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A study on Histological changes in the fresh water fish, *Channa orientalis* exposed to the *Madhuca indica* toxicant.

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Abstract

Many plant species are used to catch fish by giving them plant extract, and if the extract is poisonous to fish, it is known as a piscicide. *Madhuca indica* is a piscicidal plant, and the extract of the dry flower of this plant is toxic to fishes after exposure to the extract i.e. LC50/5 at regular intervals of 24 hours, and the histological effect on the liver of the freshwater fish *Channa orientalis* was studied. Hepatic cells are loosely arranged or compactly arranged, central vain is inflated and dilated, portal vain is bordered by coagulated blood, and the hepatic artery is decreased or swollen, and other abnormalities seen in the liver. Gills showed severe lamellar fusion, hyperplasia, hypertrophy and epithelial lifting, swelling and deformed lamella, in some parts sloughing off and curving of lamellae was also observed. Kidney showed Necrosis, epithelial tubuli contraction, glomerular injury, reduction of renal hematopoietic system, tissue damage, glomerular constriction and proliferation of connective tissue. Fish have been found to be poisoned by the extract.

Key words: Madhuca Indica, Histology, LC50, gills, liver, kidney.

Introduction:-

Now a day there is a wide range of insecticide, pesticide and herbicide available in market for controlling pest in agriculture, these toxicants affect our environment. Our environment is very dynamic and interdependent system in which each part is related with other part of system or change in one part of system affect the other part of system. The living things are the part of environment and their relationship with land, air, water, soil, plants, animals and other biotic factors which affect the growth and development of man.

Over last several decades, botanical extracts have been shown much interest in aquaculture to control fish parasites, fish fry predators and unwanted fishes from aquaculture ponds as attempts to replace chemical pesticides and piscicides, since extensive and indiscriminate use of these non-biodegradable synthetic chemicals results in harmful impact on aquatic environment and presents high risk to the non-targeted organisms (Kumar et al. 2010; Das 2013). Plant extracts are considered promising agents because of their eco-friendliness, ease of availability, high efficiency, rapid biodegradability and reduced toxicity to non-targeted animals (Yunis Aguinaga et al. 2014). To date, a good number of plants have been investigated in different countries to evaluate their pesticidal (Rahuman et al. 2008; Dubey et al. 2010) and piscicidal activities (Murthy et al. 2010). However, commercially available plant products are still limited, and hence, efforts should be made to find out new sources of botanical pesticides and piscicides for rapidly growing pisciculture (Ramanujam and Ratha 2008). Plant extracts are referred to piscicides if they exert toxicological effects on fishes and cause death to these aquatic animals (Burkill 1995). Plant piscicides are obtained from a variety of plants belonging to different families and species that may vary considerably not only for their taxonomic variations but also for the plant parts used (leaves, barks, fruits and seeds), mode of use, mode of extraction and species of target fishes (Neuwinger 2004). Botanical materials contain a number of bioactive compounds that work either individually or synergistically as piscicides (Obomanu et al. 2007; Ramanujam and Ratha 2008). The degree of toxicity as well as piscicidal activity of any plant extract can be assessed by exposing fishes to it and subsequent estimation of the median lethal concentration (LC50) (Ramanujam and Dominic 2012). One of the common toxicological effects of plant extract is to bring about changes in hematological and biochemical parameters of fishes (Winkaler et al. 2007; Kavitha et al. 2012), which affect biological and physiological activities of fish.

Capturing of fishes from river and streams by the fishermen's is the old practice in the history of mankind. Fish contain very good and cheap source of high quality of animal protein. Capturing of fish by the fisherman's is not only by the nets and traps but also the various plants as fish poisons in tribal and rural areas of India.

