

Assessment of *Datura stramonium* and Chlorpyrifos-Induced Cytotoxicity in Liver of *Catla catla*



Anjali Namdeo^{1*}, Manju Tembhre², Ritu Sharma³

¹Deptt. of Zoology, Govt. College, Mohangarh, Tikamgarh, MP, India

²M K Ponda College, Bhopal- 462028, India

³ Department of Zoology, Govt. Autonomous P. G. College, Chhindwara, (M.P), India

*Email: anjali_namdeo156@yahoo.co.in

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Abstract : Excessive use of pesticides in agriculture to improve crop variety results in pollution of water through agricultural run-off affecting non-target aquatic organisms. These organophosphate pesticides are non-degradable and persistent in the environment. Considering the harmful effects by synthetic pesticides, biologically active compounds of plants are used to produce biopesticides. In the present study, the histopathological alterations were studied in the Liver tissues of freshwater fish *Catla catla* exposed to sub lethal concentration of an organophosphate pesticide Chlorpyrifos and ethnolic extract of a medicinal herb *Datura stramonium* for 96 hrs. The result showed degenerative changes, vacuolation, bile ductular proliferation, hypertrophy and coagulative necrosis in hepatocytes.

Keywords: *Catla catla*, Chlorpyrifos, *Datura stramonium*, Histopathology, Liver.

Introduction

Organophosphate pesticides pose a global public health and environmental problems as it causes water pollution which leads to hazard to aquatic life. Even at relatively low levels, some organophosphates may be hazardous (Rodrigues and Fanta, 1998). The agricultural run-off is the major cause of water pollution due to pesticides which affects non-target organisms. They affect various organs including Liver which is a large vital organ and has a wide range of functions like detoxification, protein synthesis, production of biochemicals necessary for digestion (Paulo, 2012; Mostakim *et al.*, 2015). Liver is very vulnerable to exposure by organic and inorganic compounds and causes life threatening conditions as it accumulates over time. Because of its capacity of detoxification and storage it is often used as an environmental biomarker (Reddy, 2012). Chlorpyrifos is an organophosphate pesticide used to control foliage and soil-borne insect pests on cotton, corn, almond and fruit trees including apples and oranges.

In many countries of the world, variety of herbs is used to cure disease. The medicinal properties of herbs depends on the presence of active compound includes alkaloids, tannins, flavinoids, saponins and phenolic compounds (Edeoga *et al.*, 2005). However, some herbs have adverse effects on organ system of animals and man as well. Therefore, herb-induced liver injury has drawn attention due to serious public health problems (Jing J and Teschke, 2018). *Datura Stramonium* (D.s) is a common weed along roadsides. The *Datura stramonium*, a member of the family Solanaceae, is often called Dhatura or Devils apple possesses both poisonous and medicinal properties having hallucinogenic and anticholinergic effects. It has been reported to contain alkaloids, atropine, hyoscyamine and scopolamine, tannins, carbohydrates and proteins (Soni *et al.*, 2012). Investigations have been made to assess its pharmacological activity and showed its anti-asthmatic, anti-cholinergic, acaricidal, repellent & oviposition deterrent properties, anti-microbial activity, anti-cancer activity, anti-inflammatory activity and anti-fungal activity (Soni *et al.*, 2012; Tasneem *et al.*, 2016). Till today

no research has been done on the effect of *Datura stramonium* on the histological alterations in the liver of fish. The present study was undertaken to investigate the effect of *Datura stramonium* on the Liver tissues of Indian Major Carp *Catla catla* and compare it with chlorpyrifos-induced liver injury to know whether *Datura stramonium* could be a botanical alternative to synthetic pesticides.

Materials and Methods

Experimental Animal

Catla catla fingerlings ranging 100 ± 10 gm in weight collected from the fishpond near Kolua village, Raisen road, Bhopal. The fishes were stocked in glass aquaria of 60 liters supplied with tap water (temperature 22.7 ± 0.61°C, hardness as CaCO₃ 212 ± 4.8 ppm, pH 7.3 ± 0.05, chlorides 87.62 ± 2.39 unit, total alkalinity as CaCO₃ 165 ± 1.15 ppm). They were acclimatized for 15 days prior to the experiment. Fishes were fed daily with commercial dry feed pellets (Tokyu, Spirulina, Japan).

