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Research Article

Ameliorative Potential of *Allium sativum* against Chlorpyrifos-Induced Toxicity in Swiss Albino Mice (*Mus musculus*)

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ABSTRACT

Background: Chlorpyrifos (CPF) is one of the most widely used organophosphate pesticide in agricultural fields and is known to induce toxicity by inhibiting acetylcholinesterase, leading to oxidative stress, hepatotoxicity and metabolic disbalances. **Objectives:** The impact of CPF and ameliorative effect of *Allium sativum* on Swiss albino mice (*Mus musculus*) were assessed through biochemical markers, including lipid peroxidation, total serum protein, albumin, cholesterol and liver enzymes (ALT, AST, ALP). **Results:** CPF exposure significantly increased oxidative stress, as evidenced by elevated MDA levels (216.75%), disrupted liver function by increasing ALT (42.27%), AST (24.07%) and ALP (32.49%) levels. CPF exposure also reduced total protein and albumin levels (26.23% and 37.93% respectively) and also induced hypercholesterolemia. The co-administration of *A. sativum* (200 mg/kg) effectively protected the toxicity of CPF by reducing oxidative stress (165.99%), normalising liver function enzyme levels and restoring serum protein, albumin and cholesterol levels. The lower dose of *A. sativum* (100 mg/kg) provided partial protection and its efficacy was found to be statistically insignificant, whereas the higher dose showed a more considerable ameliorative effect. **Conclusion:** This study highlights the strong antioxidant, hepatoprotective and hypolipidemic properties of *A. sativum*, supporting its potential as a natural therapeutic agent against pesticide-induced toxicity.

Keywords: Chlorpyrifos, Organophosphate, Hepatotoxicity, Garlic.

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INTRODUCTION

Chlorpyrifos (CPF) is a broad-spectrum chlorinated organophosphate which is most widely used as an insecticide, acaricide and nematicide.[1] CPF induces its toxic effect by inhibiting acetylcholinesterase, causing continuous excitation of nervous system due to accumulation of acetylcholine.[2] CPF induces several adverse outcomes such as haematotoxicity, reproductive toxicity, developmental toxicity, cardiotoxicity and hepatic dysfunction.[3-7]

Allium sativum (garlic) has long been utilized as a natural cure for various diseases, indicating its wide-ranging therapeutic capabilities. Garlic's adaptability and efficacy in traditional medicine are supported by its health-promoting properties.[8,9] Garlic effectively manages lifestyle-related diseases like high blood pressure and hypercholesterolemia and is globally used as a functional food.[10] The principal bioactive components of garlic are sulphur-containing compounds such as allicin, diallyl disulfide, diallyl trisulfide, and S-allyl cysteine. When garlic cloves are crushed or chopped, the enzyme alliinase is activated to allicin which is responsible for garlic's distinctive odour.[11]

Lipid Peroxidation (LPO) assessment is very important biomarker in toxicology, as it reflects oxidative degeneration of lipids leading to membrane dysfunction, DNA damage and apoptosis.[12] Malondialdehyde (MDA) is a LPO biomarker and the principal oxidative product of peroxidised polyunsaturated fatty acids.[13] Pesticides can induce oxidative stress in an organism by generating free radicals that can cause LPO and membrane fluidity alterations.[14] CPF is known to cause oxidative stress in human and different animal cells.[15]

Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline Phosphatase (ALP) are essential biomarkers for liver function and hepatotoxicity.[16] Elevated levels of these enzymes indicate hepatic damage, oxidative stress and impaired metabolic functions.[17] ALT is primarily liver-specific, whereas AST reflects both hepatic and extrahepatic damage and ALP is associated with biliary dysfunction.[18] Albumin which is synthesized in the liver, serves as an indicator of hepatic protein

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synthesis, while alterations in total protein levels indicates systemic toxicity and organ dysfunction.[19] Total cholesterol assessment is essential to understand lipid metabolism disruptions and oxidative stress.[20] Hence, in the present study, the toxic effect of CPF on *Mus musculus* and the ameliorative potential of *A. sativum* against CPF induced mice were investigated.