Since long ago Tribal people were using the various plant toxins for capturing fishes such as leaves of *khinna (Sapium insigne), rambans(Agave Americana), stem bark of jamun (Syzygiumcumini), tatex of surai (Euphorbia royleana), leaves and bark of akhrot (Juglansregia), bark of agali (Acacia pennata), fruits of chilla (Casearia elliptica) andmaindul(Catunaregamspinosa)* (K.S. Negiand K.S. Kanwal 2008)

Many plant species are used for catching the fish by giving them plant extract are called botanical and if it is poisonous to fish are called piscicides . such plants contains some active ingredients like alkaloids, resin, tannin, saponin, nicotine, diosgenin etc. and these ingredients are toxic to fish at high concentration. Application of such plant extract to the river or streams also affect to the ecological system due to the poisoning of water bodies the larval forms of the fishes, crabs, molluscs also died, and due to the flow of the river or stream the lower level water bodies also contaminated and the necessary animals, macro

vertebrates also killed by the plant toxins and it lead to contamination and disturbed of food chain. These pollution is disturbed the mutual balance of the environment.

The Piscicidal plants which contain the active ingredients which cause the physiological effect in fish most of the plants have the medical values. Piscicidal plants may be useful in developing eco-friendly method to eradicate fishes from agriculture pond without using any harmful chemicals. So that there is need to explore more environment and health friendly fish toxicants from plants to superfy and catch fishes.

Mahua (*Madhucaindica*) is one of the piscicidal tree used for capturing the fishes in India, the mahua tree is found in all over in Maharashtra and other part of India. Flower of these trees are used for fishing by the tribal's. Such as stem bark extract, Leaves extract, seed extract. These extractions are made by the people near the river or stream, and it used in stagnant water or in the pond where the water is stagnant. (K.S. Negiand K.S. Kanwal 2008). Madhuca longifolia (Madhuca indica; Sapotaceae) is an indigenous tree to the Indian subcontinent. It is a deciduous tree abundantly distributed in Jharkhand, Chhattisgarh, Madhya Pradesh, Orissa and Bihar and parts of Gujarat, Andhra Pradesh, Rajasthan and TamilNadu.It has various vernacular names, namely, Mahua or Mowarh (North), Mahul (Orissa) and Illipi (Southern part of India). Sanskrit versions of Mahua are Madhuka, Madhupuspa, Madhudruma, Madhusakha and Gudapuspa (Ranjana et al., 2018). M. Indica plant is a storehouse of numerous phytochemicals which are used by the tribal people for ethno-medicinal purposes. In ayurvedic system of medicine, the flowers find application as aphrodisiac, astringent, demulcent with cooling properties. They are uses as general tonic and to treat helminths, acute and chronic tonsillitis, pharyngitis as well as bronchitis (Chandra, 2001). The flowers are also recognized to possess antioxidant and anti-microbial properties (Sinha et al.,2017). It is observed that Mahua flowers are open dried under sun before storage. The flowers get spoiled easily by absorbing atmospheric moisture because of their hygroscopic nature. The tribal people are bound to sell their collections immediately with little value addition or without value addition.

Material and Method:-

The toxicity will be observed by obtaining the parts of different plant. For the experimental purpose the fishes of same size about 3 to 4 inches in length and 15 to 20 gms.in weight is being collected from nearly situated water bodies the fish were observed, if pathological symptom's shown they were transfer in 1% KMNO4 solution for 10 minute before keeping in aquarium so that it to avoid any dermal infection. The fishes is being rinse wither and then acclimated in laboratory condition for 15 days in separate aquarium. During acclimatization the fishes were fed daily on natural aquarium food for avoiding malnutrition effect. The test water is changed after 24 hrs. if mortality occurred in these condition date fishes were removed immediately. Only active fishes were chosen for experimentation and don't fed them 48 hr. before the start experiment to avoid any changes in toxicity of plant Extract from Excretory product. The procedure for bio assay was adapted from the standard method of APHA (2005).

