SYNERGISTIC EFFECT OF SODIUM FLUORIDE AND CYPERMETHRIN ON THE SOMATIC INDEX AND HISTOPATHOLOGY OF ALBINIO MICE TESTES

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SUMMARY: Male 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 single 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 single dose of LD_{50} 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 a significant ($p<0.05$) decrease in body, testis, and epididymis weights, along with histopathological changes in the testis, especially after 30 days. The changes were greater in combination than separately, possibly because of a synergistic effect of cypermethrin and NaF.

Keywords: Cypermethrin; Fluoride synergism; Mouse testis histology; Somatic index.

INTRODUCTION

Pyrethroids are natural insecticides derived from yellow chrysanthemum, *cinerarifolium* and *Tanacetum cinerariifolium*, and are among the oldest known insecticides, first used in the 1800s.[1] In addition, numerous synthetic derivatives known as pyrethroids have been produced and marketed that have greater chemical stability than the natural pyrethrins. Liberal use of pyrethroids has increased the risk of intoxication for non-target species, such as birds, animals, and organisms present in the environment. Several studies have indicated that pyrethroids induce oxidative stress.[2] Pyrethroid insecticides, including cypermethrin, are widely used against pests all over the world to increase the production of food grains and other agricultural products. These insecticides can produce unintended adverse effects on non-target organisms including both vertebrates and invertebrates living in an exposed area affected by the insecticides.[3]

In 1977, cypermethrin was allowed for use as a very active synthetic pyrethrin insecticide, effective in the control of many pest species in agriculture, animal breeding, and the household.[4] In spite of their low mammalian toxicity, retention of pyrethroids in mammalian tissues may be potentially dangerous.[5] Fluoride when ingested beyond the limit of tolerance may cause the environmental disease known as "fluorosis."

To determine the pathological response of an animal to pyrethroid insecticides, histological study helps to show how these substances cause injury to tissues. For microanatomical study of specific tissues, histology has been successfully employed as a diagnostic tool in medical and veterinary science since the middle of the nineteenth century.[6] Since certain pesticides are toxic to mammals even at very low concentrations, they can impair the metabolism in animal physiology and tissue structure, especially when they enter the body after oral ingestion. Often

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such compounds are then transported to all parts of the body in their unmetabolised form.[7] Obviously, as an aid to the diagnosis of resulting disorders and diseases, knowledge of their resulting histological effects is useful to distinguish normal cells from abnormal or diseased ones.[8]

Previous investigations have revealed that fluoride (F) affects the structure and function of various tissues and organs of rats and mice, including liver, muscle, kidney, brain, endocrine glands, and both male and female reproductive organs. There is also evidence of F-induced oxidative stress in the testis and ovary as well as in other organs.[9]

MATERIALS AND METHODS

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

Animal model: Seventy 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,[10] 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 $1/10^{th}$ single-dosage LD_{50} level of cypermethrin and NaF (i.e., 8.5 mg/kg bw and 5.6 mg/kg bw, respectively) for individual administration and $1/20^{th}$ the single-dosage LD_{50} 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	16th
III	Treated with cypermethrin (8.5 mg/kg bw)	30	31st
IV	Treated with NaF (5.6 mg/kg bw)	15	16th
V	Treated with NaF (5.6 mg/kg bw)	30	31st
VI	Treated with cypermethrin + NaF (4.25 mg/kg bw + 2.8 mg/kg bw)	15	16th
VII	Treated with cypermethrin + NaF (4.25 mg/kg bw + 2.8 mg/kg bw)	30	31st

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.

Tissue somatic index: The body weight of the mice was taken before they were sacrificed by cervical dislocation. Afterward the testes and accessory sex organs were dissected and then, after removing the blood and other adhering tissue mass, they were weighed to the nearest milligram on a Shimadzu electronic balance (model No: BL-220H). The tissue indices were calculated using the formula:

$$\text{Tissue index} = \frac{\text{Weight of the tissue in grams}}{\text{Weight of the body in grams}} \times 100$$

Histopathological examination: Following the method of Humason[11] histological examination of the tissues was conducted after removal from the mice. The 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.[12] 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

As seen in Table 2, when the mice were treated with different dosages of cypermethrin and NaF separately and in combination, they exhibited a significant persistent decrease ($p<0.05$) in body weight, testes, and epididymis indices when compared to the controls.