Test Chemical

Chlorpyrifos is an organophosphate insecticide used to control insect pests. It is used mainly on Cotton, Corn, Nut and fruit trees. Chlorpyrifos was first registered in 1965 and marketed by Dow Chemical Company under trade names Dursban, Lorsban. IUPAC Formula: O, O, Diethyl-O-(3, 5, 6 trichloro-2- pyridyl) phosphorothioate.

Plant Material

Preparation of ethanolic leaf extract

Fresh leaves of *Datura stramonium* were collected from botanical garden, authenticated, and thoroughly washed in water to remove dust and shade dried. Dried powder of *Datura stramonium* leaves (40 gm) with 400 ml of 90% ethanol was extracted using Soxhlet apparatus for about 12 hrs following standard method (Harborne, 1973). The extract was kept at room temperature to evaporate ethanol and a semi solid mass was obtained.

Experimental Design

After acclimatization, fishes were divided into four groups.

Group I: served as Control; Group- II: exposure to sub lethal concentration 0.00073 mg/L was based on the 96 h LC₅₀ value (0.0034 mg/L) of Chlorpyrifos for *Catla catla* was selected for the test. The fishes were exposed to this concentration daily for 96 hrs with replenishment of water at every 24 hrs; Group-III: Fishes were exposed to 100 mg/L of *Datura stramonium* leaf extract for 96 hrs; Group-IV: The fishes were pre-treated with 100 mg/L of *Datura stramonium* extract for 96 hrs followed by the exposure to 0.00073 mg/L chlorpyrifos for 96 hrs.

Preparation for Histological Examination

Fishes from these stocks were removed, euthanized. They were dissected out and Liver was removed from fish of each group. Tissues were cleaned in 0.9% saline, fixed in freshly prepared aqueous Bouin's fluid in glass vials for 24 hrs, washed in running tap water for 7-8 hrs and then dehydrated through graded series of alcohol, cleared in xylene, infiltrated in paraffin wax and then embedded in paraffin wax. Sections were cut at 5-6 micron thickness with the help of rotary microtome. The sections were affixed on glass slides using Mayer's albumin, stained

using Haematoxylin-Eosin stain, mounted in DPX. Binocular microscope (Olympus) was used to closely examine the stained slides for any alterations in the normal structure of tissue. Selected fields of Liver were microphotographed at 10X and 40X magnification with the help of computer-aided microscope.

Results

The microphotograph of the Liver of healthy fish *Catla catla* showed large polygonal cells containing spherical nucleus arranged in cord-like manner around the hepatic vein. The bile duct, capillaries and sinusoids were irregularly distributed (Fig. 1).

Hepatic cells of the fish exposed to sub lethal concentration 0.00073 mg/L chlorpyrifos showed several alterations as hypertrophy of hepatocytes and bile ductular proliferation (Fig. 2&3). Exposure to 100 mg/L of *Datura stramonium* ethanolic leaf extract for 96 hrs showed coagulative necrosis and shrinkage in vein (Fig. 4) Necrosis was seen in the fishes pretreated with *D.s* followed by the exposure of CPF (Fig. 5)

