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Research Article

EFFECT OF *ZIZIPHUS JUJUBE* LEAF EXTRACT ON KIDNEY OF FRESHWATER FISH, *OREOCHROMIS MOSSAMBICUS*

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ABSTRACT

The effects of *Ziziphus jujube* leaf extract on the histopathology of kidneys of the freshwater *Oreochromis mossambicus* were investigated. The LC50 after 96 hrs of exposure to the aqueous extracts of elanthai *Ziziphus jujube* were 0.11 ml/l and 0.33 ml/l, respectively. Histopathological changes of kidneys were recorded when effects of *O. mossambicus* were exposed to 10% and 30% of 96 hrs LC50 of each aqueous extract for 15 days. Fish kidneys exposed to sub lethal concentration of 10% (0.11 ml/l) *Ziziphus* leaf extract slightly changed the structure of Bowman's capsule, Glomerular shrinkage, Neurotic changes, 30% (0.33 ml/l) of *Z. jujube* leaf extract affect the level of severity of lesions increase in exposure time. Renal capsule, Tubular, Distal convoluted tubule; Erythrocytes in glomerular capillaries were severely damaged. Therefore the present study concluded that the aqueous extracts of *Z. jujube* should be used with precaution in the agricultural fields. Nevertheless these plants can be recommended as an environmentally sound alternative for synthetic processes for fish. It will provide new opportunities.

Keywords: LC50, Bowman's capsule, Glomerular shrinkage, Neurotic Changes, Tubular.

INTRODUCTION

Plants may provide leaf extract for the control of pests as they constitute a rich source of bioactive chemicals (Kim *et al*., 2011). Indiscriminate and extensive use of insecticides to protect crops possesses a serious threat to humans and the surrounding environment (Padmapriya *et al*., 2017). Recent studies have demonstrated that insecticidal properties of chemicals derived from plants are active against specific target species; biodegradable to non-toxic products and potentially suitable for use in integrated pest management (Markouk *et al*., 2000). Many plants contain chemicals which have traditionally been used to harvest fish and also to monitor various pests in almost all parts of the world (Ayoola *et al*., 2011). Recent studies have proved that extremely low quantities of pesticides which enter the aquatic environment can affect productivity of organisms to kill eggs and larvae (Jayalakshmi *et al*., 2017) though the plant products are toxic they are degraded easily within 7-12 days and safe for users (Tiwari & Singh, 2004). Aquatic ecosystems that run through agricultural or industrial areas have a high probability of being contaminated by runoff and ground water leaching by a variety of toxic pesticides which pose a potential direct threat to freshwater organisms, particularly to sensitive animals, such as fish (Usha *et al*., 2017).

Recently the applications of medicinal plants from different families in the management of aquaculture ponds are gaining momentum because they are safe, effective, widely available and inexpensive. Also, to produce fish free from any fish predators. (Martinez *et al*., 2004) stated that aqueous extract of *Ziziphus jujuba* leaves and other *Z. jujube* based products have been extensively used in fish-farms as alternative for the control of fish fry predators such as dragon-fly larvae. Although *Z. jujuba* has been used successfully in aquaculture systems to control fish predators (Dunkel & Richards, 1998). Indiscriminate and extensive use of insecticides to protect crops possesses a serious threat to humans and the surrounding environment (Padmapriya *et al*., 2017). *Oreochromis mossambicus is* a tilapiine cichlid fish native to southern Africa. It is a popular fish for aquaculture. Dull coloured, the *Mozambique tilapia* often lives up to a decade in its native habitats. Due to human introductions, it is now found in many tropical and subtropical habitats around the globe,

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where it can become an invasive species because of its robust nature. These same features make it a good species for aquaculture because it readily adapts to new situations. Heavy metals enter into aquatic habitats by a number of routes and cause hazardous effect on their morphology and physiology. This may lead acetate to the destruction of beneficial species either indirectly through breaking the biological food chain or directly by affecting the aquatic forms of life (Pichaimani *et al*., 2017). Environmental pollution is tangled with the unsustainable anthropogenic activities, resulting in substantial public health problems. Khan & Sharma, (2012) reported that the U.S population from infectious disease to disease such as cancer, birth defects, and asthma, many of which may be associated with environmental exposures.

