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Synergistic Effect of Cypermethrin and Sodium Fluoride on Liver Histo Pathology of Albino Mice

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Abstract

The present study was aimed to investigate the hepatotoxicity induced by cypermethrin and sodium fluoride (NaF) separately and combined in albino mice. Albino mice were treated at 48-hr intervals with cypermethrin and sodium fluoride (NaF), separately and in combination, for 15 and 30 days with 1/10th of the LD_{50} dosage of cypermethrin and NaF for individual administration by oral gavage (i.e., 8.5 mg/kg bw and 5.6 mg/kg bw, respectively) and 1/20th of the LD_{50} dose of cypermethrin and NaF for combined administration (i.e., 4.25 mg/kg bw and 2.8 mg/kg bw, respectively). Separate or combined treatment resulted in histopathological changes in the liver tissue such as cytoplasmic degeneration, cellular disarray, binucleated condition, vacuolar and nuclear degeneration in the hepatocytes were observed. The changes were greater in combination than individual treatment, this may be because of a synergistic effect of cypermethrin and NaF.

Key Words: Synergism, Cypermethrin, Sodium fluoride, Liver, Albino mice

Introduction

Water is the most precious natural resource that exists on our planet. It is a key component in determining the quality of our lives. Today, people are concerned about the quality of the water they drink. Water dissolves numerous substances in large amounts, pure water rarely occurs in nature. Pesticides are one of the most common causes of water pollution. Pesticides from farms and individual home owners run off into streams and rivers. Among minerals, fluoride is one of the contaminants of water. Fluoride is an essential trace element for human beings and animals. In small amounts fluoride is beneficial as it is believed to impart stability to bone and enamel, thereby preventing dental carries and osteoporosis to some extent but its higher concentration is highly toxic to humans and animals alike. Chronic exposure to fluoride above the permissible limits, it causes a disease called "Fluorosis". Fluorosis is an important clinical and public health problem in several parts of the world. Exposure higher than permissible levels of fluoride (>1.5 mg/L) may lead to serious health problems (WHO, 2017). Vital organs such as liver, kidney, reproductive organs and endocrine glands are reported to be adversely affected by high fluoride intake (Chinoy, 1991; ATSDR 2001). Some metabolic activities are also disturbed due to alteration in regulatory enzymes and biomolecules after exposure to fluoride (Kumar et al., 2007). Tripathi et al. (2009) has describe of severity of fluorosis.

The study of abnormal cells and tissues is histopathology (Aughey and Frye, 2001). It is a structural science and serves to compliment the knowledge gained from the anatomy, physiology and pathology and it gives insight into the functioning of tissues and organs. Histopathology helps in diagnosing the damages of the tissues of an animal subjected to toxic stress. The knowledge of the histology is useful to distinguish normal cells from abnormal or diseased ones, which helps in diagnosis of many diseases (Majumdar, 1980). Even though biochemical studies may give an idea of the pathological state of the animal, a clear picture of cytoarchitectural changes produced during the chemical intoxication can be produced during the chemical intoxication can be traced by histopathological studies.

Several workers reported on the pesticides and fluoride toxicity separately, the present study was designed to investigate the synergistic effects of cypermethrin and sodium fluoride (NaF) on hepatic histological architecture in albino mice.

Materials And Methods

Test chemicals: Cypermethrin technical (92% purity; cis:trans isomers ratio 40:60) was obtained from Tagros Chemicals India Limited, Chennai. Sodium fluoride (NaF) (99%) was supplied by BDH Chemical Division, Bombay.

Animal model: Healthy adult male albino mice of the same 75±5-day age group and weight (35 g) were taken from parental stock obtained from the Veterinary College, Bangalore and maintained as a colony. They were kept in well-cleaned and sterilized cages and were maintained at 26±2ºC with a 12-hr light/dark photoperiod throughout the study. The mice were fed on commercial rodent feed supplied by Hindustan Lever Limited, Bombay, and tap water was supplied ad libitum.

