

Therapeutic Effect of Antioxidant Extract of *Ocimum Gratissimum* on Liver Toxicity Model in Rats

MUHAMMAD SHAHID JAVED¹, SOHAIL IQBAL², NIDA QASIM HAYAT³, ATTYA ZAHEER⁴, FATIMA QAISER⁵, FAIZA IRSHAD⁶, AMAL SHAUKAT⁷

¹Assistant Professor Department of Physiology Sargodha Medical College Sargodha

²Associate Professor Department of Pharmacology Muhammad College of Medicine Peshawar

³Associate Professor Department of Anatomy Women Medical College Abbottabad

⁴Assistant Professor Department of Anatomy, Rashid latif medical college.

⁵Professor Department of Anatomy, Rashid latif medical college.

⁶Associate Professor Department of Anatomy M.Islam Medical & Dental College Gujranwala

⁷Assistant Professor Department of food science and technology Faculty of life science University of central punjab

Correspondence to: Muhammad Shahid Javed

ABSTRACT

Background: Lead harms haematological, biochemical, and hepatic parameters, hence studies have concentrated on antioxidants with therapeutic potentials.

Objective: Induced hepatotoxicity and hemato-biochemical parameters in adult Wistar rats with *Ocimum Gratissimum* extract.

Methodology: 42 adult Wistar rats were divided into seven groups of five. Group A was the control, Group B had 120 mg/kg lead, and Group C got 300 mg/kg OG. D and E had 120 mg/kg lead before 300 mg/kg and 600 mg/kg OG. Group F had 300 mg OG extract, then 120 mg lead, whereas Group G got 120 mg lead and 1000 mg ascorbic acid. The animals were then slaughtered and blood and liver tissues were taken for biochemical and histological investigation.

Results: The outcome revealed a rise in Control but a drop in B. ALT, AST, GGT, and ALP levels were higher in Group B than in Control and other treatment groups ($p \leq 0.05$). As in Groups D, E, F, and G, histological investigation of liver tissues revealed degenerative alterations with localised necrosis and aggregated inflammatory cells in B.

Conclusion: The extract of OG has the potential to be employed as a medicinal agent in the treatment of lead poisoning.

Keywords: Hepatobiochemical; Lead; Liver; *Ocimum gratissimum*; Wistar rats; Parameters

INTRODUCTION

Lead can be found in a variety of organic and inorganic compounds. The nervous system, kidneys, and liver are all affected by this type of lead poisoning. According to Kim et al., lead causes anemia by inhibiting three key hemoglobin-producing enzymes, which they claim is the case. The Centers for Disease Control and Prevention (CDC) recommended that adults have a lead level of 5 g/dL or less and children have a lead level of 10 g/dL or less. Adults who are exposed to lead at their place of employment should have blood lead levels below 10 g/dL. It is the liver that has the greatest lead storage capacity among soft tissues. Lead has a detrimental effect on tissues and metabolism. Lead damages DNA by increasing the production of reactive oxygen species and the occurrence of oxidative stress. The antioxidant properties of natural plant remedies have been used to treat tissue damage that has been harmful to the patient. *Ocimum gratissimum* is the source of this material. Nutritional antioxidants have been extensively studied as potential therapeutic and disease-prevention agents in both animal and human studies. Flavonoids, phenolics, limonoids, carotenoids, coumarins, phytosterols, and other bioactive plant components are all examples of phytoactive plant components. An adult population was studied to see if *Ocimum gratissimum* leaf extract had any effect on hepatobiochemical markers. The purpose of this study was to assess the effect of *Ocimum gratissimum* leaf extract on lead-induced alterations in hepatobiochemical parameters in adult Wistar rats, which was carried out in the laboratory.

MATERIALS AND METHODS

Plant collection, identification and extraction

From Market bought *Ocimum Gratissimum* (OG) leaves. The leaves were washed with running water, rinsed with clean water, dried in the shade, and pulverised into powder. A rotary evaporator condensed 200 g of the fine powder in 1000 ml of distilled water for an hour. The paste was kept below 38°C until utilized in tests.