Environmental pollution can cause biological changes in organisms. These changes can be measured and used as indicators of environment pollutants, which are called biomarkers .These biomarkers enable rapid assessment of the health of organisms and warn about possible environment risks (Van der Oost *et al*., 2003). Histology is the microscopic examination of tissue in order to study the manifestation of disease. The histological changes provide a rapid method to detect effects of irritants, especially chronic ones, in various tissues and organs (Bernet *et al*., 2001). Histological studies on fish have revealed that various toxicants have produced various histological changes in the tissues such as micro biotic changes in the liver, tubular damage of kidney, gill and lamellar abnormalities (Ramalingam *et al*., 2000). Histopathological investigations have long been recognized to be reliable biomarkers of stress in fish. These can be used as biomonitoring tools or indicators of health in toxicity studies since they provide early morning science of disease and the health of the aquatic environment (Rand & Petrocelli, 1985).

MATERIALS AND METHODS

Collection of fish

Fishes were collected from Chumbath farm, brought to the laboratory in well packed polythene bags containing oxygenated water. Chumbath has an average weight of 15 - 20 gram (10cm long). In the present study, fishes were exposed to different concentrations of *Ziziphus jujube* aqueous leaf extract on the kidney of freshwater fish, *Oreochromis mossambicus* for histological studies.

Preparation of aqueous leaf extract

Fresh leaves of *Z. zuzuba* were collected and washed in tap water and dried in shade for ten days. After complete drying, the leaves were pulverized to fine powder in an electric blender. 50% of aqueous leaf extract was prepared by dissolving 50 grams of powdered leaves in 1 liter of distilled water and kept at room temperature for 24 hrs, with intermittent shaking. After 24 hrs the mixture was filtered and the extract was immediately collected into beaker (Saravanan *et al*., 2011).

Experimental Setup

Z. jujuba leaf extract was dissolved 100mg/L of water. The fishes were grouped into three groups such as group I, II and III. The group I fishes were maintained as control. Group II and III were exposed to the extract of 10% and 30% concentration of *Z. jujuba* for 15days. At the end of the experimental period, the fishes were sacrificed and samples were collected for further analysis. Kidney separated from the experimental fish was used for histopathological analysis of tissue sample. Kidney was excised from the fish of control and experimental groups were fixed with 10% formalin solution. After proper dehydration of graded alcohols paraffin blocks were prepared. It was cut into 4-5 µm thick ribbons in a rotator microtome and was stained with Eosin and Hematoxylin. The histological changes were observed and photographed, (Humason, 1972). Histological methods in life science (Tamizhazhagan & Pugazhendy, 2017).

RESULTS AND DISCUSSION

The present study investigated the histological changes on kidneys of *O. mossambicus* in normal condition and exposed to experimental conditions by long term exposure to leaf extract. This leaves has water soluble, which is known as “Ber” in Iran and India. It is widely used for medicine as a laxative and blood purifier. In China, it is used as a taste enhancer and is recommended for treating fatigue, loss of appetite and diarrhea. It is believed the dried fruits of *Z. jujube* are anodyne; anticancer, pectoral, sedative, stomachic, styptic and tonic and immune response enhancers. Some of the compounds isolated from the seed of *Z. jujube* exhibit significant pharmacological activities. In India it is used as food, fodder, nutrient, medicine, construction material and fuel. *Z. jujube’s* medicinal properties are attributed to a diverse group of secondary metabolites such as alkaloids flavonoids, terpenoids, saponin, pectin, triterpenic acid lipids (ie) *Jujuboside* (saponin) isolated from jujube is reported to have hemolytic, sedative and sweetness inhibiting properties. Kidneys are the core organs which come in contact with environmental pollutants, Paradoxically, they are highly vulnerable to toxic chemicals because firstly, their large surface area facilitates greater toxicant interaction and adsorption and secondly, their detoxification system is not as robust as that of liver (Franklin *et al*.,1987; Mallatt, 1985). Additionally, absorption of toxic chemicals through the Kidney is rapid and therefore toxic response in the kidney is also rapid. Kidney has frequently been used in the assessment of impact of aquatic pollutants in marine as well as freshwater habitats (Haaparanta *et al*., 1997; Mir *et al*., 2012).