Experimental design: The albino mice were divided into seven groups with ten animals in each group. The toxicity of cypermethrin and NaF in mice was evaluated by the static bioassay method of Finney (1971), and the single-dosage of LD_{50} of cypermethrin and NaF to albino mice was found to be 85 mg/kg bw/24 hr and 56 mg/kg bw/24 hr, respectively. A 1/10th single-dosage LD50 level of cypermethrin and NaF (i.e., 8.5 mg/kg bw and 5.6 mg/kg bw, respectively) for individual administration and 1/20th the single-dosage LD50 level for combined administration

were selected. The treatments were by oral gavage and the first group of mice was treated as controls, as shown below in the experimental protocol in Table 1.

Table 1. Experimental protocol

Group	Treatment	Duration (days)	Day of sacrifice
I	Controls	-	-
II	Treated with cypermethrin (8.5 mg/kg bw)	15	16
III	Treated with cypermethrin (8.5 mg/kg bw)	30	31
IV	Treated with NaF (5.6 mg/kg bw)	15	16
V	Treated with NaF (5.6 mg/kg bw)	30	31
VI	Treated with cypermethrin + NaF (4.25 mg/kg bw + 2.8 mg/kg bw)	15	16
VII	Treated with cypermethrin + NaF (4.25 mg/kg bw + 2.8 mg/kg bw)	30	31

The second and third groups were treated for 15 and 30 days with cypermethrin, respectively, at 48-hr intervals. The fourth and fifth groups were treated with NaF for 15 and 30 days at 48-hr intervals. The sixth and seventh groups were treated with combined dose of cypermethrin and NaF for 15 and 30 days at 48-hr intervals.

Histopathological examination: Following the method of Humason histological examination of the tissues was conducted after removal from the mice. The liver tissues were gently rinsed with a physiological saline solution (0.9% NaCl) to remove blood and adhering debris. They were then fixed in 5% formalin for 24 hr, and the fixative was removed by washing overnight with running tap water. After dehydration through a graded series of alcohols, the tissues were cleared in methyl benzoate and embedded in paraffin. Sections were cut by a microtome to a thickness of 6 µm and stained with hematoxylin as described by Harris et al (2006). and counter-stained with eosin dissolved in 95% ethanol (H&E). After dehydration and clearing, sections were mounted with DPX (digital picture exchange) and observed under a microscope.

Results

Normal histology of mice liver

The liver of normal mice comprises of continuous mass of hepatic cells with cord like formation. The cells are large in size with more or less centrally placed nucleus and homogenous cytoplasm. There is no clear division of the hepatic cells into lobules. The hepatic cells are hexagonal in their nature. The bulk of the hepatic lobule comprised of polyhedral epithelial parenchyma cells containing round nuclei and a prominent nucleolus. A fine network of vascular capillaries, sinusoids running in between the parenchyma cells, the nucleus in hepatocytes consists one or more nucleoli were noticed. (Figs. A-C).

Experimental mice liver

The mice exposed to cypermethrin, sodium fluoride separately and their combination for 15 days and 30 days have shown remarkable changes when compared to control (Figs. D - I). These changes include – cytoplasmic degenerative changes in hepatocytes, cellular degeneration, vacuoles, congestion, cellular disarray, nuclear fragmentation, nuclear degenerative changes, binucleated condition, pushing of nucleus to periphery of hepatocytes, severe necrosis in hepatocytes, haemorrhage in central vein and pycnotic nucleus (Figs. D -I). In 15 days cypermethrin showed more changes compared to sodium fluoride. The mice which received cypermethin and sodium fluoride in combination have exhibited more changes compared to the mice received the chemicals separately. In the case of 30 days the sodium fluoride intoxicated animals showed more changes than cypermethrin. With the combination of these two chemicals more severe changes were observed than sodium fluoride and cypermethrin intoxicated mice.

