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Insights into the role of *Moringa oleifera* in ameliorating acetaminophen induced hepatotoxicity in Nile tilapia, *Oreochromis niloticus*

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Abstract

The study was aimed to investigate the hepato-protective effect of Moringa oleifera in *Nile tilapia (Oreochromis niloticus) exposed to acetaminophen. Fishes exposed to sub-lethal concentration of acetaminophen for 96 hours, were fed on feed incorporated with moringa leaf, for 21 days. Histological studies of liver of fish fed with M. oleifera leaf incorporated feed , for 21 days after 96-hour acetaminophen exposure showed significant reparative changes when compared to the control. The experiment indicate that dietary supplementation of moringa leaf had hepatoprotective effect in Nile tilapia exposed to acetaminophen.*

Keywords: *Acetaminophen*, *Moringa oleifera, histopathology, hepatoprotection*

Running title: Role of *Moringa oleifera* in ameliorating acetaminophen induced hepatotoxicity

The presence of pharmaceuticals and active pharmaceutical ingredients (APIs) in the environment have become an emerging problem in recent decades (Heberer, 2002). Acetaminophen (N-acetyl-para-aminophenol, paracetamol, APAP) is one of the most widely used over-the-counter analgesic and antipyretic agent. Although acetaminophen has a good safety profile at therapeutic levels, it can cause severe hepatic and renal damage when administered in high dose in both in experimental animals and in humans (Agrawal and Khazaeni, 2020; Ghosh and Sil, 2007). Liver being the major organ involved in detoxification shows alterations in response to exposure to harmful components. Conventional hepatoprotective drugs used for the treatment of such adverse reactions are often inadequate. Traditional herbal drugs are significant repositories of chemical constituents which are hepatoprotective in nature. There is an increasing demand for phyto-drugs and therefore it is essential to explore new medicinal plants to ameliorate the hepatotoxic effects of acetaminophen.

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Moringa oleifera, commonly known as drum-stick, is found mostly in Asia, Africa and South America. It is also known as miracle tree due to its medicinal properties. All parts of this tree such as seed, leaf, root, bark, flower etc. have medicinal value. *Moringa oleifera* is used as an anti-inflammatory, antioxidant, antidiabetic, antiproliferative, anti-ulcer, hypolipidemic and hepatoprotective agent (Al-malk and El Rabey, 2015; Saini *et al*., 2016; El Rabey *et al*., 2018; Elbakry *et al*., 2019). The main objective of this study was to examine the ameliorative and hepatoprotective effect of *M.oleifera* leaves in acetaminophen induced toxicity in Nile tilapia.

Materials and methods

Moringa oleifera leaves collected locally from Ernakulam district, were washed in clean water and dried under shade for 7 days. Using a mechanical homogenizer the leaves were powdered. The fine powder was stored in a clean and dry container at room temperature. Acetaminophen exposed fishes were fed with feed mixed with drum stick leaf powder, at a rate of 2.5g/day/kg for 21 days.

The male *O. niloticus* of mean length 15 ± 1 cm weighing about 100 g were collected from fish farm at Maliankara, Ernakulum district in Kerala, India. The fishes were brought to the laboratory and acclimatized for a week (pH 6.5, temperature 28°C, dissolved oxygen 3.73mg/L).

The entire experiment was divided into two phases. In the first phase, a control group of 15 fishes after acclimatization, was kept in dechlorinated tap water (Group I). Another group of 15 fishes (Group II) were kept in water containing 1/5th LC_{50} of acetaminophen (0.33 ml of acetaminophen per litre of water) for 96 h. The xenobiotic level was maintained constant, after renewal of water every day. After four days, 5 fishes each from Group I and II were sacrificed (Rema, 1995), liver dissected and separately pooled. The liver tissue, washed and wiped thoroughly with blotting paper to remove blood and other body fluids, was then fixed in Bouins fluid for 24 h and processed further to form the wax blocks. Blocks with the tissue were cut into thin section of five micrometer thickness and stained with haematoxylin and eosin. The sections were observed under phase contrast microscope with attached photomicrography (NIKON ECLIPSE 80 i) under 400 X magnification.

During the second phase, the remaining 10 fishes from Group II were again grouped into two and was subjected to feeding study. One group was fed on normal fish feed (proximate composition: protein 30%, fat 5%, fiber 5.5% and moisture 11.5%) and formed the control for the second phase of experiments. The second group was fed with f normal fish feed incorporated with moringa leaf powderfor 21 days. After the period of feeding, liver was carefully dissected out and processed as described earlier.