Histology: Testes of a control mouse showed seminiferous tubules with spermatids and mature spermatozoa with an outer membrane, theca albuginea alongside Leydig cells lying between seminiferous tubules (Figures A and B). Cypermethrin and NaF-treated mice testes exhibited clumped spermatozoa, degenerative changes in seminiferous tubules with increased lumen of seminiferous tubules, vacuoles and severe necrosis in seminoferous tubules. Beside these, increased size of lumen, degenerative changes in spermatids, atrophied seminiferous tubules, necrotic changes in theca albuginea and scattered spermatids (Figures C–H) were also observed.

Table 2. Effect of cypermethrin and NaF on body weights, and the testis and epididymis tissue indices of male albino mice. Values are means of six individual observations in each group

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Body Weight (g)							
Mean	35.423	33.810	32.125	34.272	31.501	31.655	30.240
SD±	0.142	0.075	0.101	0.017	0.153	0.092	0.120
PC[a]		(-4.55)	(-9.31)	(-3.24)	(-11.07)	(-10.63)	(-14.63)
Testes tissue index							
Mean	0.700	0.611	0.538	0.647	0.504	0.548	0.459
SD±	0.011	0.019	0.009	0.009	0.006	0.009	0.004
PC[a]		(-12.71)	(-23.14)	(-7.57)	(-28.00)	(-21.71)	(-34.42)
Epididymis tissue index							
Mean	1.342	1.202	0.956	1.251	0.798	1.163	0.663
SD±	0.024	0.034	0.033	0.033	0.037	0.033	0.047
PC[a]		(-10.34)	(-28.76)	(-6.78)	(-40.53)	(-13.33)	(-50.59)

SD± = Standard Deviation

[a]PC = percent change from the control. All the values differ significantly (p<0.5) from the controls

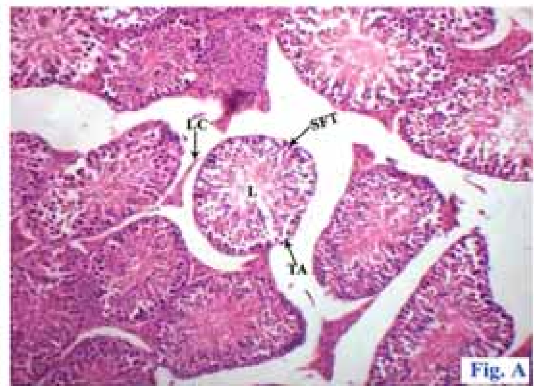

Figure A. Microphotograph of control group mouse testis showing seminiferous tubules (SFT) with spermatids (SPD) and mature spermatozoa (MSP) with a outer membrane, theca albuginea (TA), and Leydig cells (LC) in between seminiferous tubules.
H&E staining. 100 ×.

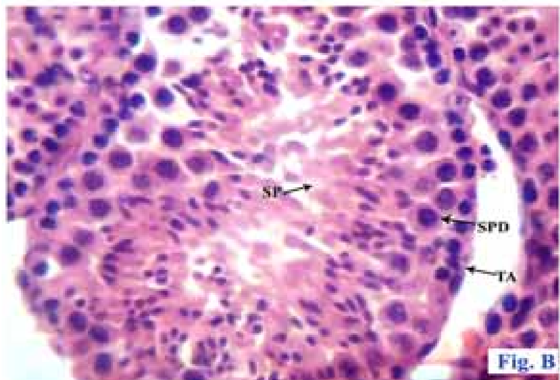

Figure B. Same as Figure A but at higher magnification.
H&E staining. 400 ×.

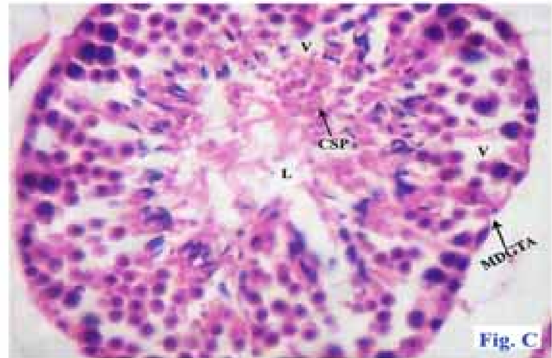

Figure C. Microphotograph of Group II mouse testis after 15 days of cypermethrin treatment showing vacuoles (V), clumped spermatozoa (CSP) besides increase of lumen (L) space in seminiferous tubules, and mild degenerative changes in tunica albuginea (MDGTA). H&E staining. 400 ×.