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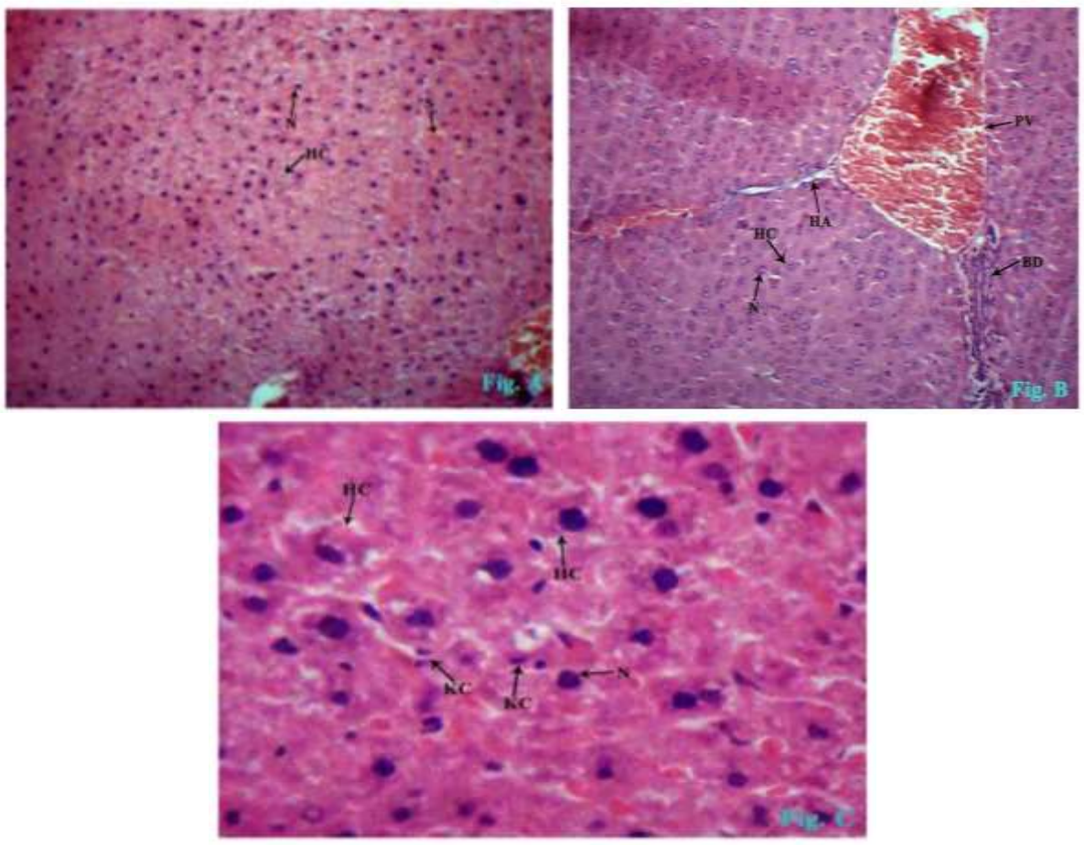

Figs. A &B : Microphotograhs of control liver of mouse showing hepatocytes (HC) with centrally placed nucleus (N) besides portal vein (PV) with bile duct (BD) and hepatic artery (HA) – H & E. 100 X.

Fig. C : Microphotograph of control mouse liver at higher magnification showing hepatocytes (HC), Nucleus (N) and Kuffer cells (KC) - H&E. 400 X.

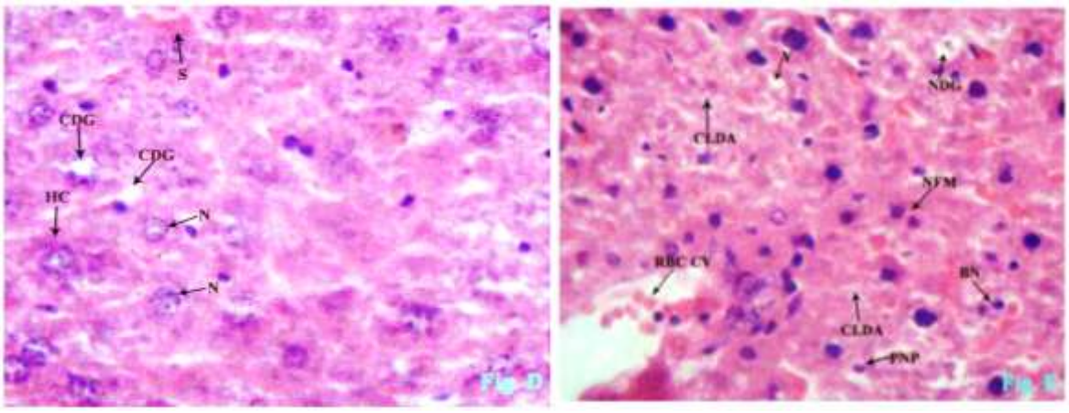

Fig. D: Microphotograph of mouse liver under 15 days of cypermethrin showing cytoplasmic degeneration (CDG) - H&E. 400 X.

