ORIGINAL ARTICLE

ROLE OF ACTIVATED CHARCOAL IN MITIGATING LEAD-INDUCED HEPATOTOXICITY IN ALBINO WISTAR RATS

Mozna Talpur, Shahnaz Bano Memon, Roomi Memon*, Ali Abbas, Tanveer Talpur, Naveen Lohana*****

Department of Pharmacology, *Physiology, **Internal Medicine, ***Isra University, Hyderabad, Pakistan

Background: Lead is a pervasive environmental toxin known for its severe impact on human health, particularly targeting vital organs like the liver through mechanisms involving oxidative stress and inflammation. This study was conducted to evaluate the hepatoprotective effects of activated charcoal against lead-induced hepatotoxicity in albino Wistar rats. **Methods:** This quasi-experimental study was conducted from Feb to Aug 2024. Thirty-six male albino Wistar rats were divided into 3 groups (n=12): Group A (control group); Group B (lead-acetate group); and Group C (lead-acetate+activated charcoal group). Blood samples were collected by cardiac puncture and analysed for liver function tests. Liver tissues were harvested for histopathological analysis under high power after H&E staining. **Results:** Group B showed significant increases in AST (79.31±9.79 U/L), ALT (38.28±2.29 U/L), ALP (209.09±8.49 U/L), bilirubin (0.98±0.43 mg/dL), and reductions in albumin (2.88±0.28 g/dL) and total proteins (6.92±0.22 g/dL) (p<0.001). Group C had intermediate values between Groups A and B for these parameters. Glutathione peroxidase and superoxide dismutase levels were highest in Group A, lower in Group B, and intermediate in Group C (p<0.001). Histological examination revealed significant vacuolar degeneration and lymphocyte infiltration in Group B, while Group C exhibited milder histological changes with fewer infiltrations. **Conclusion:** Activated charcoal significantly reduces lead-induced hepatotoxicity in albino Wistar rats, evident from biochemical and histological improvements.

Keywords: Charcoal, Heavy metals, Lead, Lead-acetate, Oxidative Stress

Pak J Physiol 2025;21(2):29−33, **DOI:** https://doi.org/10.69656/pjp.v21i2.1761

INTRODUCTION

Lead, a commonly occurring and non-biodegradable heavy metal, causes several physiological as well as biochemical alterations in the body and is a major cause of toxicity in the environment.[1] Over 140,000 deaths occur each year from lead exposure, which is still a serious public health concern in both industrialized and developing nations. Lead is ranked among the top 10 most deadly compounds by the WHO.[2,3]

Lead exposure primarily occurs via the gastrointestinal tract and the respiratory systems, after which it is metabolized in liver and distributed to other organs of the body such as the brain, kidneys, testes, etc.[2] Owing to its metabolic role and its importance in the detoxification of toxins, liver is the first organ which gets affected when lead is either ingested through the gastrointestinal tract or inhaled via the respiratory system.[4] Chronic lead exposure can lead to liver dysfunction and has been implicated in the development of chronic liver disease (CLD) as it causes oxidative stress, inflammation, and fibrosis within liver tissues.[5,6]

Like all heavy metals such as cadmium, nickel, chromium, etc., lead-induced toxicity is attributed to its potential to disrupt the body's homeostatic mechanisms and cause oxidative stress.[4] Lead causes inhibition of the antioxidant enzymatic defences of the body leading to a build-up of free radicals.[2]

Chelation therapy, the administration of substances that find and stick to metals in the bloodstream (ethylenediaminetetraacetic acid, EDTA), dimercapto succinic acid (DMSA), etc., is the treatment of choice for heavy metal poisoning, especially lead.[7]

Activated charcoal, a black, finely split powder, is made by pyrolyzing carbonaceous materials like wood, coconut shells, or petroleum and then oxidizing the substance at high temperatures of 600–900 °C with either air or steam.[8] It can adsorb a wide array of substances, ranging from heavy metals like lead to various industrial pollutants, including dyes and harmful organisms.[8,9] Activated charcoal mitigates lead toxicity primarily through adsorption in the gastrointestinal tract, preventing systemic absorption and interrupting enterohepatic recirculation of lead. Although it does not chelate substances like EDTA or DMSA, its non-specific binding reduces lead bioavailability and indirectly lowers oxidative stress by limiting toxin-induced reactive oxygen species (ROS) production.[10]

Since most antidotes of lead poisoning are biological products, their limited availability and efficacy can be attributed to factors such as their cost, production processes, immunogenic response potential, generation time, and instability issues, which makes it difficult to provide prompt treatment. Therefore, research into easily accessible and natural antidotes is necessary for treating lead poisoning.