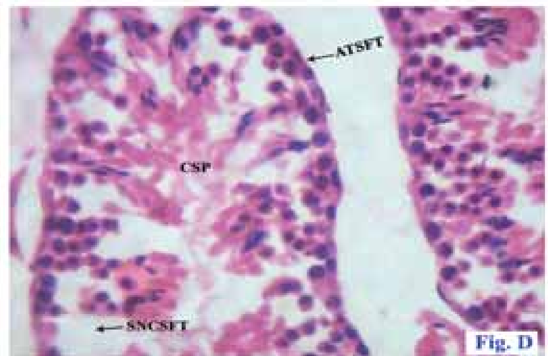

Figure D. Microphotograph of Group III mouse testis after 30 days of cypermethrin treatment showing atrophied seminiferous tubules (ATSFT), clumped spermatozoa (CSP), and severe necrosis in seminiferous tubules (SNCSFT). H&E staining. 400 ×.

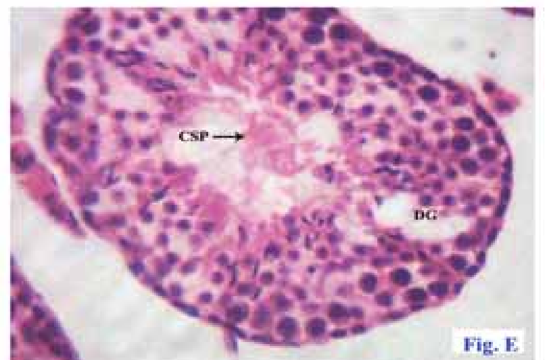

Figure E. Microphotograph of Group IV mouse testis after 15 days of NaF treatment showing clumped spermatozoa (CSP) and degenerative changes (DG) in spermatids with formation of vacuoles (V). H&E staining. 400 ×.

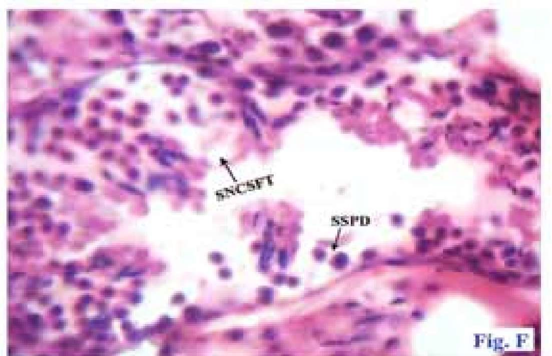

Figure F. Microphotograph of Group V mouse testis after 30 days of NaF treatment showing severe necrotic changes in seminiferous tubules (SNCSFT) and scattered spermatids (SSPD). H&E staining. 400 ×.

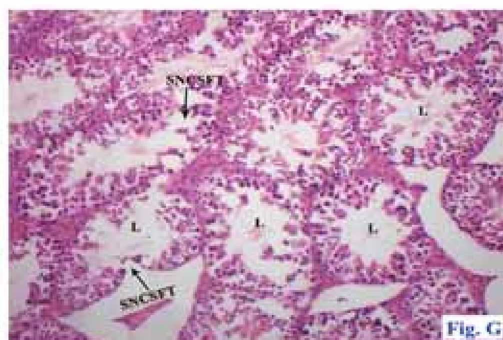

Figure G. Microphotograph of Group VI mouse testis after 15 days of combined cypermethrin and NaF treatment showing severe necrosis in seminiferous tubules (SNCSFT), increased lumen (L) of seminiferous tubules, and necrotic changes in the theca albuginea (NCTA). H&E staining. 100 ×.

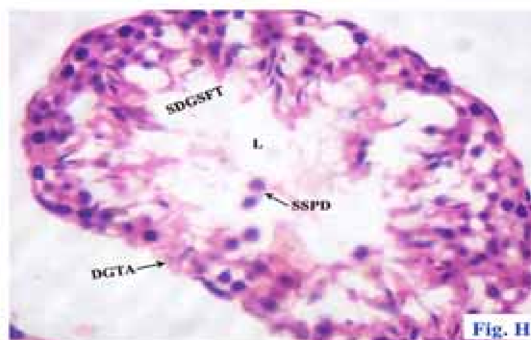

Figure H. Microphotograph of Group VII mouse testis after 30 days of combined cypermethrin and NaF treatment showing more degenerative changes in the theca albuginea (DGTA), seminiferous tubules, and separation of spermatids (SSPD). H&E staining. 400 ×.

