TOXIC EFFECTS OF FLUORIDE AND CHLORPYRIFOS ON ANTIOXIDANT PARAMETERS IN RATS: PROTECTIVE EFFECTS OF VITAMINS C AND E

Naseer Ahmad Baba,[a] R Raina,[b] Pawan K Verma,[c] M Sultana,[b] S Prawez,[c] Nisar Ahmad Nisar[a]

R. S. Pura, Jammu, India

SUMMARY: In continuing our studies on the effects of fluoride (F) on the toxicity of pesticides, we investigated the interaction of 1 ppm and 10 ppm F in the drinking water of rats orally administered 1 and 10 mg chlorpyriphos/kg bw/day, alone and in combination for 28 days. Changes in antioxidant parameters, along with protective effects of vitamins C, and E, were examined. Effects on superoxide dismutase, catalase, glutathione S transferase, glutathione peroxidase, glutathione, and lipid peroxidation were measured in the blood. Significant ($p<0.05$) alterations in these antioxidant indices were observed with repeated exposure of the rats to both toxicants alone and more so in combination. However, simultaneous oral administration of the antioxidant vitamins C and E in amounts of 60 and 100 mg/kg bw/day, respectively, afforded only partial protection against the subacute toxicity of F and chlorpyriphos alone and in combination.

Keywords**:** Antioxidant vitamins; Chlorpyrifos; Fluoride intoxication; Oxidative stress; Rat intoxication.

INTRODUCTION

Chlorpyrifos (*O*-*O*-diethyl-*O*-[3, 5, 6 trichloro-2-pyridyl]-phosphorothioate) (see Figure) is a widely used organothiophosphate pesticide for domestic and agricultural applications throughout the world.[1] Chlorpyrifos (CPF) induces deleterious effects primarily through acetylcholinestrase inhibition and produces symptoms characteristic of cholinergic overstimulation like salivation, nausea, vomiting, tremor and convulsions in mammalian species including human beings.[2-3] Chronic exposure to CPF elicits a number of other toxic affects including hepatic dysfunction, immunological abnormalities, embryotoxicity, genotoxicity, teratogenicity, neurotoxicity, and neurobehaviourial changes.[4-7]

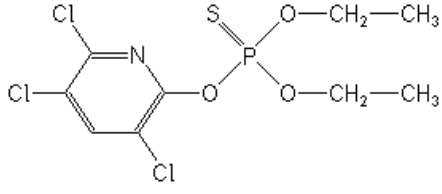

Figure. Structure of Chlorpyrifos

Fluorosis endemic in different areas of the world seriously affects the growth and production of animals.[8] Fluoride (F) intoxication causes damages to osseous tissue and other tissues such as liver, brain, etc. Various laboratory studies show that excessive ingestion of F has been found to induce free radical injury and oxidative damage in various tissues.[9-13] Previous studies undertaken in our

[a]PhD Scholar, [b]Professor, Division of Veterinary Pharmacology and Toxicology, Faculty of Veterinary Sciences & Animal Husbandry, R.S. Pura. [c]Assistant Professor, Division of Veterinary Pharmacology and Toxicology, F. V. Sc. & A.H., R.S. Pura. Correspondence: Dr. Pawan K Verma, F. V. Sc. & A. H., Division of Veterinary Pharmacology & Toxicology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, R.S. Pura, 181102, J&K, India; Email: drpawankv@yahoo.co.in

laboratory have shown that exposure to different pyrethroids like deltamethrin,[14] cypermethrin,[15] and bifenthrin,[16] induces oxidative stress in rats due to excessive generation of free radicals and reactive oxygen species (ROS). Unfortunately, relatively few investigations have assessed such potential hazards posed by simultaneous exposure to more than one toxicant, especially at lower doses.[17] As is well known, antioxidants protect against cellular damage stress by either preventing the uncontrolled formation of free radicals or directly scavenging them or inhibiting their disruptive reaction with sensitive biological sites. Thus the present study was undertaken to investigate the interactive effect of CPF and F on antioxidant parameters in rats and their protection by simultaneous administration of the antioxidant vitamins C and E.

