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Chlorpyrifos-induced oxidative stress in rat's brain and protective effect of grape seed extract

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Abstract

Oxidative stress is one of the possible mechanisms resulted from chlorpyrifos toxicity. Therefore, the aim of this study is to evaluate the in vivo effects of chlorpyrifos (7.5 mg/kg, s.c., for 28 days, 1/10 LD50 of CPF) on tissues antioxidant system of wistar rat and the efficacy of grape seed proanthocyanidin extract (GSPE; 100 mg/kg/day body weight) as polyphenols to antagonize this response. The parameters were acetylcholinesterase (AChE), levels of malondialdehyde (MDA) as a marker of lipid peroxidation; reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) were estimated in brain tissue. Administration of CPF for 28 days induced a significant increase in LPO levels and inhibition in brain AChE activity. Also, results showed significant decreases in GSH content, CAT and SOD activities in brain. Supplementation with grape seed proanthocyanidin extract to treated animals significantly ($P< 0.05$) attenuated the toxicity and oxidative stress evoked by CPF.

Keywords: Chlorpyrifos, Grape seed proanthocyanidin extract, Acetylcholinesterase, Oxidative stress, Antioxidant system.

Introduction

Chlorpyrifos [O, O-diethyl-O-(3, 5, 6-trichloro-2-pyridyl)-phosphorothioate, CPF], is a member of the most commonly used organophosphorus insecticide. Like other organophosphate (OP) compounds, chlorpyrifos is known to produce toxic effects through the inhibition of acetylcholinesterase (AChE) activity.[1] As a result of this activity, large quantities of acetylcholine (ACh) are accumulated in synaptic clefts[2], leading to a progression of noxious signs, including hypersecretions, convulsions, cognitive dysfunction and ultimately death due to inhibition of respiratory center in medulla oblongata.[3, 4]

As demonstrated by epidemiological studies, oxidative stress has been described as a co-lethal factor in both acute and chronic intoxication with OP-induced poisoning which is manifested by changes in antioxidative enzymatic activities in humans and rats.[5, 6] Moreover, these authors have shown the effects of oxidative stress in the form of increased concentration of e.g. malonyldialdehyde (MDA), being a lipid peroxidation marker as well as increased level of ROS.[7, 8] Exposure to chlorpyrifos increased levels of lipid peroxides in the rat liver, kidney, brain, and erythrocytes and altered antioxidant enzymes in rat blood, liver, and lung.[9-14] Moreover, administration of CPF to pregnant rats induced oxidative stress and altered antioxidant system in liver, kidney, brain, and fetus.[15]

Brain accessible phytochemicals potentially defend against oxidative damage.[16] A large amount of synthetic and natural antioxidants have been revealed to induce beneficial effects on human health and disease prevention. Grapes and grape products are good sources of dietary flavonoids, have long been documented to possess many properties, including antioxidant, anti-inflammatory, anticarcinogenic, platelet aggregation inhibiting, and metal chelating properties, etc. [17] Yamakoshi et al.[18] showed that grape seed extracts are non-toxic to rats. Moreover, grape seeds polyphenols (proantho-cyanidins) were found to be highly bioavailable providing significantly greater protection against free radicals, free radical-induced lipid peroxidation and DNA damage than that observed with vitamins C, E and β-carotene, possibly due to its broad spectrum of health benefits.[19] Thus, many recent studies suggest that in-vivo grape seeds proanthocyanidins exposure may protect multiple organs from a variety of toxic assaults.[19, 20]

Thus the aim of this study was to investigate the effect of grape seed extract on chlorpyrifos-induced oxidative stress in rats.

Material and Methods

Animals

Age matched male Wistar rats, weighing 200-250g, were employed. Rats were fed on standard chow diet (Ashirwad Industries, Ropar, India) and water ad libitum. The animals were maintained at an ambient temperature of 25+20C and 55-65% relative humidity less than 12 h/12-h light/day cycles. The experimental protocol used in the current study was approved by the Institutional Animal Ethical Committee.