Animal grouping:

The study's 42 rats were divided into 7 groups of 6 animals each. The animals were housed for 15 days to acclimate. Rats ate Protocol for study For the extract dose, the LD50 was 1200 mg/kg body weight. The high and low doses were made by dissolving 600

g of extract in 100 ml of distilled water, respectively. Group A received only feed and water. Group B got 120 mg/kg lead and Group C received 300 mg/kg OG. Group D got 120 mg/kg lead and 300 mg/kg OG extract, whereas Group E got 600 mg/kg OG extract. According to the procedures, rats in Group F received 300 mg/kg OG extract for two weeks, followed by 120 mg/kg lead for one week.

Biochemical analysis

Alanine Aminotransferase was examined using conventional feed and water ad libitum during the study period. ALT, AST, GGT, and ALP were measured spectrophotometrically using commercial available kits.

The activity of ALT was measured using a commercial test kit. The concentration of pyruvate hydrazone produced with 2,4-dinitrophenylhydrazine at 550 nm was used to quantify ALT activity.

AST activity was measured in serum using a spectrophotometer. At 550 nm, the concentration of Oxaloacetate hydrazone produced with 2, 4-dinitrophenylhydrazine was monitored.

The serum was separated by centrifugation at 3000 rpm for 5 minutes to determine serum Gamma Glutamyl Transferase (GGT) levels using commercial test kits.

Using a phenolphthalein monophosphate technique to measure alkaline phosphatase in serum. alkaline phosphates alkaline phosphates alkaline phosphates The alkaline reagent inhibits enzyme activity while producing a blue chromagen that may be detected spectrophotometrically at 550 nm.

RESULTS

On the other hand, there was no significant difference between groups C and F in terms of mean weights of animals and organs. WBC increased in Group B compared to Groups A, C, D, E, and F ($p < 0.005$). Compared to Groups A and E, haemoglobin in Group B was not significantly different, however it was different from Groups C, D, F and G ($p < 0.005$).

The differential white blood cell count revealed a significant reduction in monocytes and neutrophils in Group B compared to Group A ($p < 0.005$), but no difference between Groups A and B. (C-G). ($p < 0.005$), but there was no significant change in Group B relative to Groups A, C, D, E and F. The ALP, AST, GGT, and

ALT levels were higher in Group B than in Groups A,C, D, and G (p <0.005).

Histological studies

As illustrated in Figure 1, the liver from Group A (Control) had normal hepatic anatomy with hepatocytes, central vein, and sinusoids. The liver tissues of the lead-only rats (Group B) revealed significant deformation with localised aggregation of inflammatory cells (Figure 2). As illustrated in Figure 3, Group C liver tissues had a typical hepatic anatomy with a Central Vein and Hepatocytes. Figure 4 shows modest central venous congestion in the liver tissues of Group D rats. Figures 5 and 6 demonstrate normal histological structure in the liver tissues of animals in Groups E and F, whereas Figure 7 shows normal histological structure in the liver tissues of animals in Group G.

Table 1: The mean weight of the organ & LFT in the experimental animals.

Groups	Final weight (MEAN ± SD) (g)	Liver weight (MEAN ± SD) (g)	ALP (U/L)	ALT (U/L)	AST (U/L)	GGT (U/L)
A	116.5 ± 13.80	5.39 ± 0.58	127.2 ± 2.67	81.22 ± 1.28	128.2 ± 5.94	12.7 ± 8.58
B	134.2 ± 48.12	5.77 ± 3.45	182.8 ± 4.85	89.72 ± 2.22	15.4 ± 6.06	11.42 ± 0.14
C	131.9 ± 48.02	5.97 ± 1.32	135.0 ± 6.88	68.42 ± 2.97	134.2 ± 2.01	12.12 ± 2.77
D	136.6 ± 16.01	5.58 ± 0.65	147.2 ± 4.34	72.57 ± 0.88	140.2 ± 5.25	12.22 ± 0.71
E	155.3 ± 19.33	6.88 ± 0.38	118.98 ± 6.23	67.48 ± 1.76	129.7 ± 1.38	11.21 ± 0.59
F	136.88 ± 26.32	5.03 ± 1.05	147.2 ± 5.24	70.20 ± 1.08	142.8 ± 1.09	12.00 ± 0.18
G	147.811 ± 18.94	5.01 ± 0.66	142.3 ± 9.48	70.24 ± 1.63	140.0 ± 2.08	12.18 ± 0.88

