Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **43** Issue **1** Year **2022** Page **1424 - 1431***

Restitutive Effects of *Allium Sativum* on Oxidative Stress and Hepatotoxicity in Dimethoate Induced *Mus Musculus*

Divyanshu[1], Chahat Kumari[1], Md. Equbal Ahmad[1]*

*[1]*University Department of Zoology, T. M. Bhagalpur University, Bhagalpur, Bihar*

***Corresponding Author**: Prof. (Dr.) Md. Equbal Ahmad*
University Department of Zoology, T. M. Bhagalpur University, Bhagalpur, Bihar, India,
Email: equbal.tmbu@yahoo.com

Abstract

Dimethoate (DT) is an organophosphate insecticide which is widely used in agricultural fields for the control of several types of insect pests. It causes toxicity in mammals by inducing oxidative stress and hepatotoxicity by disrupting antioxidant defences and altering liver biomarkers. The present study evaluates the restitutive potential of *Allium sativum* aqueous extract against DT-induced toxicity (16 mg/kg) in Swiss albino mice by the assessment of biochemical markers including lipid peroxidation, total serum protein, albumin, cholesterol and liver enzymes (ALT, AST, ALP). DT exposure significantly increased oxidative stress, as evidenced by elevated MDA levels (165.48%), disrupted liver function by increasing ALT (26.83%), AST (41.74%) and ALP (22.34%) levels. DT exposure also reduced total protein and albumin levels (31.97% and 30.24%) and induced hypercholesterolemia (27.33%). The co-administration of *A. sativum* (200mg/kg) with DT, effectively prevents the toxicity by reducing oxidative stress (126.9%), normalising liver enzymes levels and restoring serum protein (28.07%), albumin (19.93%) and cholesterol (23.9%) levels. The lower dose of *A. sativum* (100 mg/kg) provided partial protection and its restitutive efficacy was found to be statistically insignificant. This study highlights the strong antioxidative, hepatoprotective, hypolipidemic properties and dose-dependent effect of *A. sativum*, supporting its potential as a natural therapeutic agent against pesticide-induced toxicity.

Keywords: Dimethoate, Organophosphate, Hepatotoxicity, Garlic, Amelioration

INTRODUCTION

Dimethoate (DT) is a systemic organophosphorus (OP) insecticide that has been used worldwide in agriculture since the early 1960s to control sucking and chewing pests of fruits, vegetables and cereal crops (Singh and Walker, 2006). According to recent data of Ministry of Agriculture and Farmer's Welfare (2023), the consumption of DT has increased by around 47.84%. It exerts its toxicity by irreversibly inhibiting acetylcholinesterase (AChE) leading to cholinergic hyperstimulation (Jokanovic, 2012). Numerous studies have reported that sub-lethal exposure to DT can also cause hepatotoxicity, nephrotoxicity and reproductive toxicity along with disruption of antioxidant defence systems in experimental models (Mansour and Mossa, 2010). The liver, being the primary site of xenobiotic metabolism and vulnerable to DT-induced toxicity results in elevation of liver enzymes, histopathological alterations, lipid peroxidation (El-Shenawy et al., 2010).

