SYNERGISTIC EFFECT OF CYPERMETHRIN AND SODIUM FLUORIDE ON KIDNEY HISTO PATHOLOGY OF ALBINO MICE

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ABSTRACT

The aim of the resent study is to understand the renal toxicity, induced by cypermethrin and sodium fluoride (NaF)separately and combined in albino mice.Albino mice were treated with cypermethrin and sodium fluoride (NaF), separately and in combination, 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 kidney tissue such asdegenerative changes in bowman's capsule (DGBC), distal convoluted tubules (DGDCT),necrotic changes in glomerulus (NCG), atrophied glomerulus (ATG), necrotic change in distal convoluted tubules (NCDCT), necrotic change in bowman's capsule (NCBC) and necrosis in proximal convoluted tubules (NCPCT), severe necrotic changes in proximal convoluted tubules (SNCPCT) and distal convoluted tubules (SNCDCT) were observed.The changes were more in combination than individual treatment, this may be because of a synergistic effect of cypermethrin and NaF.

Key Words: Synergism, Cypermethrin, Sodium fluoride, Kidney, Albino mice.

INTRODUCTION

Water is known as a natural solvent. Before it reaches the consumer's tap, it comes into contact with many different substances, including organic and inorganic matter, chemicals, and other contaminants. Estimates suggest that nearly 1.5 billion people lack safe drinking water and that at least 5 million deaths per year can be attributed to waterborne diseases. Water dissolves numerous substances in large amounts, pure water rarely occurs in

nature. Today, people are concerned about the quality of the water they drink.Pesticides are one of the most common causes of water pollution. Pesticides from farms and individual home owners run off into streams and rivers.

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. As fluoride is found in small quantities in almost all foods, it enters the human body mainly through the oral route along with food and water. It can be rapidly absorbed by passive diffusion through stomach, small intestine, mouth, lungs and skin (Khandare*et al.*, 2001). 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 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).Fluoride was shown, cumulative toxic effects on various organs, including the kidneys (Abdo et al.2011). Kidney is a common target organ for toxic xenobiotics due to its capacity to extract and also, due to its large blood flow (Choi et al., 2011 and Azab et al. 2014).In areas where water with high fluoride content is used to prepare tea, So, the consumption of tea is a major risk factor for kidney disease due to its high fluoride content (Pehrsson et al., 2011 and Waugh et al., 2016).

Microtomy is the technique of using the microtome or of preparing with its aid objects for microscopic study. Histopathology is the microscopic study of unhealthy tissue and is an important device of structural pathology. 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 extreme state of the animal, a clear picture of cytoarchitectural variations produced during the chemical intoxication can be produced during the chemical intoxication can be drawn 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

Experimental design: The albino mice were divided into seven groups with tenanimals in each group. The toxicity of cypermethrin and NaF in mice wasevaluated by the static bioassay method of Finney (1971), and the single-dosage ofLD_{50} of cypermethrin and NaF to albino mice was found to be 85 mg/kg bw/24 hrand 56 mg/kg bw/24 hr, respectively. A 1/10th single-dosage LD_{50} level ofcypermethrin and NaF (i.e., 8.5 mg/kg bw and 5.6 mg/kg bw, respectively) forindividual administration and 1/20th the single-dosage LD_{50} level for combinedadministration were selected.The treatments were by oral gavage and the first group of mice was treated ascontrols, 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. Thekidney 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.

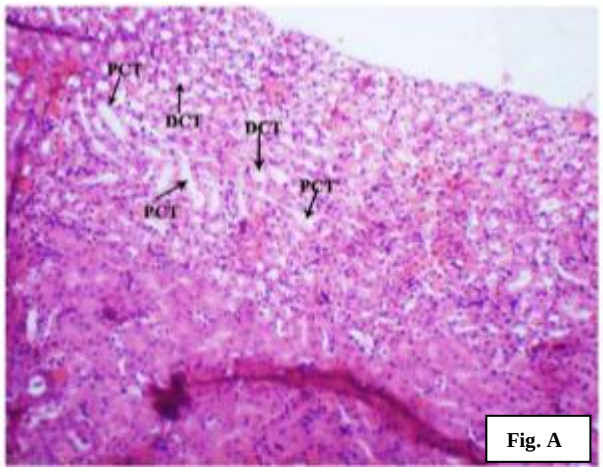
RESULTS

Normal structure of mice kidney

The kidney of albino mice is made up of more basic units, the nephrons. Each nephron consists of two major parts, the glomerulus and tubules. The intertubular spaces are filled by evenly distributed haemopoietic tissue. The cells are parenchymatous in nature, round to polygonal in shape with distinct nuclei in the centre. The tubules are divided into proximal convoluted tubules and distal convoluted tubules (Figs.A&B).