The 96hrs LC50 values of freshwater fish, *O. mossambicus* was determined in the laboratory studies 1.1ml/lit LC50 value for 96hrs was presented in the Figure 2. In this study, fish species were exposed to 0.11ml/lit and 0.33ml/lit sublethal concentrations which correspond to 10% and 30% of the 96 hrs LC 50 respectively. Effect of

Z. jujube leaf extract on the Kidney histology of the fish, *O. mossambicus*, Kidney tubules and haematopoietic cells were normal and systematically arranged in the control fish species. Control group showing compact renal mass and renal tubules. The normal renal cortex contains glomeruli, other vessels, tubules and interstitial. Normally, all the glomerular capillaries have the same thickness, which is very, thin (almost wispy). With normal cellularity, cell nuclei are not clustered or overlapping. In the cortex but not the medulla, the tubules are almost back to back .i.e. the tubular basement membranes are almost touching. There is very little interstitium in the cortex.

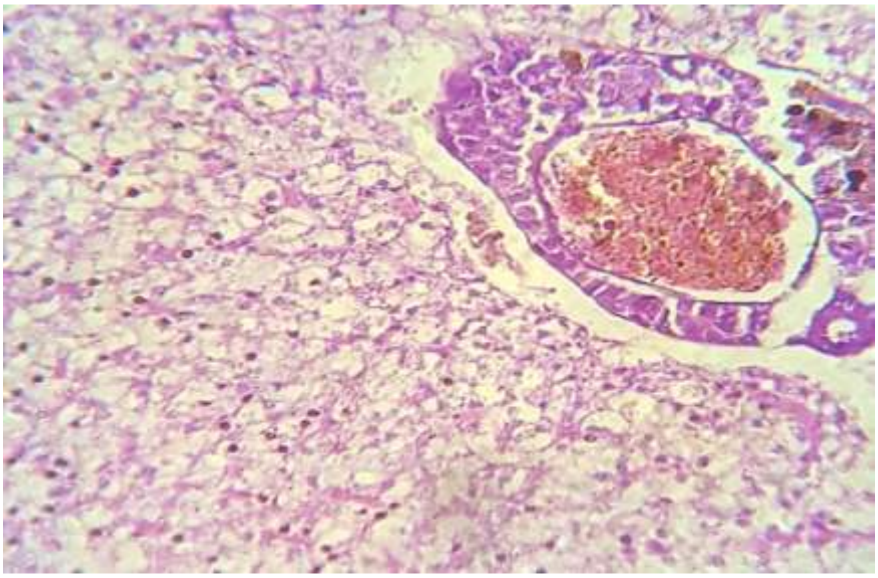

Figure 1.The normal architecture of the control fish kidney showing a clear Bowman's capsule (BC), Glomerular shrinkage (GS) and Necrotic changes (NC).