MATERIALS AND METHODS

As in our recent study on F and deltamethrin,[14] adult Wistar rats of either sex weighing 150–200 g were procured from the Indian Institute of Integrative Medicine (Council of Scientific & Industrial Research Laboratory, Jammu) and maintained under standard experimental conditions with *ad libitum* access to feed and water. The experimental design was approved by the Institutional Animal Ethical Committee. For the first phase, the rats were randomly allocated to seven groups of six rats each. Group I served as control and received only normal tap water (F level not determined) for drinking. The rats in groups II and III were provided drinking water containing 1 mg F/L and 10 mg F/L of water (from NaF), respectively, whereas rats in groups IV and V were administered 1 mg CPF/kg bw/day through oral gavage and 10 mg CPF/kg bw/day, respectively. The CPF (Tafaban Chlorpyrifos–20%, purchased from Rallis India Limited, Mumbai, India. The animals of groups VI and VII were provided drinking water and CPF with the lower and higher F concentrations and CPF dosages.

In the second phase of the experiment, amelioration studies were conducted with six groups of rats with six rats in each group. Groups I and II served as negative controls for vitamin C and E, respectively. Groups III and IV were provided 60 mg vitamin C/kg bw/day and 100 mg vitamin E/kg bw/day, respectively. Group V was treated with a combination of F, CPF, and vitamin C, while group VI received F, CPF and vitamin E. In order to minimize possible instability, both the F and CPF solutions were prepared daily. All the rats were weighed weekly to make necessary corrections in the CPF dosage as per bw. After 28 days of daily treatment, blood samples were collected from retro-orbital fossa under light anesthesia with ether using capillary tubes in aliquots containing 10 IU/mL heparin. Prior to centrifugation, 200 µL of whole blood was used for the estimation of blood glutathione (GSH).[18] Then 1 per cent of the hemolysate was used for the estimation of superoxide dismutase (SOD),[19] catalase (CAT),[20] glutathione S transferase (GST),[21] and glutathione peroxidase (GPx).[22] A third of the hemolysate was used for determination of lipid peroxidation (LPO).[23]

Statistical analysis: The results were subjected to analysis of variance (ANOVA) in completely randomized design (CRD) with statistical significance being tested using the Duncan Multiple Range Test.[24]

RESULTS

The effects of F and CPF at different dose levels on different antioxidant parameters are presented in Table 1, and the protective effects of vitamins C and E on these antioxidant parameters are presented in Table 2.

Table 1. Effects of daily oral administration of F and CPF alone and in combination at different dosage levels for 28 days on various antioxidant parameters in blood of rats. (Values are mean ± SEM, n=6)*

Activity/Groups	Control	1 ppm F in DW	10 ppm F in DW	1 mg CPF/kg bw	10 mg CPF/kg bw	1 ppm F in DW + 1 mg CPF/kg bw	10 ppm F in DW + 10 mg CPF/kg bw
SOD (Units/mg protein)	79.45^{c} ±1.76	72.33^{bc} ±1.23	64.76^{bc} ±2.07	51.34^{ab} ±2.09	49.21^{ab} ±1.07	36.45^{a} ±0.49	33.19^{a} ±0.66
CAT (µmol H_2O_2 utilized/min/mg protein)	141.21^{c} ±7.64	112.37^{abc} ±10.97	115.69^{abc} ±4.01	113.9^{abc} ±5.73	105.0^{bc} ±13.66	104.45^{ab} ±16.98	86.01^{a} ±11.51
GPx (U/mg protein)	7.70^{c} ±0.2	7.40^{bc} ±0.2	6.70^{b} ±0.4	7.20^{bc} ±0.1	6.50^{b} ±0.2	7.00^{bc} ±0.3	5.00^{a} ±0.20
GST (µmol conjugate CDNB/min/mg protein	0.007^{a} ±0.001	0.007^{a} ±0.001	0.0083^{b} ±0.001	0.007^{a} ±0.001	0.006^{a} ±0.001	0.0081^{a} ±0.001	0.0071^{a} ±0.001
GSH (nmol/mL)	71.01^{e} ±1.15	68.37^{e} ±2.22	63.16^{d} ±0.60	60.07^{cd} ±1.15	54.07^{b} ±0.86	57.13^{b} ±1.16	49.30^{a} ±1.28
MDA (nmols MDA formed/mL RBCs)	2.51^{a} ±0.20	3.34^{b} ±0.23	3.95^{bc} ±0.32	3.43^{b} ±0.19	4.37^{c} ±0.15	4.29^{cd} ±0.31	5.07^{d} ±0.29

*Means with at least one common superscript do not differ significantly ($p<0.05$).