Fig. E: Micrograph of mouse liver under 30 days of cypermethrin showing nuclear fragmentation (NFM), cellular disarray (CLDA), vacuoles (V), binucleated condition, scattered RBC in central vein (RBCCV) and pushing of nucleus to periphery of hepatocytes - H&E. 400X

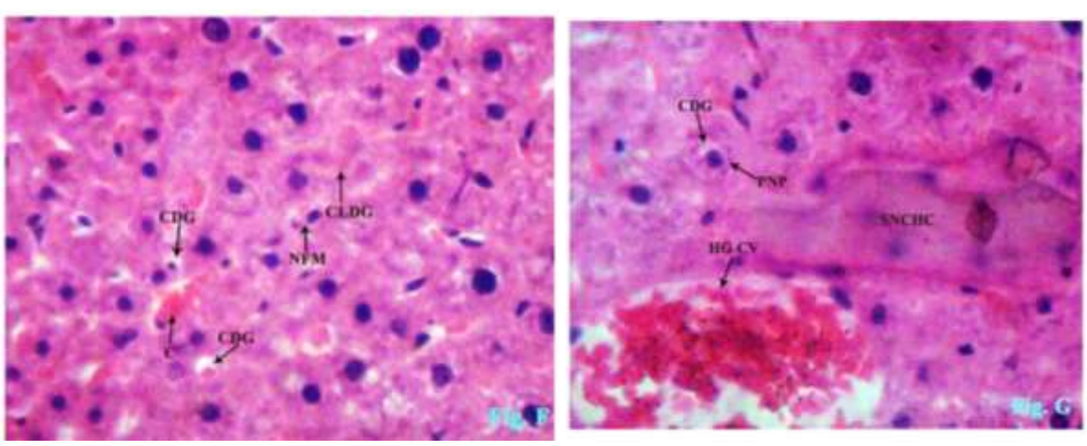

Fig. F : Microphotograph of mouse liver under 15 days of sodium fluoride showing cytoplasmic degeneration (CDG), congestion(C), Cellular degeneration (CLDG) – H & E.400 X.
Fig. G : Photomicrograph of mouse liver under 30 days of sodium fluoride showing severe necrosis in hepatocytes (SNC HC), pushing of nucleus to periphery of hepatocytes and haemorrhage (HGCV) in central vein - H&E. 400X.

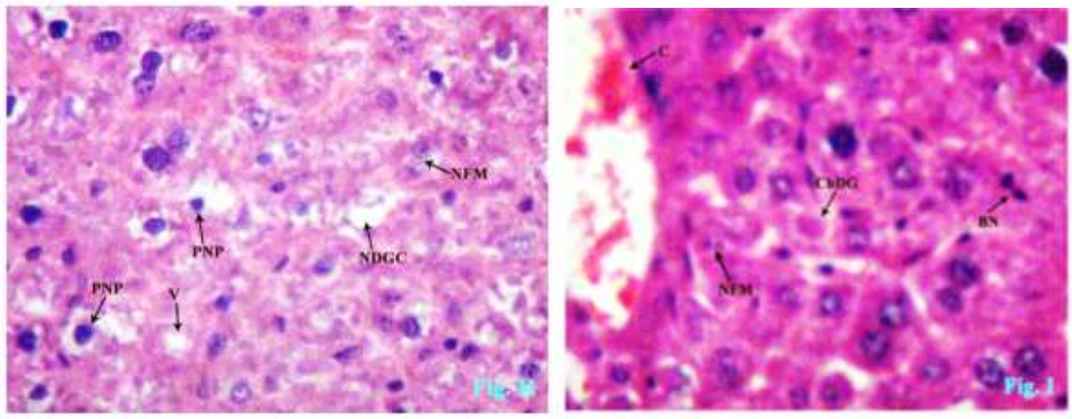

Fig. H : Microphotograph of mouse liver under 15 days of cypermethrin and sodium fluoride showing with vacuoles (V), pushing of nucleus to periphery (PNP), nuclear fragmentation (NFM) and nuclear degenerative changes (NDGC) in hepatocytes – H&E. 400 X.
Fig. I : Microphotograph of mouse liver under 30 days of cypermethrin and sodium fluoride showing binucleated condition (BN), nuclear fragmentation (NFM) and congestion (C) – H&E. 400 X