MATERIALS AND METHODS

Chemicals

Commercial-grade Chlorpyrifos (50% EC) was purchased from manufacturer Noble Crop Science (NCS), India. Other analytical-grade chemicals and reagents used in this research were obtained from Merck, Sigma-Aldrich, Himedia and Loba (India) chemical manufacturers.

Allium sativum aqueous extracts

Fresh garlic was obtained from a retail store in the local market. Garlic were peeled, washed and sliced into tiny pieces using a fine knife and 500 mg fine garlic was homogenised in 100 mL distilled water. Homogenates were filtered using 75 µm mesh and remnants were centrifuged at 3000 rpm for 10 min. The supernatant and homogenates were filtered and stored in a reagent bottle at 4°C.[21]

Model animal and treatment protocol

Healthy adult, *M. musculus* weighing 25.0±5.0 g was procured from the animal house of University Department of Zoology, T. M. Bhagalpur University. Animals were housed in polypropylene cages maintained with 12 hr light-dark cycle at 22±4°C and 45-60% relative humidity. Food and water were provided *ad libitum*. Experimental protocol was conducted following the guidelines of Institutional Animal Ethics Committee and CPCSEA, India. The mice were divided into 6 groups according to dosage and were treated orally for six weeks. (Group-1 served as control; Group-2 treated with CPF (6 mg/kg); Group-3 treated with 100 mg/kg of *A. sativum* extract (AS1); Group-4 treated with 200 mg/kg of *A. sativum* extract (AS2); Group-5 treated with CPF and *A. sativum* extract (100 mg/kg) (CPF+AS1) and Group-6 treated with CPF and *A. sativum* extract (200 mg/kg) (CPF+AS2).

Biochemical parameters

Mice tail was submerged in warm water at 40°C to dilate the vessels and blood samples were drawn from the lateral caudal vein using sterilised lancets and serum separator tubes.[22] The samples were centrifuged at 3000 rpm for 10 min to obtain serum for several biochemical analysis. Thiobarbituric Acid Reactive Substances (TBARS) were measured by method given by Okhawa *et al.*[23] LPO was expressed as nmol MDA/mL. ALT and AST activities were determined using an estimation kit according to the method of Reitman and Frankel.[24] ALP activity was measured using the kinetic method.[25] Total serum cholesterol levels were measured using the method of Zlatkis and Zak with slight modifications.[26] Total protein content in serum was measured by the Lowry's method and serum albumin was measured with bromocresol green using succinate buffer.[27,28]

Statistical analysis

Data were statistically analysed on IBM SPSS (version 26) by Analysis of Variance test and '*t*'- test followed by *post hoc* multiple comparison tests to determine significance.

RESULTS

The effect of sub-lethal dose of Chlorpyrifos (6 mg/kg) was studied in Swiss albino mice on lipid peroxidation, serum protein, albumin and cholesterol along with liver function enzymes. The protective potential of *Allium sativum* against CPF-treated group was also assessed. During the experiment no sign of mortality was observed in any treated group. However, some changes in morphological appearance and behavioural patterns were observed after CPF treatment viz., hair loss and skin darkening, decrease in body weight, excessive tearing, decreased movement and increased aggression.

LPO level

MDA is very important biomarker of oxidative lipid damage. MDA level was increased 216.75% (6.24±0.58 nmol MDA/mL) more as compared to control group (1.97±0.18 nmol MDA/mL). This elevation was statistically significant by the t-test ($p<0.05$). The principal bio-active component of *A. sativum* is allicin which has significant ameliorative potential. The administration of *A. sativum* (200 mg/kg) demonstrated a significant protective effect against LPO by reducing MDA levels. In the untreated group, *A. sativum* alone reduced MDA levels significantly in comparison to control (F=11.519). Furthermore, in CPF-treated mice, the co-administration of *A. sativum* effectively ameliorated oxidative stress, reducing MDA levels from 6.24±0.58 nmol MDA/mL (CPF group) to 2.97±0.11 nmol MDA/mL, representing a 165.99% improvement. The ameliorative potential of lower dose of *A. sativum* (100 mg/kg) was also found significant by reducing MDA level by 89.85% (Figure 1).

