Research Article www.ijcps.com

International Journal of Chemical and Pharmaceutical Sciences
2014, Mar., Vol. 5 (1)

ISSN: 0976-9390
IJCPS

Teratogenic effects of sodium fluoride and cypermethrin synergism in albino mice

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ABSTRACT

Present study was aimed to investigate the developmental defects of sodium fluoride and cypermethrin individual and combination treatment. Mice were administered with 1/10th LD_{50} of cypermethrin (8.5 mg/kg bw) and sodium fluoride (5.6 mg/kg bw) for individual treatment and 1/20th concentration of cypermethrin (4.25mg/kg bw) and sodium fluoride (2.8 mg/kg bw) for combined treatment from 5th day of pregnancy to 15th day pregnancy. A significant reduction in the number of litters delivered was observed in treated animals, skeletal and visceral weights were also reduced significantly in all treated animals. Mice litter showing wrinkles and curvature of the body, stunted growth. In combination treatment, mice delivered dead litter with the absence of one of the fore limbs, syndactyly and abnormal head. It is clearly indicates that combined treatment may cause synergism and dangerous to developing fetuses of mice, which may be equally harmful for human development too.

Keywords: Sodium fluoride, Cypermethrin, Birth defects, Mice.

1. INTRODUCTION

Fluorine is one of 92 naturally occurring elements. It is a member of the halogen family, which includes chlorine, bromine and iodine. It is the most chemically active nonmetallic element of any element and also is the most reactive electro-negative ion. Because of this extreme reactivity, fluorine isn't found in nature as an uncombined element. 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. The permissible limits of fluoride in drinking water as suggested by Bureau of Indian Standards varies between 0.6 to 1.2 ppm [1], and World Health organization [2] permits a maximum of 1.5 ppm of it. Chronic exposure to fluoride above the permissible limits causes a disease called "Fluorosis". Fluorosis is an important clinical and public health problem in several parts of the world. Earlier investigations revealed that fluoride affects the structure and function of several tissues and organs of rats and mice, including liver, muscle, kidney, brain, endocrine glands, reproductive organs in both male and female. There is also evidence of fluoride induced oxidative stress in testis and ovary as well as in other organs [3].

Majority of the chemical pesticides are harmful to man and animals, some of which are not easily degradable and tend to enter food chains, thereby spreading their toxic effects. One person is poisoned every minute by pesticides in the developing world [4]. However, most of the chemicals that are used as pesticides are not highly selective but are generally toxic to many non-target species, including man, and other desirable forms of life that co-inhabit the environment.

Cypermethrin is synthetic pyrethroid insecticide widely used against pests all over the world and there is increased risk of food being contaminated with the insecticide [5]. Thus contaminated food may harm humans and the domesticated animals. It produces reverse effects on the non-target organisms including both invertebrates [6]. Inspite of low mammalian

toxicity of pyrethroids, persistence of these compounds in mammalian tissues may be dangerous [7].

1.1. Teratogenic mechanism

The mechanisms by which different drugs produce teratogenic effects are poorly understood and are probably multifactorial. For example, drugs may have a direct effect on maternal tissue with secondary or indirect effect on foetal tissue. Drugs may interfere with the passage of oxygen or nutrients through the placenta and therefore have effect on the most rapidly metabolising tissue of foetus. Finally, drugs may have important direct action on the processes of differentiation in developing tissue [8].

A variety of mechanisms have been implicated in the causation of birth defects. Genetic as well as non genetic events have been described for compounds possessing teratogenic activity [9][. During the organogenesis it is thought that period of maximum sensitivity to teratogenic agent. Placental transfer of fluoride was demonstrated to occur under certain physiological conditions in the rat. The pregnant animals with certain amount of fluoride could be detected in the new born [10]. The limited permeability of the placenta to increased fluoride ions suggests that the placenta plays a role in the transfer of fluoride from mother to the fetus [11].

A variety of man-made contaminants and drugs have been identified [12] as teratogens which through placental barrier reach the growing fetus to affect malformations and a few of them are thalidomide [13], analgesic drug aspirin [14], organophosphorus and methyl carbamate [15], carbon tetrachloride [16], lead [17] and Malathion [18].

Several independent studies on pesticide toxicity and fluoride toxicity carried out in different parts of the world. However, not many attempts have been made to understand the combined toxic effect of pesticides and fluoride on developmet of embryos. The present study is designed to investigate the combined toxic effect of cypermethrin and sodium fluoride. Combined poisoning of cypermethrin and fluoride through drinking water is an exceptional condition and cause more severe toxicity and cause birth anomalies. In view of this, the present study is carried out in the albino mice to understand the toxic potentials of cypermethrin and sodium fluoride.

2. MATERIALS AND METHODS

2.1. Test chemicals selected

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

2.2. Animal model

Healthy adult albino mice of the same age group 75±5 days and weight (35 g) were taken from parental stock obtained from Veterinary College, Bangalore and maintained a colony. They were kept in well cleaned and sterilized cages. Mice were maintained at laboratory conditions (26±2°C; 12 hrs light and 12 hrs darkness) throughout the course of the present study. The animals were fed on rat feed supplied by Hindustan Lever Limited, Bombay and water was supplied *ad libitium*. It is approved by Institutional Animal Ethics Committee Ref No: Resoved (Resolution No. 02/ (i) /a/ CPCSCA/IAEC/SVU/ KJR-PRS/ Dt. 31.12.2008).