Results and discussion

In the present study, liver of control group showed normal arrangement of hepatic parenchyma with centrally placed nucleus and clear cytoplasm. Each hepatocyte had clear spherical nucleus. Hepatocytes were located among sinusoids and arranged as cords (Fig.1-A) Generally healthy teleost fish showed normal architecture with a typical parenchymatous appearance without pathological abnormalities (Vicentini *et al*., 2005; Figueiredo *et al*., 2007).

Liver of fish exposed to 1/5th LC_{50} of acetaminophen for 96 h showed acute congestion (Fig.1-B). In some regions, hepatocytes were hypertrophied with degeneration of cytoplasm . Necrosis of hepatocytes was seen in almost all regions of liver. Distended sinusoidal space with blood is indicative of acute congestion. Blood vessels were also engorged within the pancreatic tissue also. There was vacuolation in the periphery of hepatic parenchyma and extensive congestion with sinusoids severely expanded with blood. Surrounding the sinusoids there was a fatty infiltration. Pancreatic tissues were highly congested and bile duct was hyperplastic. Degeneration of hepatocytes, nuclear pyknosis in most cells and accumulation of metal binding proteins in its nuclei were reported in the liver of cadmium treated *O. niloticus* (Kaoud *et al*., 2011). Kavitha *et al.*(2011) reported

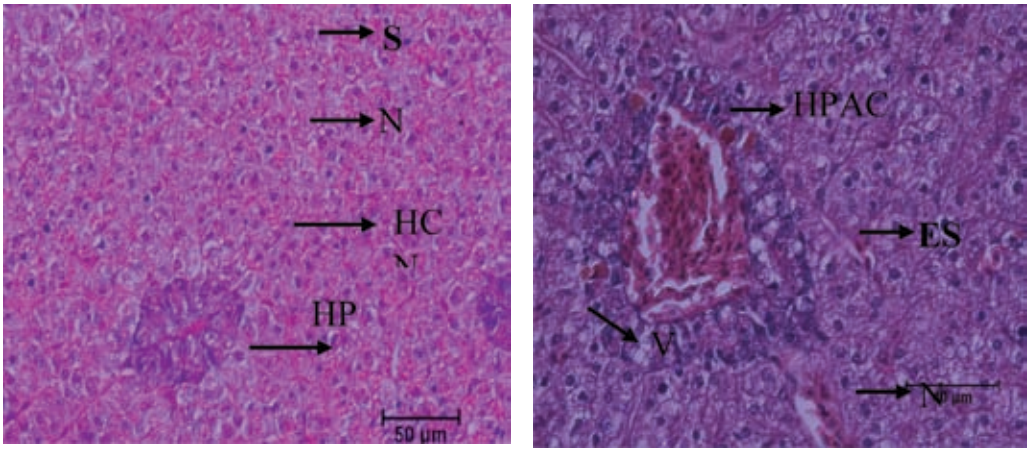

Fig. 1. Histological changes in liver of *Nile tilapia* in first phase of experiment

A-normal liver (control) showing uniform arrangement of hepatocytes (HC) with normal nucleus (N), normal sinusoids (s) and normal hepatopancreas (HP). **B**. acetaminophen treated liver ($1/5^{th}$ LC_{50} 96 h) showing acute congestion in hepatopancreas (HPAC), expanded sinusoid (ES), vacuolation (V) and necrosis (N).

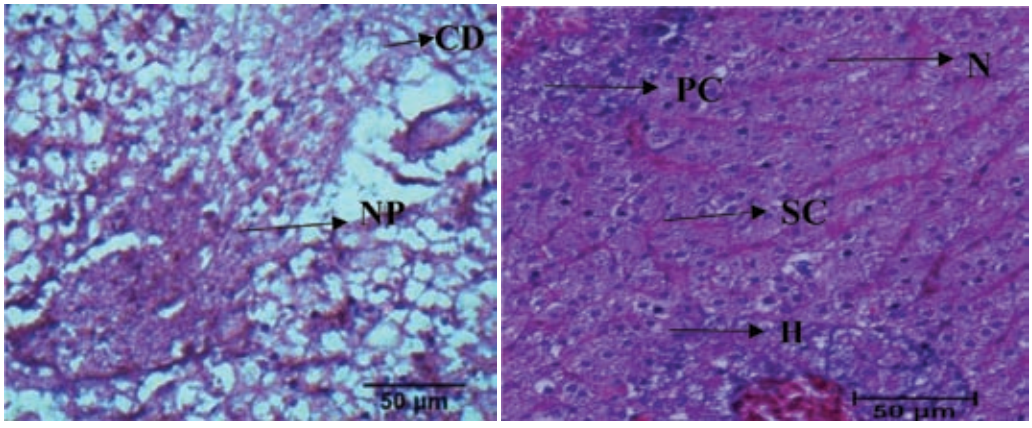

Fig. 2. Histological changes in liver of Nile tilapia in the second phase of experiment