Figure 1: A section of the liver of Group A (Control) animals showing normal hepatic structure with Central vein (CV), Hepatocytes (HC) and Sinusoids



Figure 2: A section of the liver of Group B animals (induced with only lead) showing distortion of hepatic structure, Focal Area of Necrosis (FAN), Aggregate of inflammatory Cells (AIC) and Necrosis Around Central Vein (NACV)



Figure 3: A section of the liver of group c animals administered with curcumin posttreatment only showing normal hepatic structure with central vein (cv) and hepatocytes (hc)



Figure 4: A section of the liver of Group D animals administered with 100 mg/kg Pb (200mg/kg curcumin) and 100 mg/kg Pb (200mg/kg curcumin) showing mild congestion of central vein (CV) with normal hepatocytes (HC) and aggregate of inflammatory cells (AIC). High central venous congestion and necrosis around central vein.



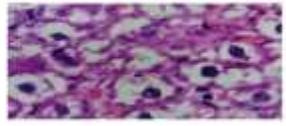
Figure 5: A section of the liver of Group E animals administered with 100 mg/kg Pb (200mg/kg curcumin) and 100 mg/kg Pb (200mg/kg curcumin) showing mild congestion of central vein (CV) with normal hepatocytes (HC) and aggregate of inflammatory cells (AIC).



Figure 6: A section of the liver of Group F animals administered with 100 mg/kg Pb (200mg/kg curcumin) and 100 mg/kg Pb (200mg/kg curcumin) showing mild congestion of central vein (CV) with normal hepatocytes (HC) and aggregate of inflammatory cells (AIC).



Figure 7: A section of the liver of Group G animals administered with 100 mg/kg Pb (200mg/kg curcumin) and 100 mg/kg Pb (200mg/kg curcumin) showing mild congestion of central vein (CV) with normal hepatocytes (HC) and aggregate of inflammatory cells (AIC).



DISCUSSION

The current investigation found that the body weight of experimental animals increased with time. During the week of the

trial, no weight loss was noticed, possibly due to the ad libitum feeding strategy. The active components in the extract may be responsible for the animals' weight gain. We saw less physical activity and more breathing, which is consistent with Philip and Gerson's findings. High levels of AST and ALT in the blood indicate hepatocellular necrosis. Because ALT accounts for 90% of all body enzymes, an elevated level indicates liver damage. ALP activity is related to the function of liver cells and has been demonstrated to increase with increasing biliary pressure. We found a substantial rise in ALT, AST, ALP, and GGT in Group B compared to control. Figure 2 shows the toxic effects of lead on the liver, including deformation, localised necrosis, and inflammatory cell aggregation, whereas portions of liver treated with OG extract showed ameliorative benefits. A central vein, hepatocytes, and sinusoids were normal in Group F vs Group G. The extract's hypoglycemic and diuretic effects may have contributed to the apparent weight loss in Group B compared to Group A. During the trial, the lead-only mice lost weight significantly compared to the control animals, possibly owing to reduced food intake. Weight loss can be caused by a lack of food, hormonal imbalances, or the direct cytotoxic action of lead. We found a reduction in PCV, RBC, and Hb content in the supplied lead alone, which may be due to anemia caused by lead's harmful effect on bone marrow, spleen, and liver. The concentration of monocytes and neutrophils decreased while lymphocytes increased.

CONCLUSION

The extract had favourable effects on hepatic toxicity in our study, and we strongly advocate its restricted usage in the therapy of hep atotoxicity.