Preparation of extract:

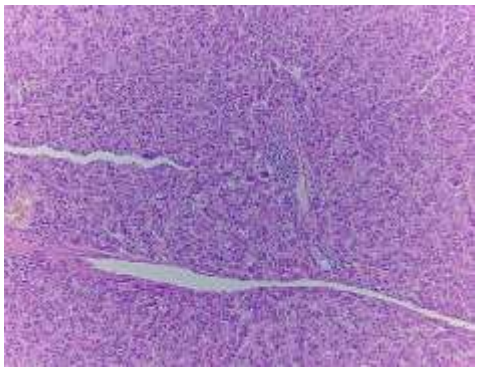
Dried Madhuca indica flower was powdered with mechanical grinder to obtain a coarse powder. The 10g powder was mixed with 50% 100ml ethyl alcohol in soxhlet apparatus at 60^0C. the solvent was completely removed by vacuum evaporator. Obtained extract was then mix with 100ml distilled water for 2 hours before use. From this concentrated extract the sub lethal dose was count and i.e. LC50, the LC50 value is 500ppm and for the experiment LC50/5 concentration of extract is used.

The two sets of fishes was made, one is experimental set which is treated with plant extract and one is controlled set was similarly maintained in tap water free from any kind of extracts. At interval of 24 hour i.e. at 24 hrs, 48 hrs, 72 hrs, and 98 hrs. the organ liver were collected from both controlled and experimental sets of fishes. To avoid post-mortem effect dissected tissue kept in saline solution and fixed in Bouins fluid overnight. After approximately 20 hrs, tissues were preserved in 70% ethanol. Dehydration was done in progressively graded alcoholic series and embedded in melted paraffin wax. The prepared blocks of embedded tissue was trimmed and get the transverse sections of 5µ thickness with the help of rotary microtome. The sections were stained with the help of double staining method and finally mounted with DPX.The prepared tissue slides were examined under microscope and photographs were taken with advance microscopic camera at x10 and x40 magnifications.

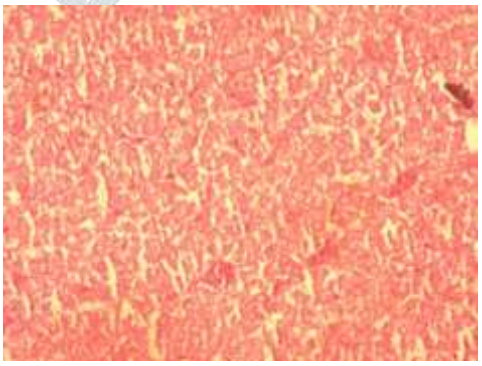
Result and Discussion :-

Histological examination of tissues is a useful method to determine the effects of plant toxicants. Since majority of toxicants accumulate in gills, major damage has been reported in this organ by most of the workers. Liver is the main metabolic organ where detoxification occurs; therefore, it would also be susceptible to damage by the toxicants. Plant extracts if toxicant produce lesions and damage the tissues.