Total serum protein and albumin

The serum protein was significantly decreased by 26.23% in CPF-treated group as compared to control. Similarly, albumin levels also decreased by 37.93% in CPF-treated group as compared to control (Figure 2). The decrease in the serum protein and albumin was statistically significant by *t*-test ($p<0.05$). Higher dose (200 mg/kg) of *A. sativum* was sufficient to improve total protein (6.17±0.19 g/dL) and albumin (3.92±0.27 g/dL) levels significantly even in the untreated group with respect to control (4.88±0.35 g/dL and 2.91±0.11 g/dL respectively) (Table 1). In the co-administered group, higher dose of *A. sativum* showed a protective effect of 15.78% on total protein (4.37±0.23 g/dL) and of 31.04% on albumin level (2.7±0.13 g/dL) with respect

to CPF-treated group. Although, higher doses of *A. sativum* significantly improved albumin levels (F=20.297), but its protective effect on total protein was found to be statistically insignificant.

Liver function

The liver function was determined by the study of several enzymes viz., ALT, AST and ALP. ALT and AST activities were increased by 42.27% and 24.07% respectively in the CPF-treated groups as compared to control. Similarly, significant elevation of ALP (32.49%) was observed in CPF-treated mice as compared to control (Figure 2). The elevation in ALT, AST and ALP was observed statistically significant by t-test ($p<0.05$). Higher dose of *A. sativum* (200 mg/kg) when co-administered together with CPF, significantly improved ALT (57.9±2.22 IU/L, 36.82%), AST (113.54±3.29 IU/L, 18.6%) and ALP (180.1±4.79 IU/L, 27.79%) with respect to CPF-treated group (Figure 2). However, the effect of lower dose of *A. sativum* (100 mg/kg) in providing hepatoprotective benefits was found to be statistically insignificant.

Serum cholesterol level

In CPF-treated group, the cholesterol level also increased about 28.17% as compared to control group (Table 1). This increase was statistically significant (t = 2.497, $p<0.05$). Higher doses of *A. sativum* significantly improved serum cholesterol levels even in the untreated group (F=4.040). The group in which CPF and *A. sativum* (200 mg/kg) was co-administered together showed a significant reduction on serum cholesterol (125.67±5.32 mg/dL, 25.12%) with respect to CPF-treated group. However, the protective effect of lower dose of *A. sativum* (100 mg/kg) on serum cholesterol was found to be statistically insignificant.

DISCUSSION

Chlorpyrifos induced toxic impact on oxidative stress, hepatotoxicity and lipid metabolism disturbances has been earlier documented on male zebrafish and adult rats.[29,30] The protective role of *A.* sativum has also been reported on several organs of adult rats.[31]

In the present study, CPF-treated mice showed a significant increase in MDA levels confirming oxidative stress-induced LPO. These findings are consistent with previous studies reporting CPF-mediated oxidative damage in liver.[32,33] The co-administration of *A. sativum* (200 mg/kg) with CPF (6 mg/kg) significantly mitigated oxidative stress, indicating a 165.99% ameliorative effect. The antioxidant potential of *A. sativum* is attributed to its organosulfur compounds, particularly allicin, which neutralizes Reactive Oxygen Species (ROS) and prevents LPO.[34]

CPF exposure significantly reduced total serum protein and albumin levels, indicating impaired protein metabolism and hepatic dysfunction. A decline in protein levels indicates hepatocellular injury, as previously reported in pesticide toxicity studies by Gupta *et al.*[35] The higher dose of *A. sativum* (200 mg/kg) significantly restored total protein and albumin levels in CPF-treated mice. The hepatoprotective effects of *A. sativum* have been linked to its ability to modulate enzymatic antioxidants and stabilize protein synthesis pathways.[36]

Hepatic damage due to CPF exposure was evident by a significant rise in liver function enzymes. These alterations align with the reports indicating hepatotoxic effects of organophosphate pesticides via oxidative damage and mitochondrial dysfunction.[37] The co-administration of *A. sativum* with CPF markedly reduced ALT, AST and ALP levels, signifying its hepatoprotective effect. These findings support the role of *A. sativum* in enhancing hepatic antioxidant defenses and detoxification mechanisms.[38]

CPF exposure induced a 28.17% increase in serum cholesterol, suggesting an alteration in lipid metabolism and hepatic cholesterol regulation. Elevated cholesterol levels due to pesticide exposure have been previously linked to disrupted lipid homeostasis and oxidative stress-induced hepatic dysfunction.[39] The administration of *A. sativum* significantly reversed this effect, proving its lipid-lowering and hypocholesterolemic properties. The hypolipidemic action of *A. sativum* is attributed to its bioactive sulfur compounds, which regulate cholesterol biosynthesis pathways.[40]

Table 1: Chlorpyrifos–induced toxicity and ameliorative effect of *Allium sativum* in different treated groups.