The objective of this study was to assess the hepatoprotective effects of activated charcoal against lead-induced hepatotoxicity in albino Wistar rats.

MATERIAL AND METHODS

This quasi-experimental study was conducted from Feb to Aug 2024, in Departments of Pharmacology and Physiology, Isra University Hyderabad. Following approval by Ethical Review Board, 36 male albino Wistar rats, aged 8–10 weeks, weighing 200–250 grams were included. All animals were procured from the Agriculture University of Tando Jam. The standard power analysis method for animal studies was employed for sample size calculation.[11–14]

The experimental animals were allowed to acclimate for two weeks under regulated conditions (temperature maintained at 22±2 °C, relative humidity 50–60%, and a 12-hour light/dark cycle). During this period, they were provided standard chow diet and clean water *ad libetum*. The animals were assigned to 3 groups, each consisting of 12 rats. Group A (control group), Group B (lead-acetate group), and Group C (lead-acetate+activated charcoal group). The doses of lead-acetate (60 mg/Kg by oral gavage) and activated charcoal (1 gm/Kg by oral gavage) were based on previous studies.[8,15] Lead-acetate (GRM756) and activated charcoal powder were procured from local resources. The body weights of all animals were noted before the commencement of the experiment.

After 30 days, all experimental animals were weighed and sacrificed by cervical dislocation following anaesthesia using chloroform-soaked cotton in a container. Blood samples were collected via cardiac puncture and transferred to gel tubes, where they were left to stand for 30 minutes before being centrifuged to separate the serum, which was then stored for analysis of liver enzymes (ALT, ALP, AST). In addition, total serum bilirubin, serum total proteins, and serum Albumin were also assessed using an automated analyser. The liver was excised and, after rinsing with saline, was preserved in 10% formalin. Using a microtome, hepatic tissue slices were created, and then they were stained with haematoxylin and eosin (H&E) for histological examination.[16]

Data were analysed using SPSS-25. The results are presented as Mean±SD. One-way ANOVA followed by post hoc Tukey's test were applied with a significance threshold of $p \leq 0.05$.

RESULTS

The pre-experimental body weights of groups A, B, and C were 240.11±8.3 gm, 242.58±6.7 gm, and 239.93±7.2 gm respectively. A significant difference was observed in the post-experimental body weight across all three groups. Animals in the control group A showed increased body weight (247.32±8.8 g), while those in lead-treated group B experienced a significant decrease (201.58±11.31 g). A similar decline was observed in group C (213.49±10.76 g), though the weight loss in group C was less severe compared to group B (Table-1).

There was a significant increase ($p<0.001$) in the levels of liver function markers, AST, ALT, ALP, and bilirubin in lead-treated animals (group B) when compared with control group A. These liver function markers decreased significantly ($p<0.001$) in group C. The serum albumin levels and total proteins were markedly lowered in group B compared to control group A ($p<0.001$). However, no significant difference was observed between group A and group C (Table-1).

There was a significant ($p<0.001$) decline in the serum levels of antioxidants including Glutathione peroxidase and Superoxide dismutase in group B when compared with control group A. similar decline was also observed in group C, though it was less severe compared to group B (Table-2).

The histological evaluation showed normal hepatic architecture in the control group A, i.e., intact hepatocytes surrounding normal central veins. However, there were marked alterations seen in group B which showed vacuolar degeneration of peripheral hepatocytes with lymphocyte infiltration in the peripheral areas. Group C showed significantly reduced histological alterations with slight infiltration of inflammatory cells around the portal areas.