Allium sativum L. (garlic), a culinary herb and traditional remedy has emerged as a potent pharmacological agent due to its organosulphur compounds (allicin, diallyl disulfide and S-allyl-L-cysteine) that collectively possess broad-spectrum antioxidant, anti-inflammatory and hepatoprotective properties (Amagase et al., 2001; Banerjee and Maulik, 2002). *A. sativum* has consistently attenuated biochemical markers of liver dysfunction and restored normal histoarchitecture in paracetamol, carbon tetrachloride and other pesticides induced several mammals (Ekor et al., 2004; Avato et al., 2020). Due to high safety margin, low cost and widespread dietary acceptance, garlic represents a promising phytotherapeutic candidate to mitigate OP-induced oxidative stress.
Lipid peroxidation (LPO) assessment is a crucial parameter in toxicological evaluations, serving as a sensitive biomarker for oxidative damage induced by various xenobiotics, including pesticides, heavy metals and pharmaceutical compounds (Ayala et al., 2014). This process is characterized by the oxidative degradation of polyunsaturated fatty acids in cellular membranes generating malondialdehyde (MDA) which is not only indicator of oxidative stress but also potent cytotoxic agent capable of modifying proteins and DNA (Esterbauer et al., 2010).
The estimation of liver enzymes, specifically alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) is very important in toxicological evaluations serving as sensitive serological markers of hepatocellular function (Ozer et al., 2008). These enzymes are predominantly localised in hepatocytes and are released into the bloodstream upon liver damage. ALT demonstrates higher liver specificity compared to AST, which is also present in cardiac and skeletal muscle tissues, while ALP primarily indicates cholestatic injury and biliary dysfunction (Ramaiah, 2007).
Albumin, the most abundant plasma protein is synthesized exclusively by the liver and exhibits a relatively long half-life, making it a reliable indicator of chronic hepatic injury, malnutrition or protein deficiency resulting from toxicant exposure, whereas alterations in total protein levels indicate systemic toxicity and organ dysfunction (Thapa and Walia, 2007). In parallel, alterations in total cholesterol levels often signify disruptions in lipid metabolism, which may occur through various mechanisms including inhibition of cholesterol biosynthetic enzymes or impairment of biliary excretion pathways (Feingold and Grunfeld, 2018). Hence, in the present study, the toxicity of DT on *Mus musculus* and the ameliorative potential of *A. sativum* against DT induced mice were investigated.

MATERIALS AND METHODS

Chemicals
Analytical reagents were obtained from Merck, Sigma-Aldrich, Himedia and Dimethoate (30% EC) was purchased from TATA enterprise, India.

Allium sativum aqueous extracts
Fresh garlic (500 mg) were peeled, washed and sliced into tiny pieces and homogenised in 100 mL distilled water, then filtered and centrifuged at 3000 rpm for 10 min. The supernatant and homogenates were filtered and stored in a reagent bottle at 4°C.

Model animal and treatment protocol
M. musculus weighing 25.0 ±5.0 g were housed in polypropylene cages maintained with 12h light-dark cycle at 22 ± 4°C and 45-60% relative humidity, after procuring from the University Department of Zoology of our university. Food and water were provided *ad libitum*. Experimental protocol was conducted following the guidelines of Institutional Animal Ethics Committee and CPCSEA, India. Sub-lethal dose of DT (16 mg/kg) was prepared in distilled water. For the experiment, mice were grouped into six as Group 1 (Control), Group 2 (Treated with DT 16 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 DT and *A. sativum* extract 100 mg/kg – DT+AS1) and Group 6 (Treated with DT and *A. sativum* extract 200 mg/kg – DT+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 (Fukuta, 2004). The samples were centrifuged at 3000 rpm for 10 min to obtain serum for several biochemical analysis. LPO was measured by Thiobarbituric acid reactive substances (TBARS) assay (Okhawa et al., 1979). ALT and AST activities were determined by Reitman and Frankel method (1957). ALP activity was measured using the kinetic method (Granstrom and Linde, 1972). Total serum cholesterol levels were measured using the method of Zlatkis et al.

(1953) with slight modifications. Total protein content in serum was measured by the method of Lowry et al. (1951) and serum albumin was measured with bromocresol green using succinate buffer (Doumas et al., 1971).

Statistical analysis

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

Table 1: Ameliorative effect of *Allium sativum* on biochemical parameters of liver in Dimethoate – induced Swiss albino mice (Mean ± Standard Error).