2.3. Experimental design

In the present investigation female albino mice were divided in four groups. I group consider as control administered distilled water. II & III groups treated with cypermethrin and sodium fluoride respectively with 1/10 of LD_{50} i.e 8.5 mg/kg bw and 5.6 mg/kg bw. IV group of animals treated with combination of both substances with $1/20^{th}$ of LD_{50} i.e 4.25 mg/kg bw and 2.8 mg/kg bw orally daily from 5^{th} day of pregnancy to 15^{th} day pregnancy. In spontaneous delivery the offsprings were examined for morphological and teratological alterations and were comparable to respective control animals in all treated animals.

2.4. Teratology

The control and experimental female mice were allowed for mating. After observing the vaginal plug they were considered to be one day of pregnancy. After 21 days they delivered the litters. The live and dead off springs were noticed.

The offspring litters were examined for gross external, visceral and skeletal anomalies and the sex was determined.

The off springs scheduled for skeletal analysis were dehydrated in 95% ethanol followed in 100% ethanol. The litters were macerated in 1% KOH and stained with Alizarin-Red-S-staining solution (Dawson, 1928). The off springs were cleared in 50% and 70% glycerine and the skeletal evaluation was done and recorded.

3. RESULTS AND DISCUSSION

In the present investigation the teratological defects in the albino mice fetuses following maternal treatment with cypermethrin and sodium fluoride by oral gavage administration was determined. These chemicals caused great growth retardation in litters. The numbers of

litters, skeletal and visceral weights were also reduced significantly ($p<0.05$) in all treated animals (Table 1). All animals were delivered on 21st day of pregnancy, but in combination the treated animal delivered on 22 and 23 day.

Table - 1: Changes in teratological parameters in albino mice exposed to sodium fluoride and cypermethrin

	Control	Cypermethrin (CYP)	Sodium fluoride (NaF)	CYP + NaF
Number of litters delivered				
Mean	8.664	6.500	4.665	4.000
SD±	1.505	1.048	1.211	0.894
PC		(-24.976)	(-46.156)	(-53.831)
Weight of litters				
Mean	1.698	1.580	1.507	1.068
SD±	0.012	0.014	0.010	0.073
PC		(-6.949)	(-11.248)	(-37.102)
Skeletal weight				
Mean	0.804	0.748	0.710	0.456
SD±	0.012	0.016	0.009	0.053
PC		(-6.965)	(-11.691)	(-43.283)
Visceral weight				
Mean	0.894	0.830	0.797	0.604
SD±	0.022	0.026	0.019	0.212
PC		(-7.158)	(-10.850)	(-32.438)

All the values are mean of six individual observations; PC – percent change over control, SD – Standard deviation; All the values are significant at $p<0.05$

Mortality was not observed in control litters. In the case of experimental animals mortality was observed. The rate of conception in the experimental animals was reduced and it was more in cypermethrin and sodium fluoride combined treated mice compared to individual treated animals. The gestation period was increased to 2 to 3 days in combined treated animals, which indicates that these compounds altered the gestation period. Maternal mortality was not observed in the present investigation. The mortality of the fetuses was noticed in cypermethrin treated and combined treated mouse litters. 30% of the cypermethrin treated litters died after delivery of 1day and 2 day. In the case of combined treated 50% of dead litters were delivered.

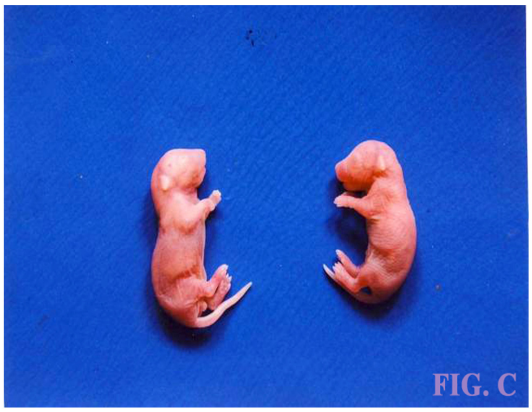
External observation in cypermethrin and sodium fluoride combined treated dead fetus showed short limbs, hyperflexion (excessively bend) of fore limbs, hyper extension (excessively straightened) of hind limbs, wrinkled, degenerated and blackened skin, cryptophthalmous (skin continuous over the eyeball) condition and right fore limb not properly developed, abnormal head and syndactyly (Figs. D-F). Skeletal examination revealed that in experimental fetuses abnormalities like domed skull, scoliosis (Lateral curvature of spinal column) was observed. Cypermethrin and sodium fluoride combined treated fetus skeleton showed syndactyly and delicate skeletal system compared to control.