Groups (*n*=5)	LPO (nmol MDA/mL)	Protein (g/dL)	Albumin (g/dL)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Cholesterol (mg/dL)
Control	1.97±0.18	4.88±0.35	2.91±0.11	54.91±2.38	107.65±2.73	172.02±6.08	124.46±2.88
CPF	6.24±0.58	3.6±0.18	1.83±0.12	78.12±5.8	133.56±3.73	227.9±9.93	159.52±13.74
AS1	1.45±0.17	5.1±0.31	3.13±0.29	53.87±2.69	102.89±1.93	163.39±3.84	112.96±6.79
AS2	0.98±0.06	6.17±0.19	3.92±0.27	51.19±2.28	93.92±1.6	152.13±6.46	106.36±2.82
CPF+AS1	4.47±0.38	3.85±0.16	2.13±0.07	72.61±4.48	122.33±3.04	214.14±9.41	140.6±7.59
CPF+AS2	2.97±0.11	4.37±0.23	2.7±0.13	57.9±2.22	113.54±3.29	180.1±4.79	125.67±5.32

All data are expressed as Mean± Standard Error.

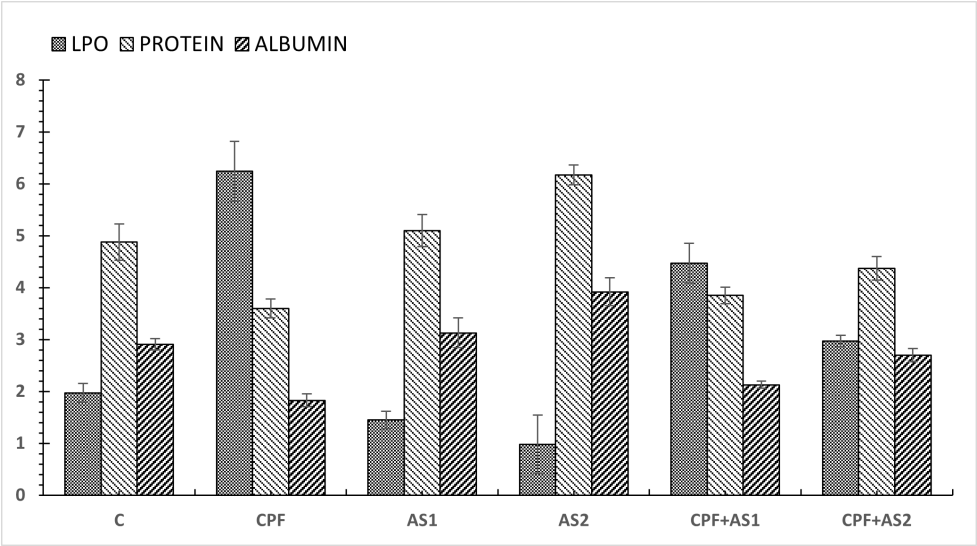

Figure 1: LPO, total protein and albumin levels in different groups.

