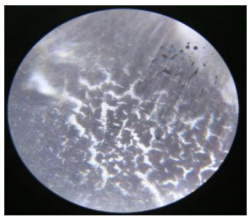


Fig.5 Treated Kidney

3) Intestine

Untreated: The outermost serosal covering consists of a single layer of epithelial cells and subserosal smooth muscle fibres arranged in a distinctive pattern: the outer being longitudinal and the inner circular. The submucosa consists of connective tissue fibres, nerves and blood vessels. The muscularis mucosa showed two layers of muscles (outer longitudinal and inner circular), and gastric mucosa epithelial coat forming the inner layer of columnar prismatic cells with basically located nuclei.

Treated: Due to adverse effects on the intestine, it shows a narrow intestinal lumen. The flattened and fused villi showed vacuolation in tissue, including disintegration and inflammation recorded at the base of the villi in tissue.

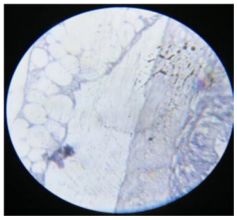


Fig.6. Control Intestine

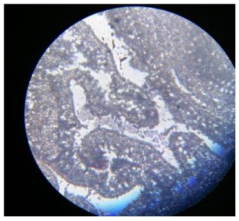


Fig. 7. Treated Intestine

IV. CONCLUSION

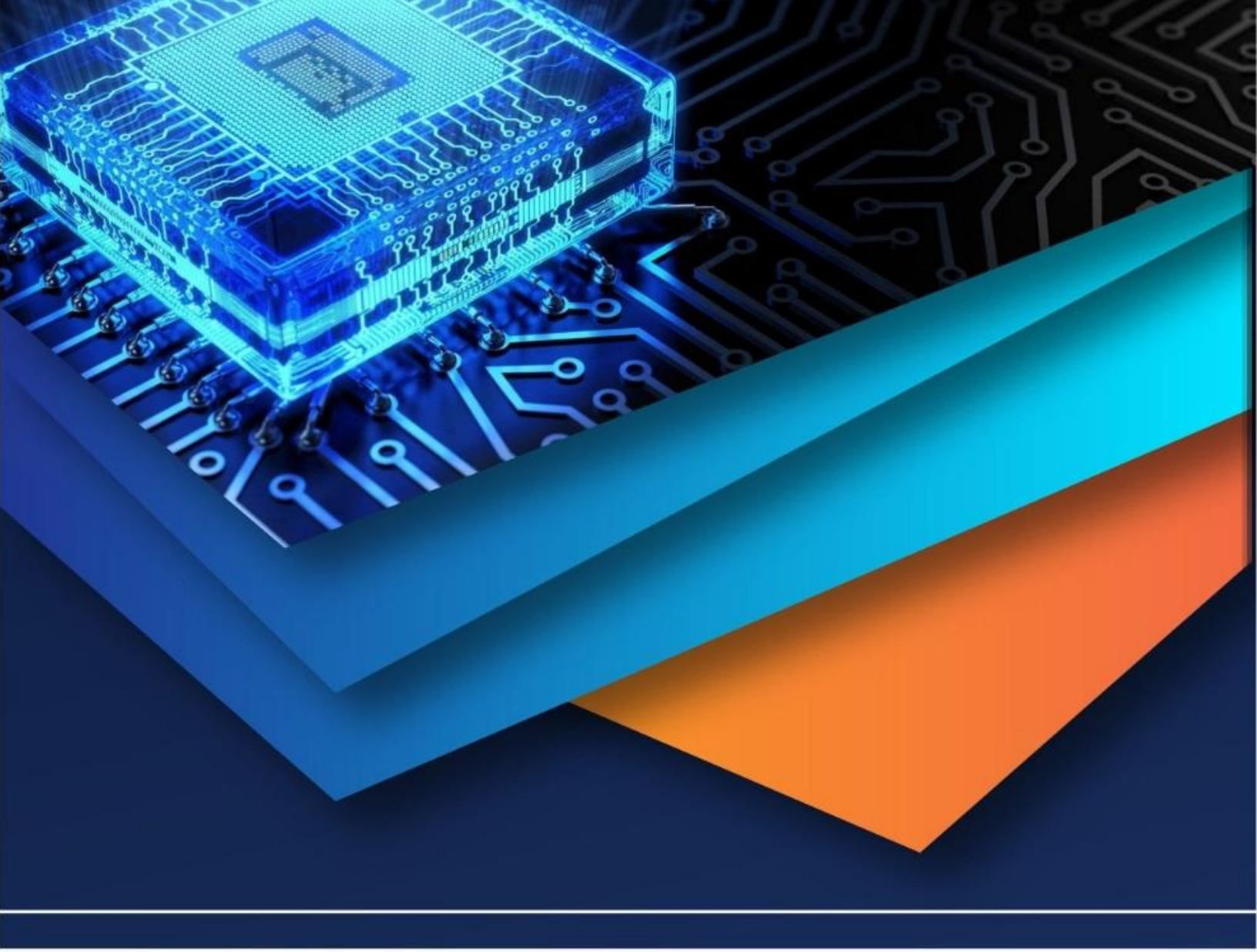
In the present study, exposure of aqueous leaf extract of *Aristolochia bracteolata L.* to common freshwater *Oreochromis niloticus,* resulted in apparent toxic effects on their kidney, which was more severe at 24 hr. Several plants have phytotoxic compounds, which have pesticide properties. Several researchers have reported this kind of study. The extract of *Aristolochia bracteolata L.* can poison fishes leading to morphological alterations in their tissues and organs.

V. ACKNOWLEDGEMENT

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