Mice kidney under experimental condition

Mice kidney under experimental condition has shown pronounced cyto architectural changes. These changes include degenerative changes in glomerulus, distal convoluted tubules, and bowman's capsule besides necrotic changes in glomerulus, tubular region, increase of lumen of distal convoluted tubules, congession, appearance of vacuoles, necrosis in glomeruli and bowman's capsule, atrophy of glomerulus (Figs. M - Q). These changes more in 30 days compared to 15 days. The combination of cypermethrin and sodium fluoride has induced severe architectural changes compared to the mice received the above chemicals individually.



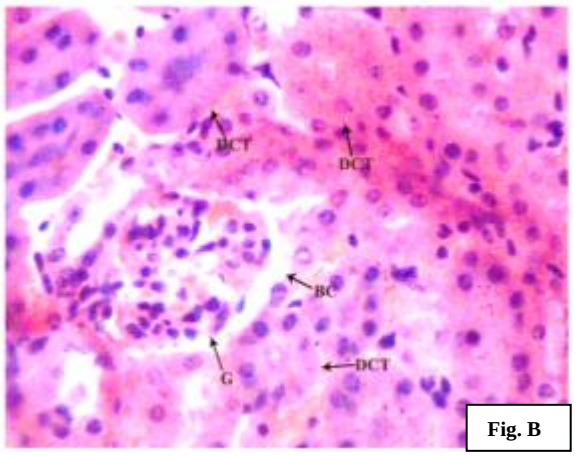
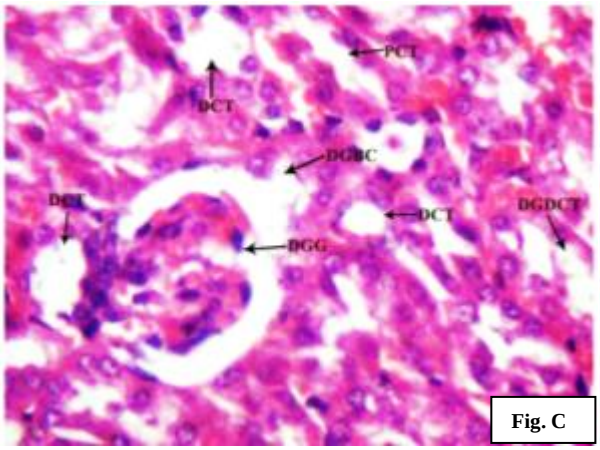


Fig. A : Microphotograph of control mouse kidney showing proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) – H&E.100 X

Fig. B : Microphotograph of control mouse kidney showing distal convoluted tubules (DCT), proximal convoluted tubules (PCT), glomerulus (G), bowman's capsule (BC) – H&E. 400 X



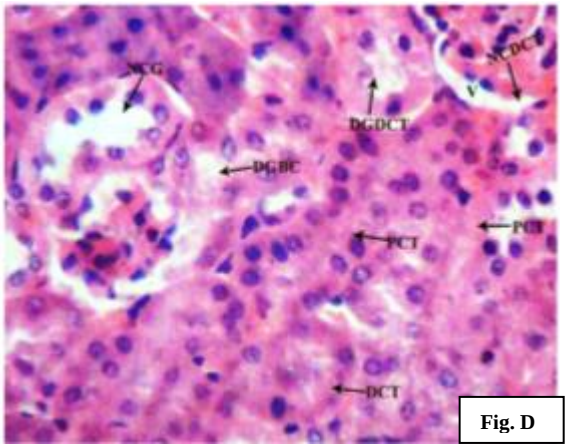


Fig. C : Microphotograph of mouse kidney under 15 days cypermethrin showing degenerative changes in distal convoluted tubules (DGDCT), degeneration in glomerulus (DGG) and degeneration in bowman's capsule (DGBC) – H&E. 400X