DISCUSSION

When foreign agents enter into a mammal, they may create several degrees of change in various organs such as (a) inducing no or very little change, (b) breaking down spontaneously, and (c) undergoing degradation by the action of specific enzymes. The classical pharmacological theory of "dose-response" assumes that after entering the body, a given xenobiotic, whether changed or unchanged will interact with various body components.[13]

As seen in Table 2, after 15 days the total body, testis, and epididymis weights were decreased more in the cypermethrin-treated mice than in the NaF-treated mice, but after 30 days the decrement was greater in the NaF-treated mice than in the cypermethrin-treated mice. The reduction in weight of accessory organs indicates atrophy of glandular tissue and also a reduction in secretionary ability. As discussed below, the decline in testis weight may also be a result of a decrease in sperm count as well as reduction in the weight of the seminal vesicles and ventral prostate, which may reflect an interference with androgen output. In the combined treatment, the decrement in testicular weight was greater in both 15 and 30 days compared to individual exposures. These decreases in the weights of the whole body, testis, and epididymis indicate that both cypermethrin and NaF exert toxic effects on these organs. Similar weight loss results have been reported by Wang et al., for moderate (10 mg/kg bw/day) and high doses (20 mg/kg bw/day) of beta-cypermethrin in male mice.[14]

Since the weight of the testis is largely dependent on the mass of differentiated spermatogenic cells, testis weight has been used as a measure of

spermatogenesis,[15] especially in view of the strong correlation that exists between the weight of the testis and the number of germ cells in it.[16] It is logical, therefore, that a reduction in the weight of the testis has been shown to occur with loss of germ cells.[17]

Harmful effects of various insecticides on male reproduction have also been reported.[18] Thus, exposure of rats to elevated doses of fenvalerate not only proved toxic to the testis and epididymis, but also decreased sperm counts as well as absolute weights in both organs.[19]

As already noted, we found that NaF treatment caused a decrease in the body weight of male mice. Similar results have been reported by others in mice fed different concentrations of F.[20] Thus, Chinoy and Sharma[21] found a decrease in body weight and caput and cauda epididymis in male albino mice treated with NaF. Moreover, Elbetieha et al.[22] observed fluoride toxicity in the male reproductive system, and Long et al.[23] noted negative fertility effects of sodium fluoride in male mice.

In related work, Elbetieha et al.[24] found a decreased weight of testis and epididymis and also sperm count and fertility rate in albino mice exposed to cobalt chloride via drinking water for 12 weeks. A high-dose of aluminium in male mice has also been shown to incur a significant decrease in testicular and epididymal weights, as well as a significant decrease in testicular and spermatid counts and epididymal sperm counts.[25] Similarly, decreased testicular weight in mice exposed to cadmium has also been reported.[26]

With respect to studies in other animals, Elbetieha et al.[27] found reduced fertility in male rats exposed to cypermethrin through effects on the level of testosterone, follicle-stimulating hormone, and the number of cell layers in the seminiferous tubules, leading to congestion and haemorrhage in the testes. In studies on the effects of novel phosphorothionate in rats, Khan et al.[28] observed loss of spermatozoids extensive derangement of cellular organization in the testes. Damage has also been reported to the sperm-producing seminiferous tubules with greatly reduced sperm diameter after exposure of rats to this pesticide.[29]

In other work, Sakr and Azab,[30] reported abnormal seminiferous tubules with many vacuoles, marked reduction in spermatogenic cells, and degenerated Leydig cells in albino rats inhaling pyrethroids. In addition, Manna et al.[31] found edema between seminiferous tubules, vacuolization, and hyalinization in the tubules of the testes of rats exposed to α-cypermethrin. In addition, a decrease in luminal sperm and apparent dilation of tubules, together with oxidative stress, have been associated with testicular damage from NaF in rats.[32]

Several independent studies on pesticide and F toxicity have been conducted in different parts of the world. However, few attempts have been reported to determine the combined toxic effects of pesticides and F. The present study was designed to investigate the combined toxicity of cypermethrin and NaF in male mice. As it turns out, combined poisoning by cypermethrin and F through drinking water appears to be an exceptional condition and is able to cause more severe toxic effects than either one alone.

In the present investigation cypermethrin and NaF separately and in combination produced irreparable architectural testicular damage in mice accompanied by decreased body weight and reduction in the somatic index of the testis and epididymis. Moreover, in combination the effects were more severe than from separate exposure, thus indicating that these chemicals exhibited synergistic effects on all parameters.

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