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
DT	5.23 ±0.53	3.32 ±0.19	2.03 ±0.1	69.64 ±2.79	152.58 ±2.76	210.51 ±8.7	158.48 ±9.18
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
DT+AS1	4.09 ±0.47	4.04 ±0.2	2.27 ±0.1	62.04 ±2.77	129.52 ±1.49	197.69 ±11.08	138.05 ±5.78
DT+AS2	2.73 ±0.42	4.69 ±0.34	2.61 ±0.12	56.47 ±2.44	118.85 ±1.57	178.33 ±5.41	128.73 ±3.41

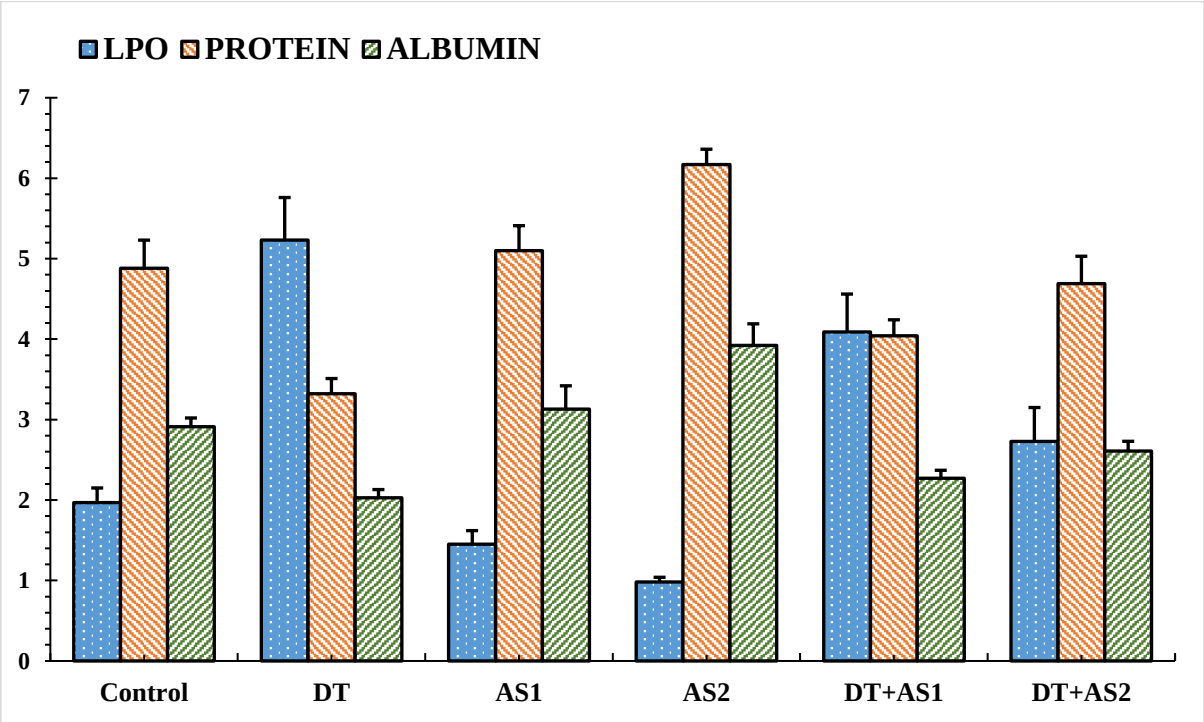

Figure 1. LPO, total protein and albumin levels in different groups of mice.