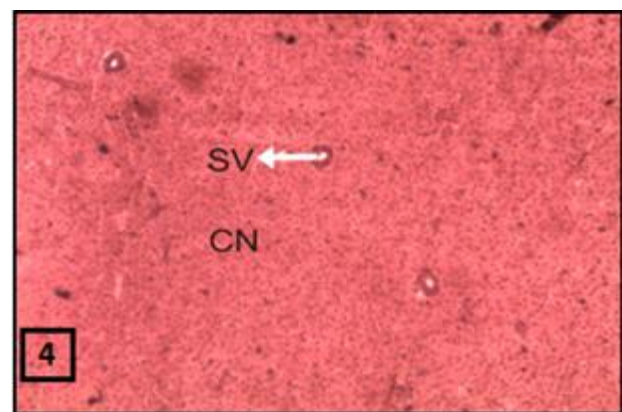
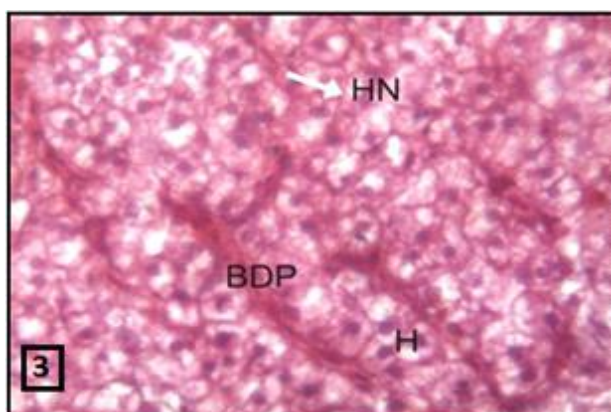
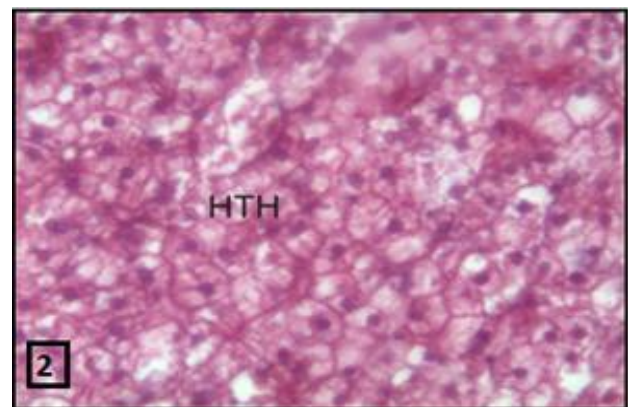
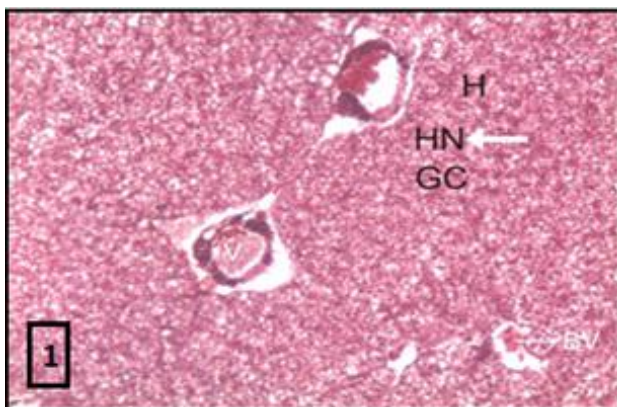


Fig.1-5. - Microphotograph of T. S. of the Liver of *Catla catla* (H & E) **Fig.1-** Control group showing hepatocytes (H); hepatocytes nuclei (HN); granular cytoplasm (GC); blood vessel (BV) (100 X); **Fig. 2-** exposed to 0.00073 ppm CPF for 96 hrs showing hypertrophy of hepatocytes (HTH) (400 X); **Fig. 3-** exposed to 0.00073 ppm CPF for 96 hrs showing bile ductular proliferation (BDP) (400 X); **Fig. 4-** exposed to 100 mg/l *D.s* extract for 96 hrs showing coagulative necrosis (CN); shrinkage in vein (SV) (100 X).

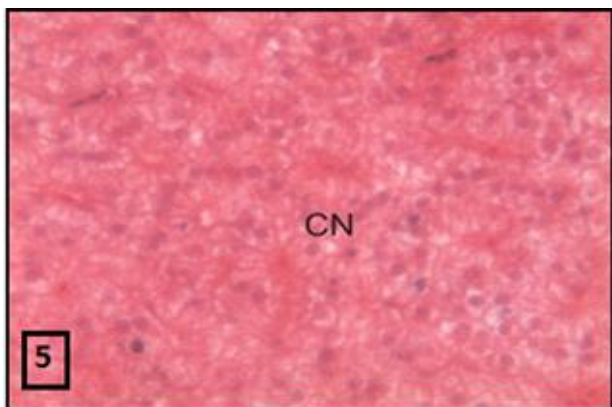


Fig. 5- Pretreatment of 100 mg/l *D.s* extract followed by CPF exposure for 96 hrs showing coagulative necrosis (CN) (400 X).

Discussion

The present study was conducted to study chlorpyrifos and *Datura stramonium*-induced histopathological alterations in liver of *Catla catla* to evaluate the extent of toxicity of synthetic pesticide and natural herbal extract. We exposed healthy *Catla catla* to 0.00073 mg/L sub-lethal concentration of chlorpyrifos alone, ethanolic extract of *D.s* leaves alone and pretreatment of 100 mg/l *D.s* leaves extract followed by the exposure of chlorpyrifos for 96 hrs to observe alterations in liver architecture. In the present investigation, in the untreated fish liver, normal structure of liver observed with well arranged hepatocytes, bile capillaries and blood spaces as reported earlier by many investigators (Hasina and Mithra, 2014; Muttappa *et al.*, 2015; Jain *et al.*, 2018).