Drugs and Chemicals

Chlorpyrifos 80% emulsion concentrate (Tafaban, Tata Rallis) was purchased from local market. Grape seed extract (GSPE) was kindly donated by Biogen Extracts (P) Ltd. Bangalore, India. All other chemicals used in present study were of analytical grade.

Animal treatment schedule

The rats were divided into two groups: control (n = 6) and experimental (n = 12). Rats in the experimental group were further divided into two treatment groups chlorpyrifos (n = 6), chlorpyrifos + GSPE (n = 6).

Control treatment

For the control group, normal saline was administered via gavage once per day**.**

Chlorpyrifos treatment

For the chlorpyrifos-only group, the rats were given chlorpyrifos dissolved in distilled water at a dose of 7.5 mg/kg, s.c., ($1/10^{th}$ LD_{50} of CPF, s.c.) for 28 days.

Chlorpyrifos plus GSPE treatment

Rats were given GSPE suspended in 0.5% carboxymethyl cellulose at a dose of 100mg/kg after two hours of CPF and were administered orally through oral cannula for 28 days. All animals were sacrificed on day 45 for biochemical estimation.

Biochemical estimation

Biochemical tests were carried out on day 45.

Tissue Preparation

Animals were sacrificed by decapitation and the brains were removed and rinsed with ice-cold isotonic saline. Brain tissue samples were then homogenized with ice-cold 0.1 mol/L phosphate buffer (pH 7.4) 10 times (w/v). The homogenate was centrifuged at 10000g for 15 min and aliquots of supernatant were separated and used for biochemical estimation.

Measurement of Acetylcholinesterase activity

The quantitative measurement of acetylcholinesterase levels in brain was performed according to the method of Ellman.[21]The assay mixture contained 0.05 ml of supernatant, 3 ml of 0.01 M sodium phosphate buffer (pH 8), 0.10 ml of acetylcholine iodide and 0.10 ml of 5,5-dithio-bis(2-nitrobenzoic acid) (DTNB) (Ellman reagent). The change in absorbance was measured immediately at 412 nm using UV-1700 spectrophotometer, Shimadzu, Japan. The enzymatic activity in supernatant was expressed as nmol per mg protein.

Estimation of Reduced Glutathione (GSH)

GSH in the brain was estimated according to the method described by Ellman.[22] 1 ml supernatant was precipitated with 1ml of 4% sulfosalicylic acid and cold digested at 41°C for 1 hour. The samples were centrifuged at 1200g for 15 min at 41°C. To 1 ml of this supernatant, 2.7 ml of phosphate buffer (0.1 mol/l, pH 8) and 0.2 ml of 5, 50-dithio-bis (2-nitrobenzoic acid) were added. The yellow color developed was read immediately at 412 nm by using the UV-1700 spectrophotometer, Shimadzu, Japan. Results were calculated using molar extinction coefficient of chromophore (1.36×104/mol/l/cm) and expressed as nmol per mg protein.

Measurement of Lipid Peroxidation (MDA)

The quantitative measurement of lipid peroxidation in the brain was performed according to the method of Wills.[23] The amount of MDA, a measure of lipid peroxidation, was measured by reaction with thiobarbituric acid at 532 nm by using the UV-1700 Spectrophotometer, Shimadzu, Japan. The values were calculated by using molar extinction coefficient of chromophore (1.56×105/mol/l/cm) and expressed as nmol per mg protein.

Estimation of Catalase (CAT)

Catalase activity was assayed by the method of Luck[24] (1971), wherein the breakdown of hydrogen peroxides (H_2O_2) was measured at 240 nm. Briefly, assay mixture consisted of 3 ml of H_2O_2 phosphate buffer and 0.05 ml of supernatant of tissue homogenate (10%), and change in absorbance was recorded at 240 nm with UV-1700 Spectrophotometer, Shimadzu, Japan. The results were expressed as micromole H_2O_2 decomposed per mg protein /min.