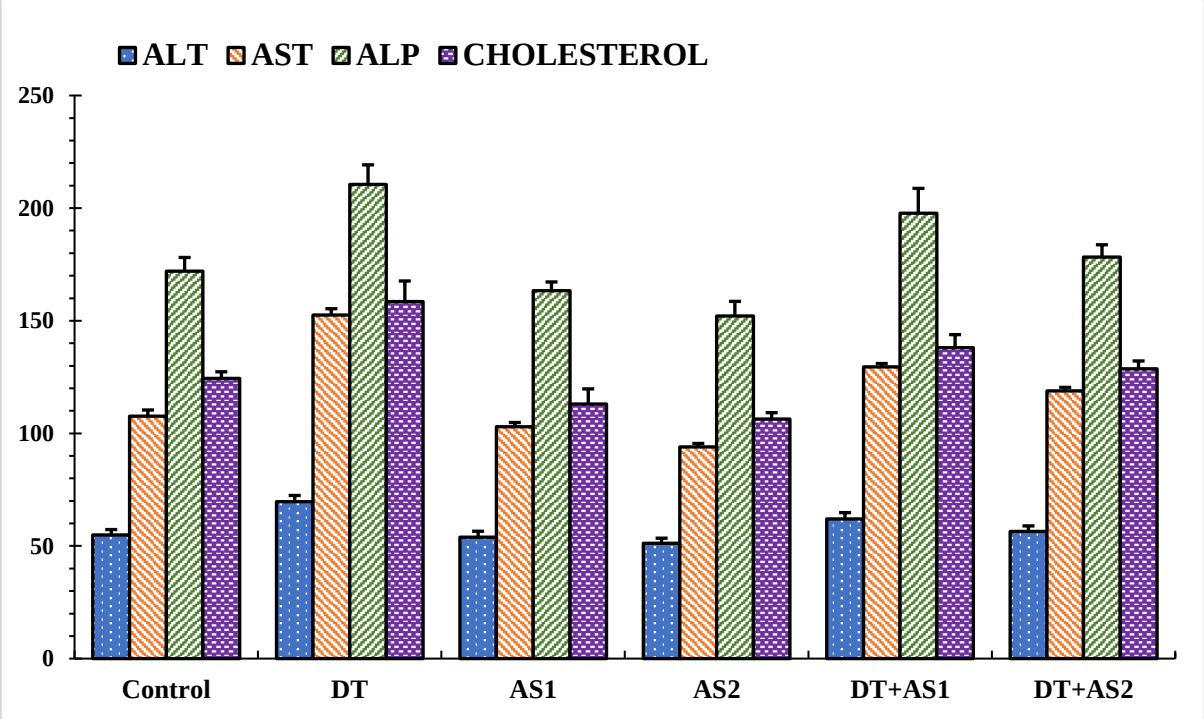

Figure 2. ALT, AST, ALP activities and total cholesterol level in different groups of mice.

RESULTS

During the treatment of sub-lethal dose of DT (16 mg/kg), no mortality was observed, however noticeable morphological and behavioural changes, including hair loss, significant reduction in body weight and decreased locomotor activity were observed.

The toxicity of DT and the ameliorative efficacy of *A. sativum* were studied in *M. musculus* by evaluating lipid peroxidation (LPO), serum total protein, albumin, cholesterol and key liver function biomarkers (ALT, AST and ALP).

LPO level

LPO which is measured as MDA concentration, showed a significant increase in the DT-treated group. The MDA level in treated mice increased to 5.23 ± 0.53 nmol MDA/mL, representing a 165.48% increase compared to the control group (1.97 ± 0.18 nmol MDA/mL), and this elevation was statistically significant by t-test (t = 5.8) (Table 1). Administration of *A. sativum* alone at a dose of 200 mg/kg significantly reduced MDA levels in the untreated group (0.98 ± 0.06 nmol MDA/mL; F = 11.52). The co-administration of higher dose of *A. sativum* (200 mg/kg) along with DT resulted in a significant protection (2.73 ± 0.42 nmol MDA/mL; F = 11.85) by lowering LPO levels by 126.9% as compared to DT-treated group (Figure 1). In contrast, the lower dose of *A. sativum* (100 mg/kg) showed only 57.87% recovery which was statistically insignificant, suggesting a dose-dependent ameliorative effect of garlic on DT-induced oxidative stress.