Table-1: Distribution of body weight and liver function markers across experimental groups

	Group A	Group B	Group C	*p*
Post-experimental body weight (gm)	247.32±8.8^{B}	201.58±11.31^{A,C}	213.49±10.76^{B}	<0.001
AST (U/L)	56.84±1.84^{B}	79.31±9.79^{A,C}	51.06±6.12^{B}	<0.001
ALT (U/L)	17.55±3.71^{B}	38.28±2.29^{A,C}	20.67±5.48^{B}	<0.001
ALP (U/L)	178.45±4.21^{B}	209.09±8.49^{A,C}	181.64±12.68^{B}	<0.001
Bilirubin (mg/dL)	0.29±0.15^{B}	0.98±0.43^{A,C}	0.41±0.58^{B}	<0.001
Albumins (g/dL)	4.19±0.27^{B,C}	2.88±0.28^{A,C}	3.83±0.39^{A,B}	<0.001
Total proteins (g/dL)	8.32±0.16^{B,C}	6.92±0.22^{A,C}	7.83±0.61^{A,B}	<0.001

Note: Different superscript letters (A, B, C) indicate statistically significant differences ($p<0.001$) between these groups

Table-2: Distribution of oxidative stress markers across experimental groups

	Group A	Group B	Group C	*p*
Glutathione peroxidase (U/L)	461.37±34.81^{B,C}	258.25±67.14^{A,C}	349.89±84.21^{A,B}	<0.001
Superoxide dismutase (U/mL)	138.16±8.32^{B}	118.48±6.81^{A,C}	135.68±3.39^{B}	<0.001

Note: Different superscript letters (A, B, C) indicate statistically significant differences ($p<0.001$) between these groups

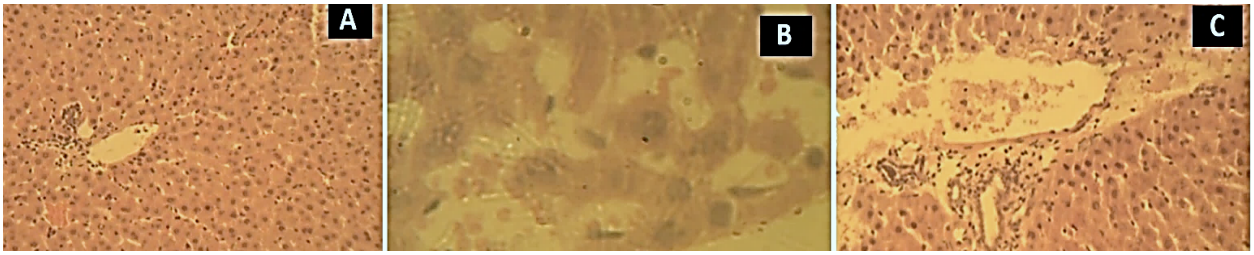


Figure-1: Photomicrographs of liver tissue of experimental groups stained with H&E

DISCUSSION

Lead is one of the most dangerous heavy metals that significantly impacts public health. Lead exposure commonly occurs through industrial emissions, contaminated water supplies, ingestion of lead-based paints or contaminated food, and occupational contact in battery manufacturing, mining, and smelting industries.[17] Contrary to popular belief, encountering a small amount of heavy metals like lead may play a far larger role in the development of chronic illnesses like diabetes, liver dysfunction, renal disease, cancer, and male infertility.[18] Chelators, the only antidote for lead exposure that is currently on the market, have several negative side effects, including gastrointestinal problems, and discomfort.[19] Certain chelators can be difficult to administer but also costly and difficult to find. Furthermore, the Centers for Disease Control advises against using chelation therapy unless necessary and the blood lead concentration is more than 45 ug/dL.[20]

The administration of lead-acetate to rats in this research resulted in a notable elevation of blood AST, ALT, ALP, and bilirubin, accompanied by a decrease in albumin and overall protein levels which is consistent with the findings of Abubakar *et al.* and Abdel Fattah *et al*.[2,21] Elevations in liver enzymes in rats administered lead-acetate indicates damage to the liver's underlying structure. This damage occurs due to hepatocyte necrosis, leading to the release of these enzymes into the bloodstream. Lead exposure disrupts the lipid composition of cellular membranes, increasing their fluidity and compromising structural integrity. This alteration facilitates the leakage of intracellular enzymes such as AST and ALT into the bloodstream. Simultaneously, lead interferes with antioxidant defence systems, promoting excessive generation of ROS. These ROS initiate lipid peroxidation, protein oxidation, and DNA damage, ultimately leading to cellular dysfunction and hepatocyte necrosis.[22] Asiwe *et al*, and Abdelhamid *et al*, also reported similar findings where lead administration was associated with marked liver dysfunction as reflected by an elevation of plasma ALP, AST, and ALT levels.[23,24]