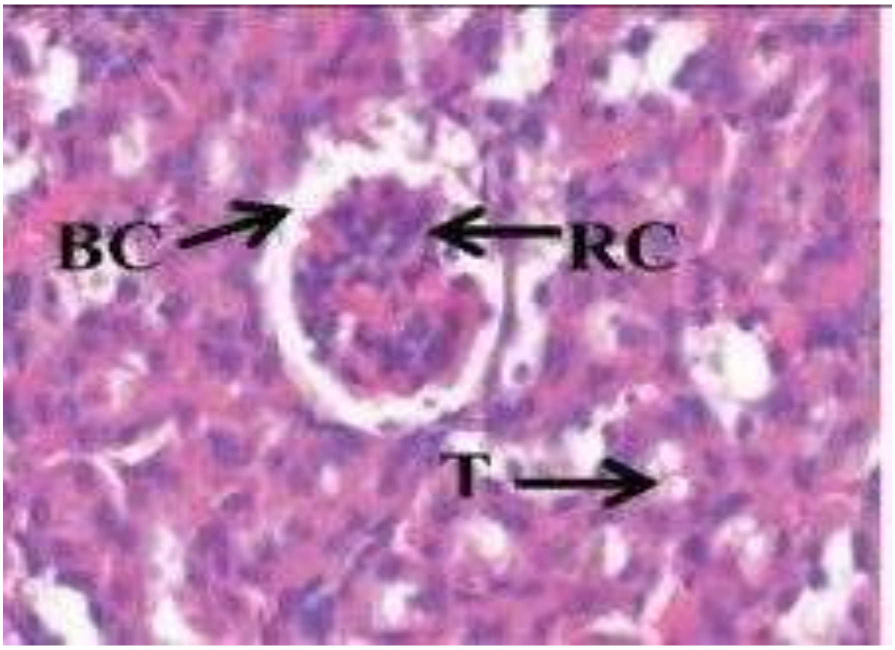

Figure 2.The kidney of the fish exposed to leaf extract for 15 days showing Bowman's capsule (BC), Renal capsule (RC),Tubular (T).

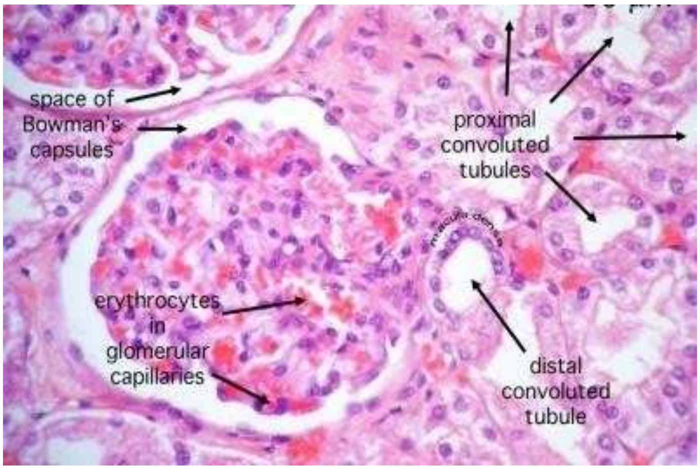

Figure 3. The kidney of the fish exposed to leaf extract for 15 days showing Space of Bowman's capsule, Erythrocytes in glomerular capillaries and Distal convoluted tubule.

10% Concentration of *Z. jujube* leaf extract treated on Kidney of fresh water fish *O. mossambicus*. On exposure for a periods of 15 days to leaf extract (0.45ppm), compared to the control, some structural changes that were observed in the kidney of the fish, *O. mossambicus* concentration in the glomerulus renal tubule, expansion of space inside the Bowman's capsule, and initiation of the degeneration of hematopoietic tissue, renal lesions, dilation of tubular lumina, tubular necrosis, epithelial desquamation, necrosis of interstitial hematopoietic tissues, tubular vacuolation and glomerular shrinkage.

The 30% concentration of *Z. jujube* leaf extract treated on Kidney of fresh water fish *O. mossambicus*. On an exposure for a period of 15 days to leaf extract (1.35 ppm) further increase in the glomerular shrinkage and in Bowman's capsule was observed. The other pathological changes that were observed are appearance of formation of pyknotic nuclei in the hematopoietic tissue. Necrosis in tubular epithelium, and desquamation of epithelial lining cell and glomerular leakage and widespread pycnosis of cells, glomerular swelling, and construction of tubular lumina and hyalinization of almost all renal cells indicated inflammatory condition of the organ. Complete damage in the structural integrity of the kidney was noticed.