Total serum protein and albumin

The total protein concentration in the DT-treated group (3.32 ± 0.19 g/dL) showed a 31.97% decrease which was statistically significant as compared to the control group (t = 3.915). Similarly, albumin levels also significantly reduced by 30.24% in the DT group (2.03 ± 0.1 g/dL) as compared to the control (t = 6.029). Administration of *A. sativum* at a higher dose (200 mg/kg) in untreated mice significantly enhanced both serum protein (6.17 ± 0.19 g/dL) and albumin levels (3.92 ± 0.27 g/dL) when compared to control (4.88 ± 0.35 g/dL and 2.91 ± 0.11 g/dL respectively). In DT-treated groups, the higher dose of *A. sativum* significantly improved total protein and albumin levels by 28.07% (4.69 ± 0.34 g/dL; F = 6.265) and 19.93% (2.61 ± 0.12 g/dL; F = 13.017) respectively (Figure 1). The effect of lower dose of *A. sativum* (100 mg/kg) was found to be statistically insignificant.

Liver function

The liver enzyme activities i.e., ALT, AST and ALP significantly elevated in DT-treated mice, indicating hepatic injury. Specifically, ALT activity increased by 26.83% compared to the control group ($t = 4.013$), AST activity showed an increase of 41.74% ($t = 11.594$) and ALP activity increased by 22.34% ($t = 3.637$) (Table 1). Co-administration of *A. sativum* at a higher dose (200 mg/kg) with DT significantly improved liver enzyme levels. ALT activity reduced by 23.99% ($F = 6.557$) and AST levels decreased markedly by 31.33% ($F = 74.538$) compared to the DT group (Figure 2). Although the same dose showed 18.71% reduction in ALP activity but the change was found to be statistically insignificant. Interestingly, the lower dose of *A. sativum* (100 mg/kg) provided a significant protective effect of 21.42% on AST levels, while its effect on ALT (13.84%) and ALP (7.45%) was statistically insignificant.

Serum cholesterol

The cholesterol concentration in the DT-treated group increased significantly by around 27.33% compared to the control group ($t=3.534$) (Table 1). In the untreated group, administration of a higher dose of *A. sativum* (200 mg/kg) alone significantly reduced cholesterol levels ($F=4.040$) (Figure 2). Furthermore, co-administration of higher dose of *A. sativum* along with DT significantly improved DT-induced hypercholesterolemia, achieving a 23.9% reduction in cholesterol levels (128.73 ± 3.41 mg/dL) compared to the DT group (158.48 ± 9.18 mg/dL) (Figure 2). In contrast, the hepatoprotective efficacy of lower dose of *A. sativum* (100 mg/kg) was found to be statistically insignificant.

DISCUSSION

Similar to present findings, several morphological and behavioural changes were also reported by Nawaz et al. (2024) in Cypermethrin treated Swiss albino mice. Such types of changes were also reported in Chlorpyrifos and Monocrotophos treated Wistar rats by Sharma et al. (2010) and Balani et al. (2011) respectively. A significant rise in LPO, as evidenced by elevated MDA levels, confirms the generation of reactive oxygen species (ROS) and oxidative stress in DT-treated mice due to inhibition of mitochondrial electron transport chain and impaired antioxidant defence mechanisms which leads to lipid membrane disruption (Abdollahi et al., 2004; Kalender et al., 2005). The marked reduction in MDA levels upon administration of higher dose of *A. sativum* indicates its antioxidative potential. This can be attributed to the presence of organosulphur compounds such as allicin and S-allyl cysteine which scavenge free radicals and upregulate antioxidant enzymes (Banerjee et al., 2003; Amagase, 2006).

Total serum protein and albumin levels are crucial indicators of hepatic synthetic function and its level significantly declined in the DT-treated group. This decline is suggestive of hepatocellular dysfunction or protein catabolism which was also reported by Verma et al. (2007) on DT-treated albino rats. The restorative effect of *A. sativum*, particularly at the 200 mg/kg dose, signifies its role in stabilising cellular integrity and protein metabolism. The protein-conserving effect of garlic in rats was also reported by Sivarajah et al. (2007).