During the treatment of cypermethrin and sodium fluoride the reproductive performances were observed in pregnant mice. Cypermethrin and sodium fluoride combined treatment influenced the implantation and caused increase embryo lethality. The embryo respiration was significant in experimental animals. However, cypermethrin is not showed external abnormalities, sodium fluoride and combination of cypermethrin and sodium fluoride showed marked changes in mice litters (Figs. A-F).

Morphological studies also supported the toxic effects of sodium fluoride and cypermethrin, sodium fluoride combination. Some cases of hydrocephaly, paddle shaped manus and pes,

deformed limbs, under developed brain and ectopia cards were noted in treated group. A highly considerable percentage of dead / resorbed fetuses was noted in combination treated groups.

PLATE -1



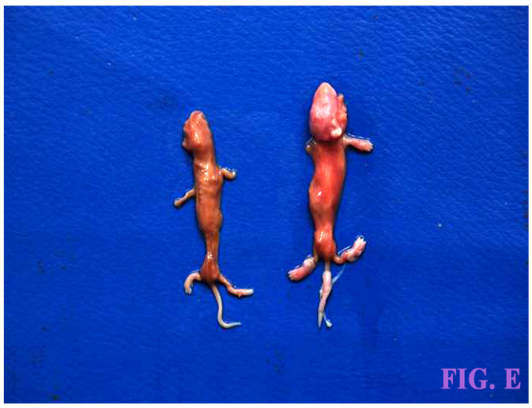


Figure A: Control litter of albino mice.

Figure B: Litter delivered from cypermethrin administered albino mouse showing wrinkles in the abdominal region.

Figure C: Litter delivered from sodium fluoride administered albino mouse showing wrinkles and curvature of the body.

Figure D: Litter delivered from cypermethrin and sodium fluoride administred albino mice showing stunted growth.

Figure E: Dead litter delivered from cypermethrin and sodium fluoride administred albino mice showing the dorsal region with the absence of one of the fore limbs, syndactyly and abnormal head.

Figure F: Dead litter delivered from cypermethrin and sodium fluoride administred albino mice showing the ventral region with the absence of one of the fore limbs, syndactyly and abnormal head.

The reduction in skeletal weight and visceral weights clearly indicates that the cypermethrin and sodium fluoride might have inhibited the organogenesis and fetal development. Reduction in skeletal weights and visceral weights might be a reflection of direct toxicity of cypermetrhin and fluoride. Prominent abnormalities were noticed in cypermethrin and sodium fluoride combined treated mice litters and it may be due to the cell death. Limbs were particularly susceptible to developmental toxic agents. Anything that interferes, directly or indirectly, with the growth process will inhibit its development [19]. Thus the cypermethrin and sodium fluoride used in the present investigation might have interfered in the terminal developmental stages resulting in abnormal development of mice.

Several authors have attributed the teratogenic effects in various animals under various pesticides treatment, in mice under dieldrin [20], endrin [21] and monochrotophos and azadirachtin [22], in rat under dieldrin, bromofenos [23], lindane and cadmium [24], Tri Phenyltin acetate (fungicide) [25]. Rupa *et al.* (1988)[26] reported still births, congenital defects, neonatal deaths and significant absorptions in 1016 couples exposed to pesticides.

High-fluoride concentrations in drinking water are also reported to be associated with decreased human birth rates [27]. Heindel *et al,* (1996) [28] investigated the developmental toxicity of fluoride and reported no adverse effect of sodium fluoride on the embryonic and fetal developments in rats or rabbits at doses of 27 mg/kg/day in the rat and 29 mg/kg/day in rabbits.

Several workers have reported that fluoride decreases body weight in rats given 10 ppm fluoride in drinking water for 7 days [29]. Verma and Sherlin (2001) [30] observed significant reduction in body weights following sodium fluoride (40 mg/kg b. wt) from 6^{th} to 19^{th} days of gestation period.

Prominent abnormalities were noticed in cypermethrin and sodium fluoride combined administered dead litters (Figs. D-F) and it may be due to the cell death i.e. necrosis. It was also reported that DNA damage results from excess fluoride in human embryo hepatocytes [31]. Previous studies have shown that fluoride induces apoptosis in human embryo hepatocytes [32]. These studies indicate that fluoride might induce apoptosis and cause abnormalities in the present investigation.

Some of the earlier reports have also attributed the teratogenic effects in various animals under various trace elements treatment. Reduction in the number of live implants and litter size, higher incidence of resorption and dead litter, malformation in both skeletal and morphological were observed in swiss albino mice treated with potassium chromate [33]. Aluminium nitrate induced maternal and embryo fetal toxicity in mice was reported by Luisa Albina *et al.* (2000) [34]. Cadmium (Cd) causes for teratogenic and embryotoxic effects in a large variety of species, including man [35]. Arsenic increased the frequency of neural tube defects and embryonic death in mice [36].

As the pesticides and metals have shown various teratological changes in various animals, cypermethrin and fluoride in the present investigation has also induced certain teratological changes such as decrement in size of fetus, decrement in body weight, skeletal weight and visceral weight and morphological, skeletal abnormalities were observed. However, these changes were more pronounced in combined treated animals which clearly indicate that repeated exposures to pesticides and fluoride cause deleterious changes on foetuses.

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