Fig. D : Microphotograph of mouse kidney under 30 days of cypermethrin showing necrotic changes in glomerulus (NCG), degeneration in bowman's capsule (DGBC), and degenerative changes in distal convoluted tubules (DG DCT) – H&E. 400 X

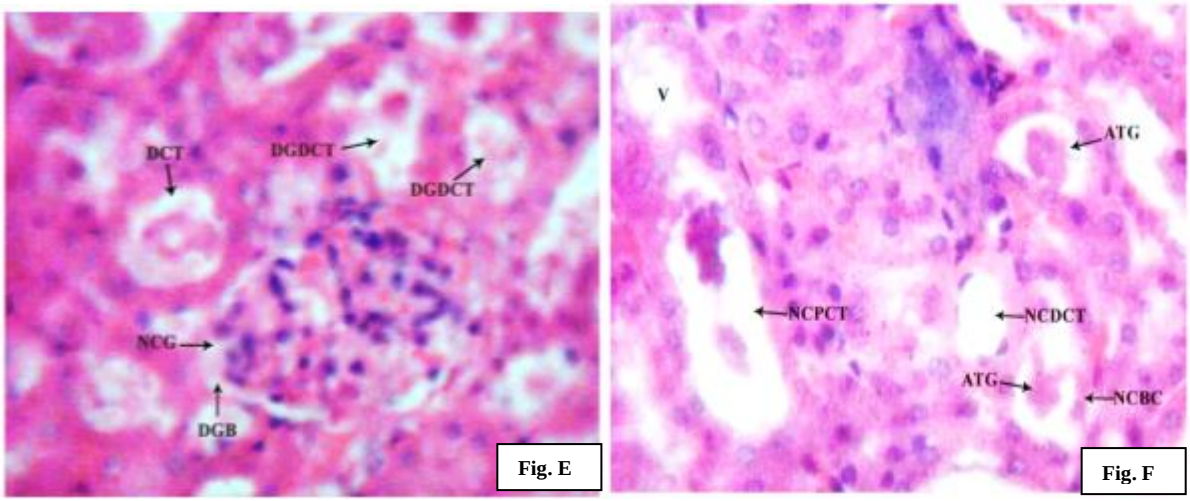


Fig. E: Microphotograph of mouse kidney under 15 days of sodium fluoride showing moderate degenerative changes in distal convoluted tubules (DGDCT) and degeneration in Bowman's capsule (DGBC) – H&E. 400 X

Fig. F: Microphotograph of mouse kidney under30 days of sodium fluoride showing atrophied glomerulus (ATG), necrotic change in distal convoluted tubules (NCDCT), necrotic change in bowman's capsule (NCBC) and necrosis in proximal convoluted tubules (NCPCT) – H&E. 400 X