The elevation in liver enzymes ALT, AST and ALP after DT exposure in the present investigation is an indicator of hepatocellular injury and membrane damage. Such elevation in ALT, AST and ALP was also reported in Swiss albino mice treated with Diclofenac (Iqbal et al., 2024), Cypermethrin (Nawaz et al., 2024) and Chlorpyrifos (Divyanshu et al., 2025). Attia and Nasr (2009) also reported regarding the hepatocellular injury in DT-treated male albino rats. The protective effect of *A. sativum* in mitigating these elevations, especially in AST and ALT levels reflects its hepatoprotective nature. The bioactive components of garlic enhance the repair of hepatic tissue by reducing oxidative stress and promoting liver regeneration as reported earlier (Sener et al., 2005; El-Shahat et al., 2008; Hassan et al., 2009; Hosseini et al., 2013).

Similarly, the significant increase in serum cholesterol in DT-exposed mice aligns with the report of Kammon et al. (2011) in Chlorpyrifos treated Sprague-Dawley rats. Cholesterol synthesis is closely linked to liver function and its imbalance further supports DT-induced hepatic stress. The ability of *A. sativum* to normalize cholesterol levels is consistent with previous studies demonstrating its lipid-lowering properties, likely through inhibition of hepatic HMG-CoA reductase and promotion of bile acid secretion (Gebhardt, 1993; Yeh and Liu, 2001).

CONCLUSION

The present study clearly demonstrates that exposure to a sub-lethal dose DT induces significant oxidative stress, hepatotoxicity and metabolic disturbances in Swiss albino mice, as evidenced by elevated lipid peroxidation, disrupted liver enzyme activity, decreased serum protein and albumin levels and increased

cholesterol. The findings support the toxic potential of DT even at lower doses, highlighting its impact on key physiological functions. *A. sativum*, particularly at a higher dose (200 mg/kg), provides a significant protective effect against DT-induced oxidative damage, hepatic dysfunction, and metabolic imbalance in Swiss albino mice. These protective effects are likely due to the antioxidant and hepatoprotective properties of its bioactive sulphur-containing compounds. Its efficacy appears to be dose-dependent, as the lower dose (100 mg/kg) showed limited or statistically insignificant protection in most parameters. These findings support the potential use of *A. sativum* as a natural therapeutic agent against pesticide-induced toxicity. Future investigations are required on its molecular mechanisms of action to establish its role as an effective bioactive compound to manage pesticide-induced toxicities.

ACKNOWLEDGEMENTS

The authors sincerely express their gratitude to the head of University Department of Zoology, T. M. Bhagalpur University for providing the essential laboratory facilities required to conduct this research.

ETHICAL APPROVAL

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.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this research.