Estimation of superoxide dismutase (SOD)

SOD was assessed by utilizing the method of Kakkar and Vishwnathan.[25] A single unit of enzyme was expressed as 50% inhibition of nitroblutetrazolium (NBT) reduction/min/mg protein by superoxide is measured at 560nm. In Brief, a mixture (1.5 ml) contained 1.0 ml of 0.01M phosphate buffer pH 7.0, 0.2 ml of tissue homogenate and 0.4 ml of 2M H_2O_2 . The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio). Results were expressed as SOD enzyme required for 50% inhibition of NBT reduction (% activity).

Estimation of Protein

Protein estimation was done by Biuret method using Bovine Serum Albumin (BSA) as standard.[26]

Statistical analysis

The data was expressed as Mean ± Standard Deviation (S.D). The statistical significance of the differences between parameters among the three experimental groups was evaluated by means of one-way analysis of variance (ANOVA) followed by Turkey's multiple comparison test. P values <0.05 was adopted as a criterion of significance. Statistical analysis was performed by means of Graph PAD prism software program (Version 5.0).

Results

AChE activity:

The present results revealed that chlorpyrifos treated rats showed a significant (P<0.05) inhibition in the activity of AChE when compared with the normal rats at the end of day 45. Supplementation with GSPE significantly countered this effect when compared with the chlorpyrifos-only group (P<0.05, Fig. 1).

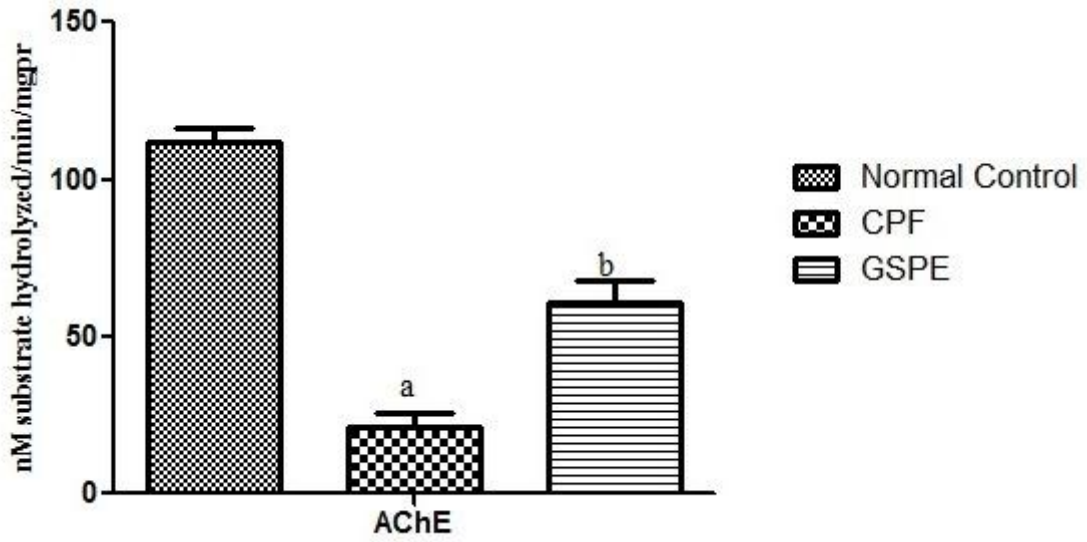

Fig. 1: Effects of subacute treatment of chlorpyrifos on AChE activity in the brain tissues of rats. aComparison of control vs CPF, bComparison of CPF vs GSPE. Values are mean ± SD of six rats in each group. Significance at P < 0.05

MDA levels:

The level of MDA was significantly increased in the chlorpyrifos only group compared with the control. However, the MDA level was significantly decreased in brain tissue at the end of the 45 day in the chlorpyrifos plus GSPE group compared with the chlorpyrifos-only group ($P < 0.05$, Fig. 2).