Histopathological alterations in the kidney were hydropic swelling and exfoliation of tubules, vacuoles accumulation in tubular cells, and many necrotic areas. Tubular degeneration and necrosis were also observed. Long term exposure to these products causes countless abnormalities and reduces the life span of organisms (Usha *et al*., 2017). These alterations were after 48 hours exposure and more severe in the acute exposure 72 and 96 hours and prolonged in chronic exposure for 3 months. In the subchronic exposure, the kidney was the first target organ and the effect was more severe than in the gills and liver (Imamura & Hasegawa, 1984). Similar result was observed in this study. Changes in glucose concentration are most often associated with renal injury (Padmapriya *et al*., 2017). The kidney of the fish exposed to leaf extract concentration showed dilation of Bowman's space and accumulation of hyaline droplets in the tubular epithelial cells of the first proximal tubule. (Omoregie & Okpanachi, 1997) studied the effects of linuron herbicides on the rainbow trout (*Oncorhyn chusmykiss*) and their results showed small cytoplasmic vacuoles, nuclear deformation in the epithelial of the second segments of the proximal tubule. The kidney cells were observed to have been massively destroyed. The renal corpuscles of the kidney were scattered resulting in their disorganization and consequently obstruction to their physiological functions. Some of the kidney cells were found clogging together while they disintegrated in some tissue of the organ. The same result came from this study. The present investigation showed that, the RBC, haemoglobin and haematocrit values are significantly decreased in cypermethrin treated fish when compared to control, (Jayalakshmi *et al*., 2017). The proximal tubule in mammals and fishes is involved in reabsorption and lysosomal degradation of macromolecules (Walsh-Monteiro *et al*., 2014). During this process the *Ziziphus* leaf extract are excreted through kidney and appears to cause Necrosis and vacuolation were observed in *Cyprinus carpio* exposed to *Z. jujube* (Pazhanisamy & Indra, 2007) observed a number of striking changes in the histological structure of the kidney of *Channa punctatus* exposed to sub lethal concentration of 0.01 ppm of the above result was observed this study *Andrographis paniculata* for a span of 3 0 days found that the shrinking of glomerulus was the visible sign of intoxication.

Fingerings infected with toxic in the kidney showed fusion and loss of secondary lamellar epithelium. The pathological effects included an increase in the thickness and the damage to the mucosa. The parasitic infection in turn disturbed the metabolic pathways (Esch *et al*., 2002). Similarly muscle of *Labeo rohita* fingerlings, infected with toxic showed fragmentation of muscle fibers and necrosis. The histopathology of muscle duplicated progressive damage in the structure of muscle with increasing concentration of the sago effluent. Similar observations were reported by (Nagarajan & Kumar, 2002) in the muscle tissue of the fish *Cirrhinus mrigala* with increasing concentrations of sago effluent. Kidney of fishes receives abundant volume of post branchial blood, and therefore, renal lesions might be exposed to be good indicators of toxicant exposure (Cengiz, 2006). Kidney of *O. mossambicus* exhibited nephrotoxic lesions, including degenerative changes in tubular epithelium (cytoplasmic vacuolation and pyknotic nuclei), dilation of tubular lumina, and casts within tubular lumina (probably resulting from protein leakage), tubular necrosis and epithelial desquamation and necrosis of interstitial hematopoietic tissues. The presence of glomerular inter-capillary thickenings noted on 8^{th} day exposure to the leaf extract, from the precursor of protein leakage culminating in the deposition of casts in tubular lumen due to the prolongation of toxic effect. Changes of this kind seemed possible as given by Ahmad, (1976). Loss of different staining with disintegration of tissue structures and cell membranes led to the sighting of basophilic areas (homogeneous mixture of granular debris that is basophilic to purple with haematoxylin and eosin) which is an indication of necrotic changes. Basophilic areas giving way to tubular vacuolation, degeneration and casts, glomerular shrinkage and capsular thickening observed on 30 day after exposure dictates the temporal increase in renal lesions due to leaf extract. Velmurugan *et al*. (2007) observed widespread changes, more similar to that registered in the present study, in the kidney of *Cirrhinus mrigala* due to exposure to the leaf extract fenvalerate. Haematological parameters can provide information on nutrient status, digestive function and routine metabolic level of fish may be confronted with stress factors such as varied water quality, pollution and disease; fish can adapt themselves to bad environmental conditions by changing their physiological activities (Pichaimani *et al*., 2017).