The liver is the main organ for detoxification. It shows morphological alterations in fish exposed to pesticides. Alterations in the cellular structure of liver may be useful as an environmental stress biomarker (Ramesh *et al.*, 2014; Rajani *et al.*, 2015). Pesticides when come in contact with fish, they act directly on the cell or causes cellular toxicity by altering its environment. The cells accordingly respond histopathologically by degeneration, proliferation, inflammation and repair (Altinok *et al.*, 2007). It has been stated that histopathological studies are important tools to assess the extent of pesticidal pollution and to measure considerable damage occurred in the cells. A number of studies have been made to study the histopathological alterations in the liver of fish exposed to various organophosphorous pesticides and botanicals (Bhatnagar *et al.*, 2007; Kunjamma *et al.*, 2008). The histopathological changes observed in the present study were pronounced in the fishes exposed to 0.00073 mg/L chlorpyrifos for 96 hrs. which includes several alterations as hypertrophy of hepatocytes and bile ductular proliferation and necrosis. This could be resulted from the excessive work by the fish to get rid of the toxicant during process (Rahman *et al.*, 2002). The liver cells were found degenerated with necrosis which appeared as focal areas with lymphocytic infiltration in *Clarias gariepinus* due to exposure of fenvalerate (Sakr *et al.*, 2005). In earlier study, chlorpyrifos showed necrosis, pyknotic nuclei in the liver

of *Channa punctatus* (Bloch) (Stalin *et al.*, 2019). Exposure of chlorpyrifos to *Oreochromis mossambicus* for a period of 7 and 21 days produced lesions in the liver in form of swollen hepatocytes and necrosis of cell with granular cytoplasm, and detached hepatic cells (Kunjamma *et al.*, 2008).

The present investigation evidenced that treatment of *Datura stramonium* leaf extract alone and pretreatment of *Datura stramonium* followed by chlorpyrifos had significant adverse impact on the histology of liver of *Catla catla*. Thus, it has lead to hepatocytes swelling, hypertrophy, vein shrinkage, bile ductular proliferation and coagulative necrosis. The combined treatment pronounced these alterations. Hence, it is suggested that *Datura stramonium* leaf has negative effects on the liver of fish. It has been reported that parts of the plant *D. stramonium* although possessing medicinal properties, are found to be poisonous. It has been reported that the botanic aqueous extract of *Moringa oleifera* seed powder to Nile tilapia *Oreochromis niloticus* produces disorientation of the liver parenchyma structure, hyperplasia, vacuole formation, enlarged hepatocyte and Shrinkage of cell (Ayotunde *et al.*, 2011). Tasneem *et al.* (2014) compared toxicity of two biopesticidal plants in fish liver of *Oreochromis mossambicus* for 7 and 14 days. The 7 day exposure of aqueous leaf extracts of *Carica papaya* causes dilation of sinusoids, swelling of cells with dark nuclei and nuclear abnormality, vacuolated hepatocytes, mild pockets of infiltration of inflammatory cells. However, 14 day exposure showed vacuolated hepatocytes with various shaped nuclei, Sinusoids showing mild dilations more of hydrobic degeneration. While exposure of *N. oleander* showed cells with swollen nuclei and multinucleated cells, complete disruption of hepatocytes and vacuolar degeneration.

Many plants contain chemicals which have been employed to collect fish and also to monitor various pests in almost all parts of the world (Siememeon *et al.*, 2011). Though some of the herbs are toxic they are degraded easily within 7-12 days and safe for users (Fathy *et al.*, 2014). Several plants due to the presence of number of compounds *viz.* saponins; tannins; alkaloids; alkenyl phenols; di and tri terpenoids; etc. display high pesticidal activity are used to manage predatory fish; pathogenic insects such as mosquito larvae and harmful fresh water snails (Singh, and Singh, 2000; Tiwari and Singh, 2003). We compared toxicity of a synthetic pesticide chlorpyrifos and an herbal extract in the liver of fish and observed that *Datura stramonium* leaf extract has equally harmful effects as that of chlorpyrifos in fish organ. Therefore the present study might be of help to establish the safer usage of ethanolic leaf extract of *Datura stramonium* in the agricultural field and aquaculture farms.

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