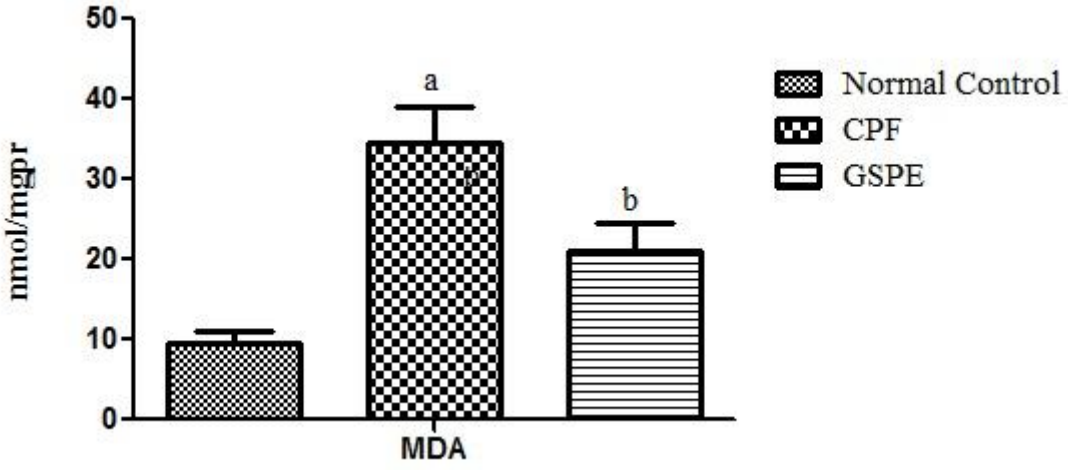

Fig. 2: Effects of subacute treatment of chlorpyrifos on MDA content in the brain tissues of rats. a Comparison of control vs CPF, bComparison of CPF group vs GSPE. Values are mean±SD of six rats in each group. Significance at $P < 0.05$

SOD activity

A significant decrease in SOD activity was observed at the end of the 45 day in the chlorpyrifos group compared with the control group. SOD activity was increased significantly in the -chlorpyrifos plus GSPE group compared with the chlorpyrifos group at the end of the 45day ($P < 0.05$, Fig. 3).

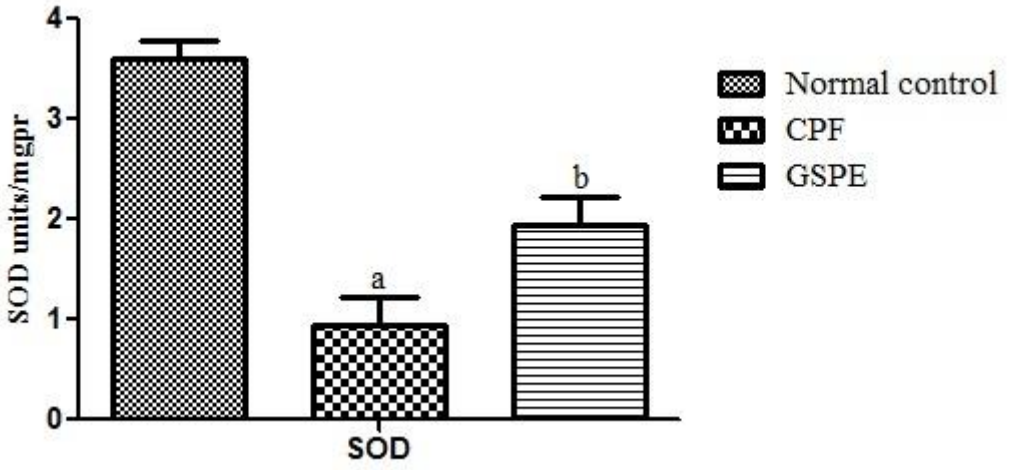

Fig. 3: Effects of subacute treatment of chlorpyrifos on SOD content in the brain tissues of rats. aComparison of control vs CPF, bComparison of CPF group vs GSPE. Values are mean±SD of six rats in each group. Significance at $P < 0.05$

CAT activity

The chlorpyrifos group showed a significant decrease in CAT activity compared with the control group. Yet, the chlorpyrifos plus GSPE treated rats showed statistically increased levels of CAT activity compared with the chlorpyrifos-only rats ($P < 0.05$, Fig. 4).