Kidney is a highly dynamic organ in most of the vertebrates. Kidney receives about 20% of the cardiac output. Any chemical substances in the systemic circulation are delivered in relatively high amounts to this organ. Thus a nontoxic concentration of a chemical in plasma could become toxic in the kidney. The kidney of the receives largest proportion of post branchial blood, and therefore renal lesions might be expected to be good indicators of environmental pollution (Joseph & Raj, 2011). In the present study the most evident changes observed in the kidney of leaf extract treated groups were glomerular congestion, pyknotic nuclei and renal tubular architecture loss m-cresol treated group showed histopathological alterations such as necrosis and vacuolation of tubular epithelial cell. It was also observed that in both the treated groups epithelial cells have become swollen and basophilic. (Virk *et al*., 1987) observed lesions in the kidney tissues of fish exposed to endren and carbaryl characterized by degeneration in the epithelial cells of renal pyknotic nuclei in the hematopoietic tissue, dilation of glomerular capillaries, degeneration of glomerulus, intracytoplasmic vacuoles in epithelial cells of renal tubules with hypertrophied cells and narrowing of the tubular lumen. (Cengiz *et al*., 2001) kidney may be also due to deficiently as a result of kidney degeneration and to the vascular dilation and intravascular haemolysis with subsequent stasis of blood. Thus in our present investigation we have recorded degeneration of kidney tubules and haematopoietic cells, necrosis, pyknosis and haemorrhage. That means if the process of exposure of the leaf extract will continue then ultimately the whole architectural change will be noticed. This will finally hamper the normal anatomy and functioning of the kidney. All the histological observation methods in life science (Tamizhazhagan & Pugazhendy, 2017) indicates that the exposure to sublethal concentration of leaf extract caused destructive effects in the kidney tissue of *O. mossambicus*.

CONCLUSION

The present study revealed that the Kidney are susceptible to high degree of damage even to a very low amount of toxicant present in the water. As the low concentration of the toxicants in the water may not be detectable by ordinary water analysis techniques and may be considered as within the safe limits, but in actual practice the vital organs especially the Kidney are adversely affected. The histological changes observed especially in fish used in this study shows that pollution is affecting freshwater fish living in these aquatic bodies and that this is seriously threatening an agricultural region. Urgent measures must be taken to correct this situation before it becomes a critical issue for the region.

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REFERENCES

Ahmad Anees, M. Intestinal pathology in a freshwater teleost, *Channa punctatus* (Bloch) exposed to sub-lethal and chronic levels of three organophosphorus insecticides. *Acta Physiologica Latino Americana*. 1976; 26(1), 63-67.

Ayoola, S. O., Kuton, M., Idowu, A., & Adelekun, A. (2011). Acute Toxicity of Nile Tilapia (*Oreochromis Niloticus*) Juveniles exposed to aqueous and ethanolic extracts of *Ipomoea aquatica* leaf. *Nature and Science*, 9(3), 91-99.

Bernet, T., Ortiz, O., Estrada, R. D., Quiroz, R., & Swinton, S. M. (2001). Tailoring agricultural extension to different production contexts: A user-friendly farm-household model to improve decision-making for participatory research. *Agricultural Systems, 69*(3), 183-198.