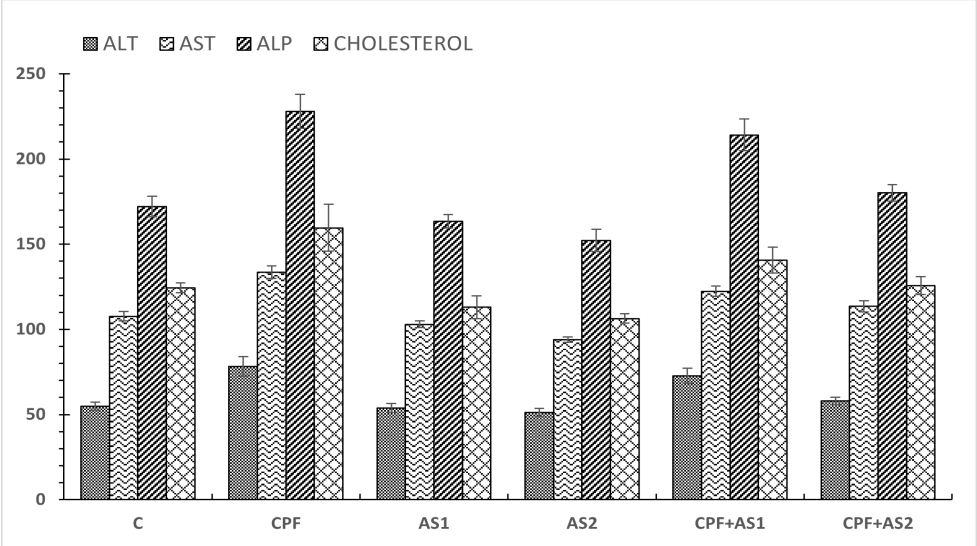

Figure 2: ALT, AST, ALP activities and total cholesterol in different groups.

CONCLUSION

The present investigation provides considerable evidence that CPF exposure induces significant oxidative stress, hepatotoxicity and lipid metabolism disturbances in *M. musculus*, as reflected by elevated Lipid Peroxidation (LPO), altered liver function biomarkers (ALT, AST and ALP) and increased serum cholesterol levels. The observed biochemical alterations confirm the detrimental effects of CPF on hepatic and metabolic functions.

Importantly, the co-administration of *A. sativum* (200 mg/kg) demonstrated a remarkable ameliorative effect by significantly reducing LPO levels, restoring total protein and albumin concentrations, improving liver enzyme activities, and normalising serum cholesterol levels.

These findings highlight *A. sativum* as a natural therapeutic agent with significant protective effects against CPF-induced toxicity. Further investigations are needed at molecular level and long-term safety profile to establish its role as an effective bioactive compound to manage pesticide-induced toxicities.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CPF: Chlorpyrifos; **LPO:** Lipid peroxidation; **MDA:** Malondialdehyde; **ALT:** Alanine aminotransferase; **AST:** Aspartate aminotransferase; **ALP:** Alkaline phosphatase; **ROS:** Reactive oxygen species; **AS1:** *Allium sativum* extract 100 mg/kg; **AS2:** *Allium sativum* extract 200 mg/kg.

CONTRIBUTION DETAILS

Divyanshu-Experimental studies, Data acquisition and Manuscript preparation.

Md. Sarfaraz Nawaz-Literature search and Data analysis.

Md. Equbal Ahmad-Statistical analysis, Manuscript editing and Manuscript review.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

All experimental procedures in the study were conducted in accordance with the guidelines of the Institutional Animal Ethics Committee (IAEC) and the Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA), India. The study was reviewed and approved by T. M. Bhagalpur University under the registration number 795/2022. Proper care and handling of animals were ensured to minimize any potential distress, following the ethical principles outlined by CPCSEA.

SUMMARY

This study investigates the toxic effects of Chlorpyrifos (CPF) and the ameliorative potential of *Allium sativum* in Swiss albino mice (*Mus musculus*). CPF exposure led to significant oxidative stress, hepatotoxicity and metabolic imbalances, as evidenced by increased lipid peroxidation (MDA levels), elevated liver enzymes (ALT, AST, ALP) and hypercholesterolemia. CPF also reduced serum protein and albumin levels. The co-administration of *A. sativum* (200 mg/kg) with CPF (6 mg/kg) significantly protected from the toxic effect induced by CPF by reducing oxidative stress (165.99%), restoring liver function markers and normalizing protein and cholesterol levels. The lower dose of *A. sativum* (100 mg/kg) provided partial protection but was statistically insignificant. These findings indicate the strong antioxidant, hepatoprotective and hypolipidemic properties of *A. sativum*, suggesting its potential as a natural therapeutic agent against pesticide-induced toxicity. Further investigation is recommended to explore its molecular mechanisms and long-term safety in managing environmental toxicant exposure.

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