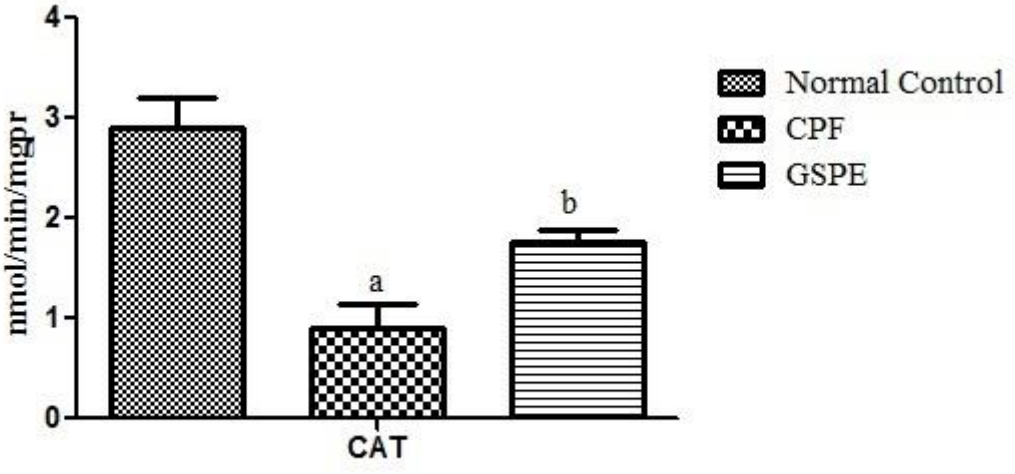

Fig. 4: Effects of subacute treatment of chlorpyrifos on CAT content in the brain tissues of rats. aComparison of control vs CPF, bComparison of CPF group vs GSPE. Values are mean±SD of six rats in each group. Significance at $P < 0.05$

GSH activity

GSH activity was significantly decreased in the chlorpyrifos only group relative to the control, Compared with the chlorpyrifos only rats, however, GSH activity increased significantly in the chlorpyrifos plus GSPE treated rats ($P < 0.05$, Fig. 5).