Cengiz, E., Ünlü, E., & Balcı, K. (2001). The histopathological affects of Thiodan® on the liver and gut of mosquitofish, Gambusia Affinis. *Journal of Environmental Science and Health, Part B, 36*(1), 75-85.

Cengiz, E. I. (2006). Gill and kidney histopathology in the freshwater fish *Cyprinus Carpio* after acute exposure to deltamethrin. *Environmental Toxicology and Pharmacology, 22*(2), 200-204.

Dunkel, F. V., & Richards, D. C. (1998). Effect of an azadirachtin formulation on six nontarget aquatic macroinvertebrates. *Environmental Entomology, 27*(3), 667-674.

Esch, G. W., Barger, M. A., & Fellis, K. J. (2002). The transmission of digenetic trematodes: style, elegance, complexity. *Integrative and Comparative Biology, 42*(2), 304-312.

Franklin, D. R., Evans, A. D., & Hansen, W. J. (1987). Energy-Recycling Scissors Lift: Google Patents.

Haaparanta, A., Valtonen, E., & Hoffmann, R. (1997). Gill anomalies of perch and roach from four Lakes Differing In Water Quality. *Journal of Fish Biology, 50*(3), 575-591.

Humason, G. (1972). Animal Tissue Technique. 3rd Ed. W.H. Freeman and Co. *San Fransisco*, 1-492.

Imamura, T., & Hasegawa, L. (1984). Role Of Metabolic Activation, covalent binding, and glutathione depletion in pulmonary toxicity produced by an impurity of malathion. *Toxicology and Applied Pharmacology, 72*(3), 476-483.

Jayalakshmi, S., Pugazhendy, K., Tamizhazhagan, V., Sakthidasan, V., Jayanthi, C., & Sasikala, P. (2017). therapeutic efficacy of *Aloe vera* against the effect of cypermethrin toxicity in the fresh water fish *Cyprinus Carpi*. *International Journal of Zoology and Applied Biosciences, 2*(6), 386-391.

Joseph, B., & Raj, S. J. (2011). Pharmacognostic and phytochemical properties of *Ficus carica* linn an overview. *International Journal of Pharmtech Research, 3*(1), 8-12.

Khan, S., & Sharma, N. (2012). A study on enzymes acid phosphatase and alkaline phosphatase in the liver & kidney of fish gambusia affinis exposed to the chlorpyrifos, an organophosphate. *International Journal of Pharmaceutical Sciences Review and Research, 13*(1), 88-90.

Kim, J., Lee, K. W., & Lee, H. J. (2011). Cocoa (*Theobroma cacao*) seeds and phytochemicals in human health nuts and seeds. In: *Health and Disease Prevention,* 351-360.

Mallatt, J. (1985). Fish gill structural changes induced by toxicants and other irritants: a statistical review. *Canadian Journal of Fisheries and Aquatic Sciences, 42*(4), 630-648.

Markouk, M., Bekkouche, K., Larhsini, M., Bousaid, M., Lazrek, H., & Jana, M. (2000). Evaluation of some moroccan medicinal plant extracts for larvicidal activity. *Journal of Ethnopharmacology, 73*(1-2), 293-297.

Martinez, C., Nagae, M., Zaia, C., & Zaia, D. (2004). Acute morphological and physiological effects of lead in the neotropical fish *Prochilodus lineatus. Brazilian Journal of Biology, 64*(4), 797-807.

Mir, F. A., Shah, G. M., Jan, U., & Mir, J. I. (2012). Studies on influences of sublethal concentrations of organophosphate pesticide; dichlorvos (Ddvp) On Gonadosomatic Index (Gsi) of female common carp, *Cyprinus carpio* Communis. *Am-Euras Journal of Toxicology Science, 4*(2), 67-71.

Nagarajan, K., & Kumar, R. (2002). Effect of sago effluent on selected physiological aspects of the fresh water fish *Labeo rohita*. *Journal of Ecotoxicology and Environmental Monitoring, 12*(3), 233-239.