Discussion

Liver is the largest organ of the body comprising 2-3% of the total adult body weight, is primarily concerned with the metabolic activity of organisms (Sheila and Dooley, 1993). It is also the central site for the biotransformation of xenobiotic chemicals and therefore is involved in the detoxification mechanism. Pesticides causes for the architectural damage to liver in various animals (Shukla et al., 2001 and Jacobdoss et al., 2007). Fluoride is a toxic agent that can permeate cell membrane and disturb the cell homeostasis. High amount of fluoride may disturb liver function and homeostasis (Wang et al., 2000) and produced abnormalities such as degenerative and inflammatory changes (Djouadi and Derouiche, 2017).

Several authors reported histopathological changes in liver in different animal models under pesticidal toxicity. Luty *et al.* (2000) observed infiltrations of individual mononuclear cells and parenchyma degeneration of hepatocytes in liver of mice exposed to α-cypermethrin. Slight inflammatory infiltrations around single necrotic hepatocytes, composed of the hyper plastic kupffer cells and single lymphocytes in the liver of mice exposed to deltamethrin (Tos-Luty *et al.,* 2001). Wade *et al.* (2002) observed hypertrophied hepatocytes with many highly vacuolated cells in liver tissues of rats exposed to complex mixture of persistent contaminants.

Choudhary *et al.* (2003) reported congestion, vacuolar degeneration and accumulation of fat in centrilobular area, focal to extensive necrosis, hyperplasia of kupffer cells, dilation of sinusoids, nuclear aberrations, cytoplasmic degranulation and pycnotic nuclei in the liver tissues of rats exposed to endosulfan. Tos-Luty *et al.* (2003) observed parenchymatous degeneration of hepatocytes with slight infiltration in the liver of rats dermal exposed to malathion. Hypertrophy of hepatocytes with pyknotic nuclei, vacuoles and hyalinization, hepatocytes with dilation of central vein in albino mice treated with carbosulfan (Ksheerasagar and Kaliwal, 2006). Congestion and fatty changes in liver of rats exposed to deltamethrin (Manna *et al.,* 2005). Sarkar *et al.* (2005) found significant changes as hyperplasia, disintegration of hepatic mass, focal coagulative necrosis in *Labeo rohita* exposed to cypermethrin. Hepatic lesion in the liver tissues of *Cirrhinus mrigala* exposed to fenvalerate were characterized by congestion, cloudy swelling of hepatocytes and focal necrosis (Velmurugan *et al.,* 2007). Liver showing blood streakes fibrosis and vacuolated hepatocytes with pyknosis nucleus in H. fossilis treated with cypermethrin (Pratap Sing and Vandana Sing, 2008).

Hepatocellular necrosis, degenerative changes, hepatic hyperplasia, extensive vacuolization in hepatocytes, degenerative and necrotic changes in liver and kidney of rabbits have been reported with high concentration of NaF (Shashi and Thapar, 2000; Shashi et al., 2002). Chinoy *et al.* (1993) observed hyalinized hepatic tubules with loss of cells and the vacuolized cytoplasm and zonal necrosis in the liver of sodium fluoride treated rats. High dose of NaF elicited hepatic abnormalities such as ballooning, hypertrophy, hepatocellular necrosis, infiltration of mononuclear cells, deformed central vein, sinusoidal dilation, and binucleated cells in albino mice (Prakash et al., 2018). Necrosis, and mononuclear cells infiltration in the liver of mice that were treated with NaF (Bouaziz et al., 2006). Chinoy *et al.1991* observed

pyknosis of nuclei, zonal necrosis, and disintegration of the organization of hepatic cords in fluoride administer. Degenerated blood sinus in Gill and liver of fishes exposed to acute and chronic concentration of sodium fluoride showed several drastic histopathological changes (Kale, 2021).

Conclusion:

Several independent studies on pesticide and fluoride toxicity have been conducted in different researchers. However, few attempts have been reported to determine the combined toxic effects of pesticides and fluoride. The present study was designed to investigate the combined toxicity of cypermethrin and sodium fluoride in mice. Combined toxicity by cypermethrin and fluoride through drinking water appears to be an exceptional condition and is able to cause more severe toxic effects than either one alone. Moreover, in Combination the effects were more severe than from separate exposure, thus indicating that these chemicals exhibited synergistic effect.

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