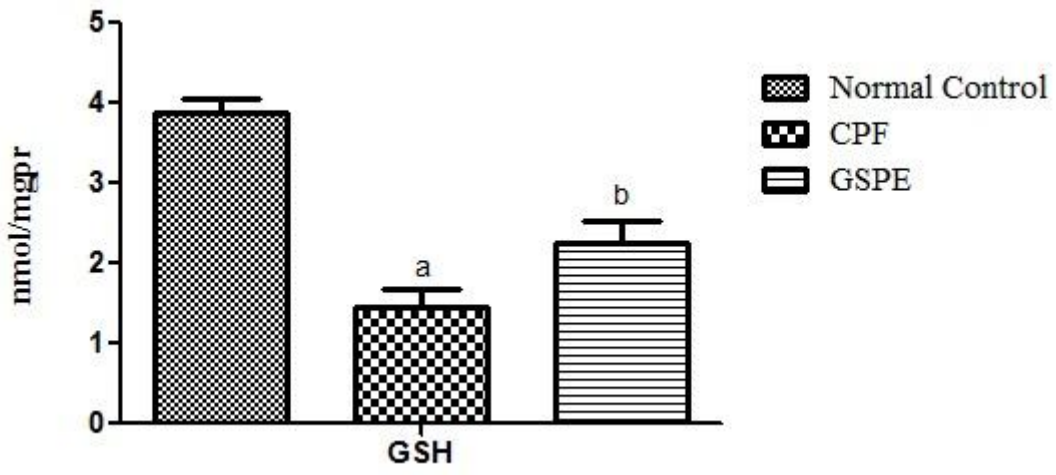

Fig. 5: Effects of subacute treatment of chlorpyrifos on GSH content in the brain tissues of rats. a Comparison of control vs CPF, bComparison of CPF group vs GSPE. Values are mean±SD of six rats in each group. Significance at $P < 0.05$

Discussion

Chlorpyrifos is one of the most widely used OP pesticides throughout the world including India. In *vitro* and in *vivo* studies shown that the main mechanism of OPs are related to buildup of acetylcholine within the cholinergic synapses resulting inhibition of acetyl cholinesterase by active oxon.[27, 28] The present study is in agreement with the pervious finding. CPF causes reduction in AChE activity as a marker of OPs toxicity. Grape seed extract treatment restored ChE activity near to control level indicating their ameliorating effect. In line with the earlier studies we found reduction in protein thiol due to CPF administration, which might be owed to increased degradation of protein or increased consumption of this antioxidant in stress environment which confirm the role of OPs in disruption of body total antioxidant capacity.[29-31] Our results indicated that supplementation with GSPE can reduce both the toxicity and oxidative stress of CPF treatment.

In OP toxicity, oxidative stress is an important patho-physiological mechanism, especially for neurotoxicity and cerebral damage. In addition, previous studies have been shown a relationship between inhibition of AChE and lipid peroxidation following subchronic and chronic exposure to OP.[8, 29] OP are known to produce oxidative stress by enhancing the production of reactive oxygen species (ROS), which play a key role, in initiating secondary brain damage.[32]

CPF-induced toxicity in the present study was also accompanied by an increase in lipid peroxidation (MDA) and a decrease in GSH contents, indicating a clear association between oxidative stress and lipid peroxidation. Lipid peroxidation, an important indicator of oxidative damage of biological tissues, was found to be induced in rats exposed to CPF.[33] Our results showed that exposure to CPF caused increased in lipid peroxidation as evidenced by increased levels of thiobarbituric acid reactive substances (TBARS), of which MDA is the most significant, reflect lipid peroxide production, decrease in GSH content, SOD and CAT activities in rat brain. The increase of free radicals and lipid peroxidation may result from the inhibition of GSH levels induced by CPF toxicity. The current findings are in agreement with other studies suggesting that accumulation of lipid peroxides has been resulted after exposure to acute dose of cholrpyrifos in rat liver[9], kidney[12], brain[10] and erythrocytes.[11] However, repetitive doses increased LPO levels as well as decreased antioxidant enzymes in blood, liver, and lungs of rat.[8, 34]

Administration of antioxidants effectively inhibited the oxidative damage induced by OPs.[12, 25, 35, 36,] Antioxidant activity of grape seed extract is due to its polyphenolic capacity which is a mixture of proanthocyanidines, which have been demonstrated to inhibit oxidative stress through modulation of metabolic functions, enhancement of detoxification pathways, and/or prevention of the interaction of xenobiotics with biological molecules.[18, 37] Previous investigation revealed the ability of grape seed extract[33] to suppress lipid peroxidation. Treatment with GSPE for a period of 28 days significantly protected against oxidative damage by modulation of LPO levels and GSH content as well as antioxidant enzymes CAT and SOD. Therefore, it can be suggested that, concomitant presence of GSPE, as a powerful antioxidant, minimize chlorpyrifos-dependent production of toxic radical species along with drastically limiting the ongoing lipid peroxidation.

Conclusion

The results obtained in the present work highlights the protective role of grape seed extract against OPs pesticides induced oxidative stress, therefore useful in the prevention of diseases in which free radicals are implicated.

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