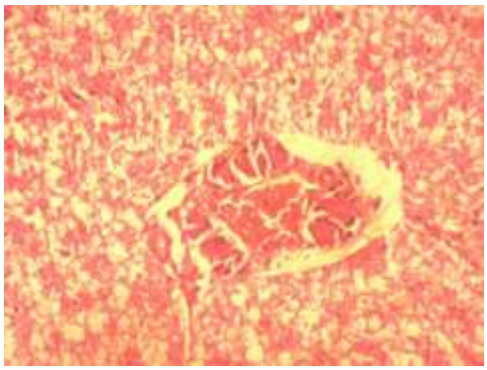
Figure:- 1



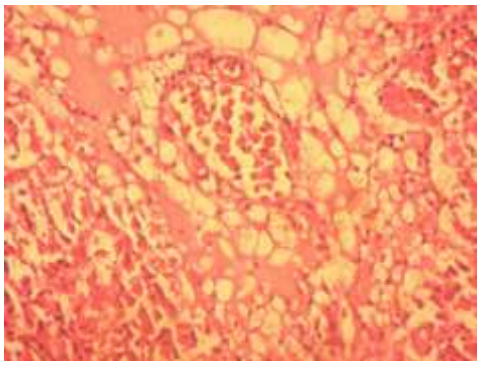
(a) Control



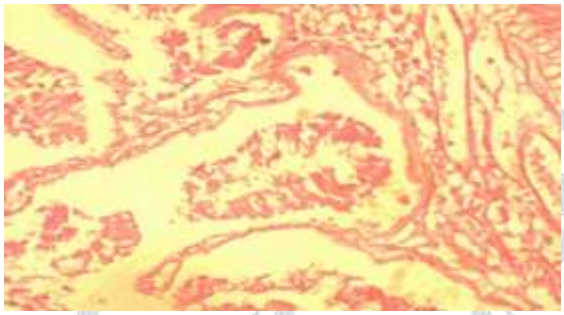
(b) 24hrs liver tissue



(c) 48 hrs liver tissue



(d) 72 hrs liver tissue



(e) 96 hrs liver tissue

Effect of *Madhuca Indica* dry flower Extract was tested at LC50/5 concentration i.e. 100ppm, at the interval of 24 hrs. on liver histology of fresh water fish *channa orientalis*.

In prepared slides found that the Hepatic cells were compact in the central region, and vacuolated at border. Blood sinusoids were extended and dispersed. The portal vain is bigger and blood was clotted inside. The Hepatic arteries were less in size and blood vessels were dilated. This effect was increased with the time of exposed of extract.

Therefore in our study, In gills of fishes, the mean lengths of primary and secondary lamellae decreased, curving of primary lamellae, separation of respiratory epithelium and lamellar fusion in secondary lamellae, loss of secondary lamellae, ballooning dilation and clavate lamellae formation at the tip of secondary lamellae were also noticed. secondary lamellae showing edematous condition, alterations in chloride and mucous cells, proliferation of cartilage in primary lamellae, pyknotic nuclei, hyperplasia, atrophy, hypertrophy, necrosis, erythrocyte were observed.

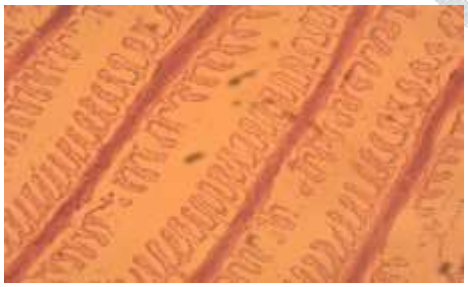
Generally, various histological lesions observed in exposed fish liver are distorted hepatocytes, vacuolization, necrosis of parenchyma, pyknosis and infiltration of leukocytes, damage to hepatopancreas, acute swelling, hemorrhage and development of large adipocytes. Such damages had been reported in liver of Clarias gariepinus exposed to fuel oil for 14 days, Channa punctatus exposed to hexavalent chromium and sugar mill effluents, Clarias batrachus to ZnSO4 (Prasanna, 2011)

It has been noticed that only toxicant exposed liver shows vacuolation and pyknosis (Karyomegaly). Degeneration of liver tissue and necrosis could be due to the infiltration of leukocytes, and according to Hughes et al., 1996 necrosis is the direct toxic effect of the pollutant and therefore most commonly reported. According to Hinton and Lauren, 1996, vacuolation in liver cells was the result of inhibition of protein synthesis, depletion of energy reserves or changes in substrate utilization. Lipidosis appears when the hepatocyte membrane disintegrates; consequently, the fat globules fuse with neighboring hepatocytes and it is irreversible (Wolf and Wolfe, 2005). Hepatic lipidosis may be correlated with lipid per- oxidation and suppression on non-enzymatic antioxidant like vitamin E (Ferguson, 1989). The livers of some fishes also contain exocrine pancreatic tissue, which is termed as hepatopancreas, aligned along the blood vessels in interstitial areas. The negative aspect of this arrangement is that it could lead to the spread of inflammatory and non-inflammatory diseases of pancreas into the adjacent hepatic parenchyma. On the other hand, the operative advantage of this arrangement has yet not been explored.

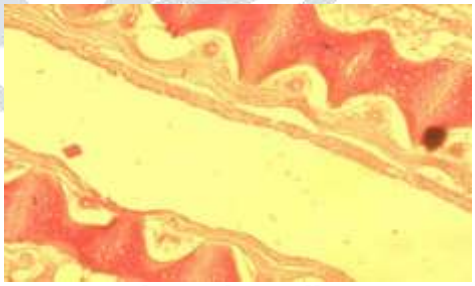
Histopathological examinations confirmed the deformities in gills, kidney and liver of the fish and results of Liver are shown in Figure 1. Figure 1 (a) Showed liver of experimental control (Channa orientalis) which had network of hepatocytes and bile duct. While Figure 1 (b and c) revealed the histological damage that occurred in liver, necrosis of parenchyma, vacuolation, congestion of blood vessel, pyknosis and infiltration of leucocytes were the major observations.