Table 2. Effects of daily administration of vitamins C and E in modulating oxidative stress parameters induced after 28 days by co-administration of F and CPF in rats. (Values are mean ± SEM, n=6)*

Activity/Groups	Tap DW without vit. C	Tap DW + corn oil without vit. E	Tap DW + 60 mg vit. C/kg bw	Tap DW + 100 mg vit.E /kg bw	10 ppm F in DW + 10 mg CPF/kg bw + 60 mg vit. C/kg bw	10 ppm F in DW + 10 mg CPF/kg bw + 100 mg vit. E/kg bw
SOD (Units/ mg protein)	80.67^{bc} ± 8.47	72.66^{bc} ±8.62	85.33^{c} ± 4.09	90.16^{c} ± 5.77	55.23^{a} ± 2.90	62.66^{a} ± 5.12
CAT (µmol H_2O_2 utilized/min/mg protein)	130.32^{bc} ± 6.13	137.33^{bc} ± 4.20	142.78^{c} ± 7.51	146.43^{c} ± 6.29	119.60^{a} ± 6.85	107.17^{a} ± 5.95
GPx (U/mg protein)	8.00^{c} ± 0.2	7.60^{bc} ± 0.5	8.60^{c} ± 0.3	8.30^{c} ± 0.2	6.30^{a} ± 0.3	6.80^{ab} ± 0.2
GST (µmol conjugate CDNB/min/mg protein	0.007^{a} ±0.001	0.006^{a} ± 0.001	0.006^{a} ±0.001	0.007^{a} ± 0.001	0.005^{b} ± 0.001	0.006^{b} ± 0.001
GSH (nmol/mL)	69.01^{ab} ± 1.29	72.61^{a} ± 1.47	83.98^{c} ± 1.15	80.01^{c} ± 1.14	62.07^{a} ± 0.58	67.08^{a} ± 1.50
MDA (nmols MDA formed/mL RBCs)	2.45^{bc} ± 0.18	2.55^{c} ± 0.07	2.12^{ab} ± 0.11	1.95^{a} ± 0.09	4.27^{d} ± 0.11	3.91^{d} ± 0.15

*Means with at least one common superscript do not differ significantly ($p<0.05$).

Changes in SOD and CAT activity: Compared to the control group, lower and higher doses of F did not produce any significant change in the activity of SOD and CAT, whereas CPF at either dose level induced a significant ($p<0.05$) decrease in the activity of SOD. The combined administration of F and CPF at low or high doses also resulted in a significant decrease in the activity of SOD. However, administration vitamin C or E with F and CPF did not reveal any significant alteration in SOD activity. CAT activity did not change appreciably with either F or CPF at different dose levels, but it showed a significant ($p<0.05$) decline with combined exposure to F and CPF at both lower and higher dosages. Simultaneous administration of vitamin C or E failed to reverse the decline in activity of CAT in the rats co-exposed to F and CPF.

Changes in GSH level, GPx and GST activity: The GSH level decreased significantly ($p<0.05$) in the rats provided with either F or CPF at the higher dosages as well as those co-exposed to these toxicants at lower or higher dosages. Vitamin C or E administered with CPF and F significantly improved ($p<0.05$) the content of GSH levels in the blood to levels similar to those in control animals. GPx activity decreased significantly ($p<0.05$) in rats exposed daily to F or CPF alone at higher dosages as well as in those with co-exposure to the higher dosages. Administration of vitamin C or E failed to alleviate the decline in activity of GPx induced by combined exposure to F and CPF at higher concentration. There was a significant ($p<0.05$) increase in activity of GST in animals exposed to different dosages of F, whereas CPF exposure showed a non-significant alteration in the GST activity. A significant ($p<0.05$) decrease in the activity of GST was observed in animals exposed to F and CPF along with vitamin C or E.