A. Paracetamol treated control liver showed cell death (CD) and nuclear pyknosis (NP). **B.** Liver of moringa leaves fed group showed comparatively mild sinusoid congestion (SC), pancreatic congestion (PC), normal nucleus (N) and normal hepatocytes (H)

hepatocellular vacuolation and blood vessel congestion in *O. mossambicus* exposed to acetaminophen at 500mg/Kg orally. Hepatic injuries like degeneration of vacuoles in the cell and necrotic aggregation of cells that was associated with oxidative stress, were observed in *Danio rerio* exposed to acetaminophen (Jyotsna, 2016).

The liver of control group in the second experiment (acetaminophen treated) showed diffused degenerative changes like extensive pancreatic and hepatocyte congestion. Signs of cell death could be observed with most cells Nuclear pyknosis was a consistently observation. Cytoplasmic architecture was also completely lost compared with the test group. Blood vessels were highly congested and filled with blood. Erythrocyte infiltration into sinusoids was observed. Loss of reticular fibres, which cross links and act as a supporting mesh in soft tissues of liver, was a common feature (Fig. 2. A). Liver is one of the main organs which regulates many important metabolic functions and hepatic injury directly affects metabolic functions of the liver (Mitra *et al*., 1998).

Histopathological evaluation plays a major role in the assessment of the harmful effects of xenobiotics (Reddy and Rawat, 2013). Van der Oost *et al*. (2003), reported that histopathological examinations have long

been recognized to be reliable biomarkers of fish exposed to stress. Hepatotoxicity is one of the very common disorders resulting in serious debilities ranging from severe metabolic disorders to mortality (Patel *et al*., 2008). Paracetamol is considered as a safe drug, if consumed at correct dose rates. But overdose may lead to dangerous and fatal conditions (Penna and Buchanan, 1991), as this drug is hepatotoxic at high concentrations. The liver is one of the major organs responsible for the metabolism of endogenous and exogenous compounds and one of the first target organs to toxic insults (Cao *et al*., 2016).

Compared to control group, liver of fish fed with feed containing moringa leaves, showed lesser signs of hepatotoxicity. In moringa leaf treated group, hepatocytes had normal arrangement with prominent nucleus. Hepatic cells maintained their round polygonal shape. The size of the hepatic cells reflects their physiological functional state. In some regions the cells showed mild enlargement, mild nuclear hypertrophy and sinusoids showed minute swelling. Mild pancreatic congestion and mild vacuolation were also observed in some regions (Fig. 2. B). Uma *et al*. (2010) and Fakurazi *et al*. (2012) observed that *M. oleifera* leaves were able to protect acetaminophen-induced liver damage by decreasing liver enzymes and hepatic lipid peroxidation as well as by increasing antioxidant enzyme levels. Many plants and their products have the potential to protect liver from toxic effects of hepatotoxins. This is due to the presence of active constituents such as alkaloids, sterols and flavonoids which have free radical scavenging activities (Anwer *et al*., 2008). In our study also, the tissues of liver of moringa fed fish showed considerable reparative changes. Ross and Kasum (2002) reported, that plant secondary metabolites such as flavonoids have much antioxidative, anti-inflammatory, anti-proliferative, radical-scavenging activity and pro-apoptotic effects in various cell types. Tekle and Sahu (2015) investigated the ameliorative effects of moringa flower on *O. niloticus* subjected to *Aeromonas hydrophila* induced stress. Moringa leaves have efficient antioxidant properties (He *et al*., 2018). The beneficial functions of *M.oleifera* are closely associated to its high content of phytochemicals such as flavonoids, glucosinolates, phenolic acids and isothiocyanates (El-Hadary and Ramadan, 2019).

Conclusion

The present study emphasises the protective role of moringa leaves against acetaminophen induced damages in liver tissue of *Nile tilapia*. Administration of moringa leaves partially restored the general structure of the liver. Moringa leaves may be considered as a potential source of natural antioxidants and phytochemicals against hepatotoxicity.

Conflict of interest

The authors declare that they have no conflict of interest.

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