Gill of control fish Figure 2 (a) showed normal structure while exposed fish sections Figure 2 (b,c,d,e) showed severe lamellar fusion, hyperplasia, hypertrophy and epithelial lifting, swelling and deformed lamella, in some parts sloughing off and curving of lamellae was also observed.

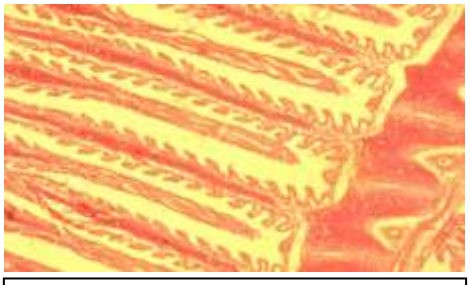
Figure:- 2



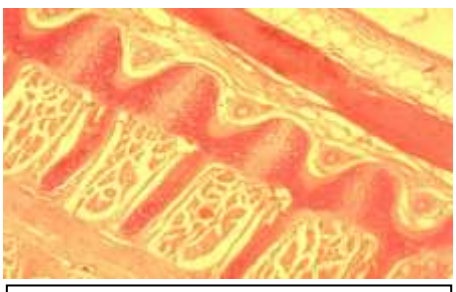
(a) Control gill



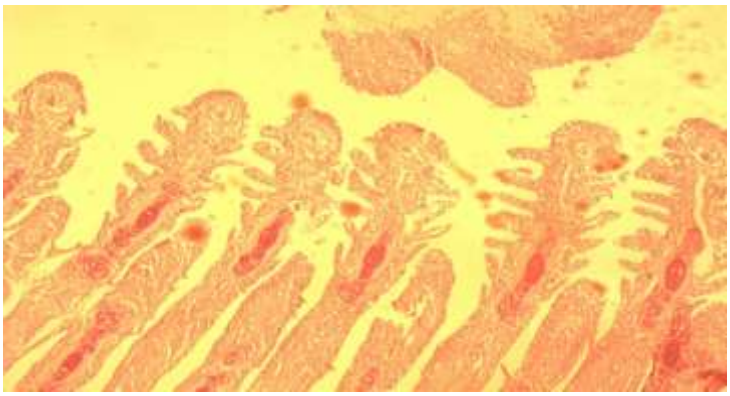
(b) 24 hrs gill



(c) 48 hrs gill



(d) 72 hrs gill

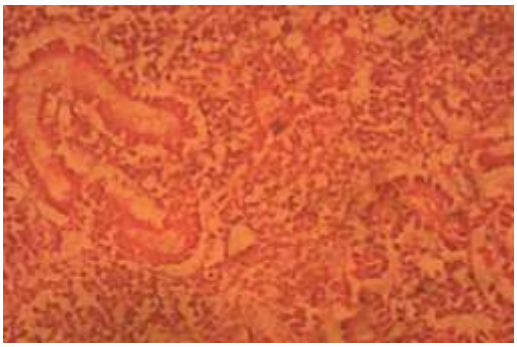


(e) 96 hrs gill

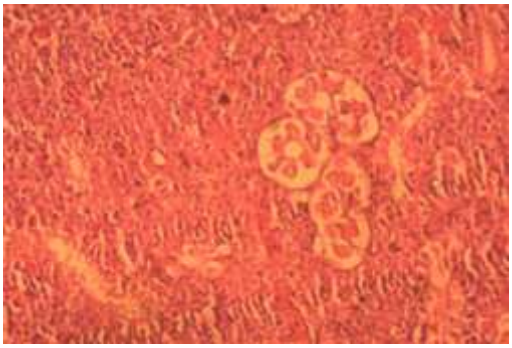
Gills are the main respiratory organ in fish and are covered by thin epithelium which is the site of exchange of gases, regulation of ionic and acid-base balance and nitrogenous waste excretion. In the current study epithelium was found to be degenerated in the exposed fishes and got separated from the lamellar tissue. The toxic exposure also induced hyperplasia, gill bridging, lamellar fusion in order to increase the distance for diffusion across the cells to reach the bloodstream. This has also been demonstrated by other investigators (Javed et al.,2015 ; Javed and Usmani 2016).