Omoregie, E., & Okpanachi, A. (1997). Acute toxicity of water extracts of bark of the neem ptarrt, *Azadirachta Indica* (Lodd) to the cichlid, *Tilapia zilli* (Gervais). *Acta Hydrobiology*, 47-51.

Padmapriya, K., Pugazhendy, K., Tamizhazhagan, V., Sakthidasan, V., & Jayanthi, C. (2017). Impact of simazine and chelate properties of *Solanam xanthopium* is the freshwater fish *Cirrhinus Mrigala* Hematological studies for the period of 120 Hours. *International Journal of Pharmacy and Biological Sciences, 7*(3), 185-195.

Pazhanisamy, K., & Indra, N. (2007). Toxic effects of arsenic on protein content in the fish, *Labeo rohita* (Hamilton). *Nature, Environment and Pollution Technology, 6*(1), 113-116.

Pichaimani, N., Pugazhendy, K., Tamizhazhagan, V., Sakthidasan, V., Jayanthi, C., & Sasikala, P. (2017). Ameliorative effect of *Solanum Virginianum* (Lin) against lead acetate toxicity in the fresh water fish *Cyprinus carpio* on hematological alteration. *International Journal of Zoology And Applied Biosciences, 2*(6), 392-398.

Ramalingam, V., Vimaladevi, V., Narmadaraji, R., & Prabakaran, P. (2000). Effect of Lead on Haematological and biochemical changes in fresh water fish *Cirrhina mrigala*. *Pollution Research, 19*(1), 81-84.

Rand, G. M., & Petrocelli, S. R. (1985). Fundamentals of Aquatic Toxicology: Hemisphere. *Methods and Applications*.1-683.

Saravanan, M., Ramesh, M., Malarvizhi, A., & Petkam, R. (2011). Toxicity of neem leaf extracts (*Azadirachta Indica* A. Juss) on some haematological, ionoregulatory, biochemical and enzymological parameters of indian major carp, *Cirrhinus mrigala*. *Journal of Tropical Forestry And Environment, 1*(1).

Tamizhazhagan, V., & Pugazhendy, K. (2017). Histological methods in life science. *International Journal Biomedical Materials Reserach, 5*(6), 68-71, 14-26.

Tiwari, S., & Singh, A. (2004). Toxic and sub-lethal effects of oleandrin on biochemical parameters of fresh water air breathing murrel, *Channa Punctatus* (Bloch.). *Indian Journal of Experimental Biology*, 42(04), 413-418.

Usha, R., Pugazhendy, K., Tamizhazhagan, V., Sakthidasan, V., & Jayanthi, C. (2017). Potential Efficacy of *Tribulus terrtri* aginst toxic impact of chlorpyrifos on haematological alteration in the fresh water fish *Oreochrommis mossambicus*. *International Journal of Zoology and Applied Biosciences, 2*(5), 232-240.

Van Der Oost, R., Beyer, J., & Vermeulen, N. P. (2003). Fish bioaccumulation and biomarkers in environmental risk assessment: A Review. *Environmental Toxicology and Pharmacology*, *13*(2), 57-149.

Velmurugan, B., Selvanayagam, M., Cengiz, E. I., & Unlu, E. (2007). Histopathology of Lambda-Cyhalothrin on tissues (gill, kidney, liver and intestine) of *Cirrhinus Mrigala*. *Environmental Toxicology and Pharmacology*, *24*(3), 286-291.

Virk, S., Kaur, K., & Kaur, S. (1987). Histopathological and biochemical changes induced by Endrin and Carbaryl in the stomach, intestine and liver of *Mystus tengara*. *Indian Journal of Ecology, 14*(1), 14-20.

Walsh-Monteiro, A., Pragana, W., Costa, E. T., & Gouveia Jr, A. (2014). Behavioral and histological effects of Rotenone in fish (Guppy, *Poecilia Reticulata*). *Psychology & Neuroscience, 7*(4), 619.