REFERENCES

- Amadi, C. N., Ofor, S. J., Frazzoli, C. & Orisakwe, O. E. Natural antidotes and management of metal toxicity. *Environ. Sci. Pollut. Res.* 26, 18032–18052 (2019).
- Neal, A. P. Mechanisms of heavy metal neurotoxicity: Lead and manganese. *J. Drug Metab. Toxicol.* 06 (2015).
- Iyer, S., Sengupta, C. & Velumani, A. Lead toxicity: An overview of prevalence in Indians. *Clin. Chim. Acta* 451, 161–164 (2015).
- Lee, J.-W. et al. Toxic effects of lead exposure on bioaccumulation, oxidative stress, neurotoxicity, and immune responses in fish: A review. *Environ. Toxicol. Pharmacol.* 68, 101–108 (2019).
- Kim, J.-H. & Kang, J.-C. Effects of sub-chronic exposure to lead (Pb) and ascorbic acid in juvenile rockfish: Antioxidant responses, MT gene expression, and neurotransmitters. *Chemosphere* 171, 520–527 (2017).
- Jaishankar, M., Tseten, T., Anbalagan, N., Mathew, B. B. & Beeregowda, K. N. Toxicity, mechanism and health effects of some heavy metals. *Interdiscip. Toxicol.* 7, 60–72 (2014).
- Kim, J. H. & Kang, J. C. The lead accumulation and hematological findings in juvenile rock fish *Sebastes schlegelii* exposed to the dietary lead (II) concentrations. *Ecotoxicol. Environ. Saf.* 115, 33–39 (2015).
- Bradberry, S. M. Metals (cobalt, copper, lead, mercury). *Medicine (United Kingdom)* 44, 182–184 (2016)
- Wani, A. L., Ara, A. & Usmani, J. A. Lead toxicity: A review. *Interdiscip. Toxicol.* 8, 55–64 (2015).
- Haridy, M., Al-Amgad, Z., Sakai, H. & Mohi-Eldin, M. Ameliorating effects of garlic, calcium, and vitamin C on chronic lead toxicity in albino rats. *Comp. Clin. Pathol.* 23, 1215–1223 (2014).
- Baselt RC. Disposition of toxic drugs and chemicals in man. 9th ed. Foster City, California: Biomedical Publications. 2011.
- National Institute for Occupational Safety and Health (NIOSH). Adult Blood Lead Epidemiology & Surveillance. 2015. Accessed 20 October, 2019.
- Mudipalli A. Lead hepatotoxicity and potential health effects. *Indian J Med Res.* 2007;126:518-527.
- Haouas Z, Sallem A, Zidi I, Hichri H, Mzali I, Mehdi M. Hepatotoxic effects of lead acetate in Rats: histopathological and cytotoxic studies. *J Cytol Histol.* 2014;5:256.
- Sivaprasad R, Nagaraj M, Varalakshmi P. Combined efficacies of lipoic acid and 2,3-dimercaptosuccinic acid against lead-induced lipid peroxidation in rat liver. *J Nutr Biochem.* 2004;15:18-23.

16. Zhang J, Wang XF, Lu ZB, Liu NQ, Zhao BL. The effects of meso-2,3- dimercaptosuccinic acid and oligomeric procyanidins on acute lead neurotoxicity in rat hippocampus. *Free Radic Biol Med.* 2004;37:1037- 1050.
17. Jurczuk M, Brzóska MM, Moniuszko Jakoniuk J. Hepatic and renal concentrations of vitamins E and C in lead and ethanol-exposed rats. An assessment of their involvement in the mechanisms of peroxidative damage. *Food Chem Toxicol.* 2007;45:1478-1486.
18. Xu J, Lian LJ, Wu C, Wang XF, Fu WY. Lead induces oxidative stress, DNA damage and alteration of p53, Bax and Bcl-2 expressions in mice. *Food Chem Toxicol.* 2008; 46:1488-1494.
19. Dawang ND. Phytochemical constituents and toxicological study of *Vitex doniana* leaf. *J Pharma Biol Sci.* 2015;10;23-27.
20. Hajjeva P, Behl C. Antioxidants as a potential therapy against age-related neuro-degenerative diseases: amyloid Beta toxicity and Alzheimer's disease. *Curr Pharm Des.* 2006;12:699-704.