Alteration in malondialdehyde (MDA) levels: The exposure to F and CPF alone or in combination induced lipid peroxidation of the erythrocyte membrane as revealed by a significant ($p<0.05$) increase in MDA levels at all dose levels. Treatment with vitamin C and E failed to prevent lipid peroxidation of the membrane induced by combined exposure to F and CPF.

DISCUSSION

In our previous studies, we have investigated the potential of pyrethroids like cypermethrin,[15] bifenthrin,[16] and deltamethrin,[14] to induce oxidative changes in animals following subacute exposure to them.[25] As we reported recently, such subtle health effects were more pronounced with co-exposure of deltamethrin and F.[14] In the present study, we investigated whether such compromised antioxidant systems are also seen with repeated co-exposure of rats to the organothiophosphate pesticide CPF and F as well as the protection of the antioxidant vitamins C and E in alleviating such oxidative stress in rats. Lipid peroxidation from oxidative stress is known to disturb the integrity of cellular membranes leading to the leakage of cytoplasmic enzymes.[26] LPO as revealed by enhanced MDA levels in blood, represents one of the most frequent reactions of free radical attack on biological membranes resulting from the disturbance of the oxidant/antioxidant balance in the biological system.[27] Higher MDA levels due to F and CPF in the present study indicate damage to the biological membranes from

an increase in free radicals and ROS generated during the metabolism of F and CPF in the body. Simultaneous administration of the antioxidant vitamins C or E exerted no protective effects on MDA levels in co-exposed F and CPF rats. Thus co-exposure to F and CPF in different dosages appears to have induced oxidative protein modifications due to either excessive oxidation of macromolecules or by decreasing the capacity of the free radical scavenging mechanisms of the body. Our previous studies have also shown that the pesticides deltamethrin[14] and cypermethrin[15] induce lipid peroxidation in animal models.

Generation of free radicals and ROS are a continuous process in the body, and to counteract their damaging effects, mammalian cells are endowed with extensive antioxidant defense mechanisms consisting of enzymatic action by SOD, CAT, GPx, and GST, along with non-enzymatic components like GSH, total thiols, carotene, etc.[6,10] SOD is the first and major line of defense against the action of $\bullet O_2^-$ and other ROS. SOD converts superoxide into H_2O_2 and O_2, and its decreased activity in the present study is suggestive of its excessive utilization for neutralizing superoxide and ROS generated by the F and CPF toxicants and their interaction.[28-31] Excess production of H_2O_2 and other hydro-peroxide radicals results in the depletion in CAT activity.[32] A similar decrease in the activity of CAT has also been found in rats treated with F[33] and deltamethrin.[14,34]

GST catalyzes the interaction of GSH sulphydryl group with electrophilic centers in a wide variety of substrates. The induction of GST in the present study could be a defensive mechanism to counter-balance the oxidative insult by utilizing endogenous GSH. This effect might be the reason for the depletion of GSH from exposure to F and CPF, especially when they were co-administered at higher dosages. Similar decreases in GSH levels have been reported in broiler chicks treated with deltamethrin,[34] rats exposed to F,[35] and rats co-exposed to NaF and katron.[27] GPx activity is dependent upon the level of GSH, and the depletion of GSH in the present study is therefore likely to be due to neutralization of excess free radicals and H_2O_2 production resulting in a significant decrease of GPx activity in the toxicant-exposed rats, especially in the co-exposed groups.

Reduced GSH levels along with the decreased activities of SOD, CAT, and GPx suggest that F and CPF exposure induced an accumulation of ROS, which can interfere with enzyme and receptor protein activities by cross-linking and fragmentation of protein strands, oxidation of amino acids like cysteine and methionine in cells leading to abnormal cellular effects. This interpretation is further supported by a recent report on chronic exposure of chicken broilers to F that caused reduced cellular and humoral immunity as exhibited by reduced cytokine IL-4, IL-6, TNF-α, and IFN-γ content in the cecal tonsil.[36]

In summary, the results of the present study clearly indicate that repeated exposure of rats to F and CPF generates an excess of free radicals and ROS responsible for oxidative stress as indicated by increased MDA levels and altered stress parameters. F thus exerted a potentiating effect on the capability of CPF to induce alterations in antioxidant indices in the rats. However, simultaneous

administration of antioxidant vitamin C or E partially attenuated the toxicity induced by exposure to F and CPF.

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