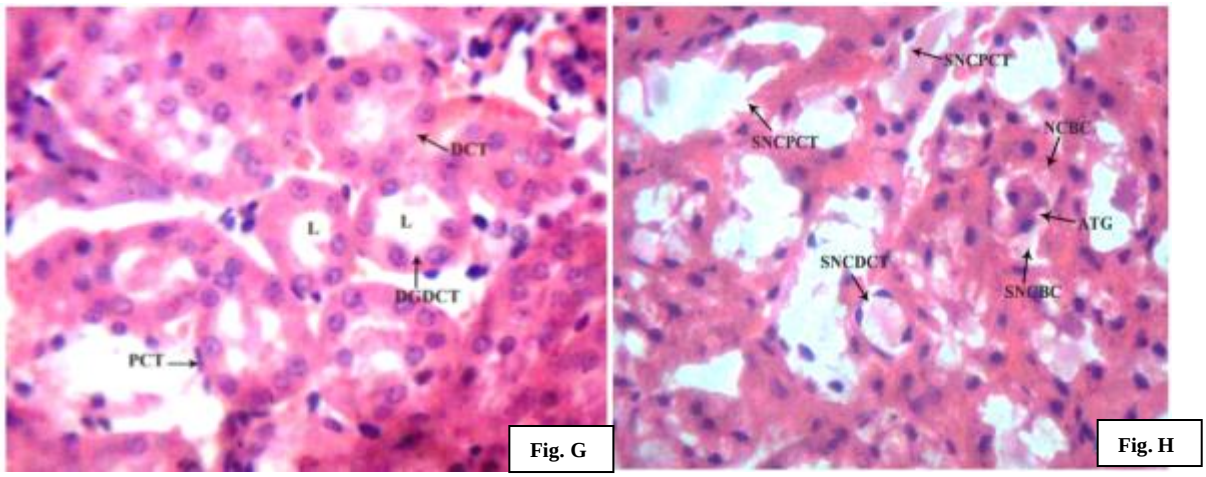


Fig. G: Microphotograph of mouse kidney under 15 days of cypermethrin and sodium fluoride showing increased area of lumen (L) of distal convoluted tubules, and degenerative changes in distal convoluted tubules (DGDCT) besides degeneration in tubular region – H&E. 400 X

Fig. H: Microphotograph of mouse kidney under 30 days of cypermethrin and sodium showing severe necrotic changes in proximal convoluted tubules (SNCPCT) and distal convoluted tubules (SNCDCT), atrophy of glomerulus (ATG) and necrosis in bowman's capsule (NC BC) – H&E. 400 X

DISCUSSION

Some toxic substances do not cause damage at the portal of entry but affect the organs systematically in which they are accumulated. Since Kidney happens to be an organ for excretion of undetoxified chemical, it is also affected badly in the present investigation showed some remarkable changes in the experimental condition which includes degenerative change in proximal convoluted tubules and distal convoluted tubules, congestion, necrosis in glomeruli and bowman's capsule, separation of glomeruli from bowman's capsule and atrophy of glomeruli (Figs. C - H).

Several authors reported histopathological changes in kidney in different animal models under pesticidal and fluoride toxicity.

Fenvalerate induced renal damage of the epithelial lining of the renal tubule, rupture of the distal tubules and enlargement of the glomerulei with hydropic degeneration (Abdeen et al. 1994). Luty et al. (1998) observed lymphocytic infiltrations of the paraglomerular region and in the outlet part of the kidney rats exposed to dichlorvos. Latuszynska et al. (1999) observed a few infiltrations of mononuclear cells between the proximal tubules or around blood vessels in the kidney of rats bare to chlorpyrifos and cypermethrin.

Atrophy of the glomerule, hypertrophy of Bowman's capsule and hyaline deposits in renal tubuli in the kidney of rats bare to deltamethrin (Tos-Luty et al., 2001). Parenchymatous degeneration of the cells of renal tubules and hyperemia of the cortical part of the kidney, especially of renal glomeruli, as well as infiltrations between the proximal tubules in the kidney of rats orally exposed to malathion (Tos-Luty et al., 2003). Choudhary et al. (2003) reported chronic glomerulonephritis, glomerulosclerosis, adenoma and glomerulus deposits in kidney tissues of rats bare to endosulfan. Manna et al. (2005) reported congestion of blood vessels in kidneys of rats bare to deltamethrin.Necrosis of tubular epithelium, pycnotic nuclei in the hematopoietic tissue, hypertrophied epithelial cells of renal tubules, narrowing of the tubular lumen, expansion of space inside the Bowman's capsule and contraction of the glomerulus were observed in kidney tissues of Cirrhinusmrigala after exposure to fenvalerate (Velmurugan et al., 2007).

Severe alterations in the kidney architectures was observed in Swiss albino mice treated with NaF (Chattopadhyay et al., 2011). Basha, and Rao, (2014) reported that exposure of albino mice to NaF results in necrosis in glomerules, Convoluted tubules and Bowman's capsule lumen in the kidney.Chronic fluoride introduction effects the kidney histological structure in young pigs (Xiu-An Zhan et al., 2006).

CONCLUSION

The present study was designed to investigate the combined toxicity of cypermethrin and sodium fluoride in mice. 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. 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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