REFERENCES

1. Abdollahi, M., Ranjbar, A., Shadnia, S., Nikfar, S., & Rezaiee, A. (2004). Pesticides and oxidative stress: A review. *Medical Science Monitor*, 10(6), 141–147.
2. Amagase, H. (2006). Clarifying the real bioactive constituents of garlic. *The Journal of Nutrition*, 136(3), 716–725.
3. Amagase, H., Petesch, B. L., Matsuura, H., Kasuga, S., & Itakura, Y. (2001). Intake of garlic and its bioactive components. *Journal of Nutrition*, 131(3), 955S–962S.
4. Annual Report 2022-23. Directorate of Plant Protection, Quarantine & Storage. Ministry of Agriculture and Farmers Welfare, Government of India.
5. Attia, A. M., & Nasr, H. M. (2009). Biochemical and histopathological effects of systemic pesticides on some functional organs of male albino rats. *Journal of King Saud University - Science*, 21(1), 163–175.
6. Avato, P., Tursi, F., & Vitali, D. (2020). Antioxidant and hepatoprotective activity of *Allium sativum* L. cultivated in Southern Italy. *Phytotherapy Research*, 34(11), 2964–2972.
7. Ayala, A., Muñoz, M.F., & Argüelles, S. (2014). Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Medicine and Cellular Longevity*, 2014, 360-438.
8. Balani, T., Agrawal, S., Thaker, A. M., & Rao, C. V. (2011). Histopathological and biochemical changes of dimethoate-induced hepatotoxicity in rats. *Toxicology International*, 18(1), 94–96.
9. Banerjee, S. K., & Maulik, S. K. (2002). Effect of garlic on cardiovascular disorders: a review. Nutrition Journal, 1(4).
10. Banerjee, S. K., Maulik, M., Manchanda, S. C., Dinda, A. K., & Maulik, S. K. (2003). Garlic-induced alteration in rat liver and kidney morphology and function. *Microscopy Research and Technique*, 62(1), 6–17.
11. Divyanshu., Nawaz, M. S., & Ahmad., M. E. (2025). Ameliorative potential of *Allium sativum* against Chlorpyrifos-induced toxicity in Swiss albino mice (*Mus musculus*). *Asian. J. Biol. Life Sci.* 14(2) In Press.
12. Doumas, B. T., Watson, W. A., & Biggs, H. G. (1971). Albumin standards and the measurement of serum albumin with bromcresol green. *Clinica Chimica Acta*, 31(1), 87–96. https://doi.org/10.1016/0009-8981(71)90365-2