Kidney sections of the reference group in Fig. 3(a) showed a normal structure such as glomeruli, bowman's space with uniform renal tubules and the interstices of the tubules contain hematopoietic tissue. However, increased bowmans space, damaged glomeruli and renal tubule, necrosis, hypertrophied renal tubule, large vacuolation, hyperplasia, constricted renal tubule and glomeruli and granuloma formation in chronologically exposed fish renal tissue (Fig. 3b–e) were the major hallmarks. Necrosis, epithelial tubuli contraction, glomerular injury, reduction of renal hematopoietic system, tissue damage, glomerular constriction and proliferation of connective tissue related with madhuca extract intoxicant water have also been documented in other reports (Al-Bairuty et al.,2013).

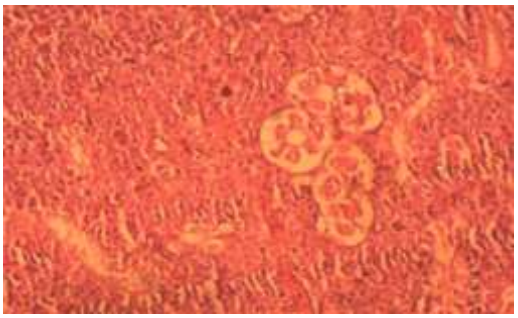
Figure:- 3



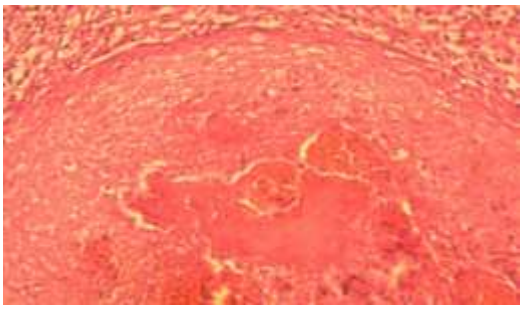
(a) Control kidney



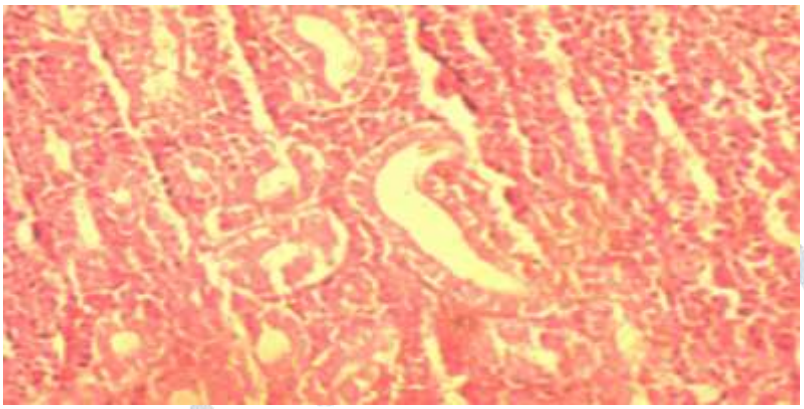
(b) 24 hrs kidney



(c) 48 hrs kidney



(d) 72 hrs kidney



(e) 96 hrs kidney

Therefore, the histopathological damage may affect the normal physiological functions of the organs.

Discussion

This study has been conducted to evaluate piscicidal activity of Madhca indica extract against a snakehead fish (Channa orientallis). To do so, effective concentrations of the madhuca extract were first determined by exposing fishes to various concentrations of the extract. Fishes were also exposed to a lethal concentration to determine the alteration in hematological parameters, since fluctuation in these parameters during exposure of fishes to a toxicant indicates the chronic toxicity in these animals (Kori-Siakpere and Ubogu 2008). However, exertion of the toxicological and hematological effects of any plant extract on fish depends on its bioactive compounds (Shahi and Singh 2011), which could vary significantly in response to various factors, particularly solvent system used for extracting these compounds from the plant materials (Tung et al. 2007).

Therefore, ethanol extract was chosen and fishes were exposed to it during toxicity and acute toxicity tests. In any aquatic system, water quality is considered one of the most important factors for its flora and fauna (Zabed et al. 2014), and any significant fluctuation in the quality attributes may affect lives in this ecosystem. When plant extract or synthetic toxicant is added to water, it causes considerable fluctuation in different quality parameters (Srivastava et al. 1995). In particular, DO level has been reported to decrease when plant extract is added to water, and consequently, fishes have encountered a hypoxic condition (Winkaler et al. 2007).

The percent mortality of fishes exposed to the extract showed significant positive correlation with doses and exposure times, which had revealed the fact that higher dose of the extract and exposure time could exert higher piscicidal activity, possibly for causing enhanced intake of the active components into fish bodies (Tiwari and Singh 2003). Increase in fish mortality over time could be due to activities of some factors that may act individually or conjointly (Singh and Singh 2002). However, excessive dose of extract and longer exposure time have negative effect on a potential agent from both economic point of view and effects on non-targeted organisms in the ecosystem. Therefore, a moderate lethal dose is expected that can exert its piscicidal activity within a prescribe period of time, and 96 h has been considered to determine lethal doses in various investigations (Kumar et al. 2010; Ramanujam and Dominic 2012).

The LC50 values of Madhuca extract against Channa orientalis varied between 12.7 mg/l for 24 h and 4.71 mg/l for 96 h in our study, which are quite comparable with those of the findings as reported earlier and conducted with various chemical and botanical extracts against different fish species. The 96-h LC50 values have been reported 124.0 mg/l for Moringa oleifera seed extract against Cyprinus carpio (Kavitha et al. 2012), 54.65 mg/l for alcoholic extract of Nerium indicum leaf against C. punctatus (Tiwari and Singh 2003), and 56.8 mg/l for alcoholic extract of Euphorbia royleana, which were much higher than that of Madhuca extract in this study. Likewise, the 24-h LC50 of neem leaf extract has been reported 4.8 g/l against Prochildu lineatus (Winkaler et al. 2007), which was again much higher than 12.7 mg/l as obtained in our study for 24 h.

This comparison have revealed higher potential of madhuca extract as piscicide due to its lower LC50 values than those of other plant extracts. Likewise, the 96-h LC50 value of this plant extract was found to be lower than the effective doses of two synthetic chemicals such as malachite green and cypermethrin studied against the Cat fish, and the values have been reported for 5.6 and 7.2 mg/l, respectively (Mishra et al. 2005).

Conclusion

This study has revealed that ethanol extract of madhuca extract is highly toxic to an air-breathing, hardy carnivore and fresh water snakehead fish (Channa orientalis) at low concentration, since the 96-h LC50 value of the bark extract for this fish species was found to be only 4.71 mg/l. Acute toxicity test on the fish specimens using a lethal concentration of the extract (96 h LC50) showed that it had brought about significant alteration in Histology of fish such as Gills, Muscles, kidney and Liver. Fluctuations in these essential attributes of fishes due to exposure to the extract indicate its mode of action as piscicide. Therefore, Madhuca extract could be a potential plant-derived fish poison to catch fishes or control unwanted species from aquaculture ponds. Therefore, C. orrientalis can suitably be used as a bioindicator for monitoring the water quality of the canal. It is hence suggested that the fish histology can be used as a model system for the study of the adverse effects of toxicants in general and Plant extracts in particular. It is especially because Vital organs like Gills, Liver, Kidneys and Muscles always remain in direct contact with the ambient

environment, counteract first to the xenobiotics, and are metabolically active organ. These plant extracts no doubt are useful to mankind, but on the other hand, they have adverse impacts on aquatic habitats, their diversity, and the livelihood of humans.

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