13. Ekor, M., Farombi, E. O., & Emerole, G. O. (2004). Modulation of carbon tetrachloride-induced hepatotoxicity by *Buchholzia coriacea* seed methanolic extract in rats: Comparison with garlic (*Allium sativum*). *Journal of Medicinal Food*, 7(4), 494–499.
14. El-Shahat, A. E., Gabr, A., Meki, A.-R. M. A., & Mehana, E. S. E. (2008). Altered testicular morphology and oxidative stress induced by organophosphate pesticide chlorpyrifos and the protective effect of vitamin C and zinc. *Food and Chemical Toxicology*, 46(6), 1764–1771.
15. El-Shenawy, N.S., El-Salmy, F., Al-Eisa, R.A., & El-Ahmary, B. (2010). Amelioratory effect of vitamin E on organophosphorus insecticide diazinon-induced oxidative stress in mice liver. *Pesticide Biochemistry and Physiology*, 96(2), 101-107.
16. Esterbauer, H., Schaur, R.J., & Zollner, H. (2010). Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biology and Medicine*, 11(1), 81-128.
17. Feingold, K.R., & Grunfeld, C. (2018). Introduction to lipids and lipoproteins. *Endotext*.
18. Fukuta, K. (2004). Collection of body fluids. In *The laboratory mouse* (pp. 543-554). Academic Press.
19. Gebhardt, R. (1993). Multiple inhibitory effects of garlic extracts on cholesterol biosynthesis in hepatocytes. *Lipids*, 28(7), 613–619.
20. Granström, G., & Linde, A. (1972). A biochemical study of alkaline phosphatase in isolated rat incisor odontoblasts. *Archives of Oral Biology*, 17(8), 1213–1222. https://doi.org/10.1016/0003-9969(72)90134-7
21. Hassan, H. A., El-Agmy, S. M., Gaur, R. L., Fernando, A., Raj, M. H. G., & Ouhtit, A. (2009). In vivo evidence of hepato- and reno-protective effect of garlic oil against sodium nitrite-induced oxidative stress. *International Journal of Biological Sciences*, 5(3), 249–255.
22. Hosseini, A., Hosseinzadeh, H., & Zakeri-Milani, P. (2013). Protective effect of garlic (*Allium sativum* L.) and its constituents against natural and chemical toxicities. *Iranian Journal of Basic Medical Sciences*, 16(9), 1031–1045.
23. Iqbal, A., Nawaz, M. S., & Ahmad, M. E. (2024). Role of Ashwagandha (*Withania somnifera*) root extract in attenuating hepatic deficits in Diclofenac sodium-induced Swiss albino mice (*Mus musculus*). *International Journal of Pharmaceutical Research and Applications*, 9(1), 563-570.
24. Jokanovic, M. (2012). Neurotoxic effects in patients poisoned with organophosphorus pesticides. *Environmental Toxicology and Pharmacology*, 33(3), 293–298.
25. Kalender, S., Uzunhisarcikli, M., Ogutcu, A., Acikgoz, F., & Kalender, Y. (2005). Effects of diazinon on pseudocholinesterase activity and haematological indices in rats: The protective role of vitamin E. *Environmental Toxicology and Pharmacology*, 20(1), 51–56.
26. Kammon, A. M., Brar, R. S., Sodhi, S., Banga, H. S., & Singh, J. (2011). Ameliorative effect of vitamin C against the cypermethrin-induced immunological and histopathological alterations in rats. *Journal of Pharmacology and Toxicology*, 6(4), 378–388.
27. Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193(1), 265–275. https://doi.org/10.1016/S0021-9258(19)52451-6
28. Mansour, S. A., & Mossa, A. H. (2010). Oxidative damage, biochemical and histopathological alterations in rats exposed to chlorpyrifos and the antioxidant role of zinc. *Pesticide Biochemistry and Physiology*, 96(1), 14–23. https://doi.org/10.1016/j.pestbp.2009.09.001
29. Nawaz, M. S., Iqbal, A., & Ahmad., M. E. (2024). Restitutive properties of *Curcuma longa* on cypermethrin induced Swiss-albino mice (*Mus musculus*). *Int. J. Adv. Res.*, 12(1), 163-171.
30. Ohkawa, H., Ohishi, N., & Yagi, K. (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*, 95(2), 351-8.
31. Ozer, J., Ratner, M., Shaw, M., Bailey, W., & Schomaker, S. (2008). The current state of serum biomarkers of hepatotoxicity. *Toxicology*, 245(3), 194-205.
32. Ramaiah, S.K. (2007). A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. *Food and Chemical Toxicology*, 45(9), 1551-1557.
33. Reitman, S., & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, 28(1), 56–63. https://doi.org/10.1093/ajcp/28.1.56
34. Sener, G., Sehirli, O., Ipci, Y., Ercan, F., Sirvanci, S., Gedik, N., & Yegen, B. C. (2005). Aqueous garlic extract alleviates ischaemia-reperfusion-induced oxidative hepatic injury in rats. *Journal of Pharmacy and Pharmacology*, 57(1), 145–150.
35. Sharma, Y., Bashir, S., Irshad, M., & Dogra, T. D. (2010). Dimethoate-induced effects on antioxidant status of liver and brain of rats following subchronic exposure. *Toxicology Letters*, 196(1), 60–66.

36. Singh, B. K., & Walker, A. (2006). Microbial degradation of organophosphorus compounds. *FEMS Microbiology Reviews*, 30(3), 428–471.
37. Sivarajah, K., Maharajan, A., & Rekha, M. (2007). Protective role of garlic extract on liver damage in albino rats with carbon tetrachloride toxicity. *International Journal of Pharmaceutical Sciences and Research*, 2(1), 1–6.
38. Thapa, B.R., & Walia, A. (2007). Liver function tests and their interpretation. Indian *Journal of Pediatrics*, 74(7), 663-671.
39. Verma, R. S., Mehta, A., & Srivastava, N. (2007). In vivo chlorpyrifos induced oxidative stress: Attenuation by antioxidant vitamins. *Pesticide Biochemistry and Physiology*, 88(2), 191–196.
40. Yeh, Y. Y., & Liu, L. (2001). Cholesterol-lowering effect of garlic extracts and organosulfur compounds: Human and animal studies. *The Journal of Nutrition*, 131(3), 989–993.
41. Zlatkis, A., Zak, B., & Boyle, A. J. (1953). A new method for the direct determination of serum cholesterol. *Journal of Laboratory and Clinical Medicine*, 41(3), 486–492.