However, these changes were reversed by administering activated charcoal in this study. This aligns with the findings of Offor *et al*[8], who reported that charcoal administration significantly reduced the elevated plasma levels of liver enzymes caused by lead toxicity. This is attributed to the ability of activated charcoal to absorb potentially harmful substances including drugs, plant-based toxic substances, and poisonous chemicals onto its surface, and in doing so preventing their absorption from the gastrointestinal tract into the bloodstream. It also interrupts enterohepatic circulation, which acts as a secondary decontamination mechanism.[25] This is also consistent with the findings of Zhao *et al*, who studied the hepatoprotective effects of charcoal-processed products and observed that all doses reduced the ALT and AST levels, especially the high dose.[26]

Lead administration was associated with marked oxidative stress in the present study as reflected by a significant ($p<0.05$) depletion of oxidative markers in group B. This is due to the underlying fact that free radical generation is one of the most important mechanisms involved in lead-induced toxicity. In doing so, lead exposure disrupts the pro-oxidant and anti-oxidant balance of the body, leading to excessive ROS production, cellular antioxidant depletion, and, consequently, DNA and protein damage, mitochondrial impairment, and ultimately apoptosis.[24] Albasher *et al*. reported a significant decline in the levels of markers of oxidative stress (SOD, GPX, etc.) in lead-treated animals, which is consistent with the findings of the present study.[27] Charcoal administration, however, was associated with significant ($p<0.05$) less oxidative stress in group C. As mentioned above, activated charcoal has the inherent ability to adsorb potentially harmful substances onto its surface, preventing their absorption from the gastrointestinal tract into the bloodstream. By binding to these substances, activated charcoal indirectly helps reduce oxidative stress by limiting the availability of toxins that can generate ROS within the body.[25,28] Offor *et al*. also reported in their study that the treatment of rats with activated charcoal caused a significant increase in Glutathione Peroxidase and Superoxide Dismutase levels as compared to lead-treated animals, which is consistent with our findings.[8]

Lead administration was also observed to cause significant alterations in hepatic histological architecture in the present study. This is similar to the findings of Abubakar *et al*, who noted that lead

administration was associated with congestion, infiltration of inflammatory cells, and activation of resident hepatic macrophages, which aligns with our findings.[2] Zou *et al*, also reported similar findings, noting that lead administration resulted in cells exhibiting degenerative and necrotic features indicative of microvesicular steatosis.[29] In contrast, charcoal administration was associated with a marked decrease in these hepatic alterations in the current study. This conforms with Offor *et al*, who observed that activated charcoal administration significantly reduced histological markers of hepatic dysfunction, such as inflammatory cell infiltration, congestion, and vacuolar degeneration.[8]

This study highlights the potential hepatoprotective effects of activated charcoal in mitigating lead-induced hepatotoxicity. These findings underscore the potential of activated charcoal as a cost-effective and accessible therapeutic agent for mitigating lead-induced hepatotoxicity. Future research should explore its protective effects across other organ systems and evaluate its efficacy in long-term exposure models. However, certain limitations should be acknowledged, including the lack of assessment of oxidative stress and inflammation markers, which could have provided further insight into the potential anti-inflammatory and antioxidant effects of charcoal. Additionally, the study focused solely on the hepatotoxic effects of lead and the hepatoprotective effects of charcoal, without evaluating other organ systems, such as renal, nervous, and reproductive systems.

CONCLUSION

Activated charcoal exerts a potent hepatoprotective effect against lead-induced hepatotoxicity in albino Wistar rats. This hepatoprotective effect can be attributed to the anti-oxidant properties of activated charcoal. In cases of persistent lead-induced liver deterioration, activated charcoal might be helpful.

REFERENCES

1. Sani AH, Amanabo M. Lead: A concise review of its toxicity, mechanism and health effect. GSC Biol Pharm Sci 2021;15(1):55–62.
2. Abubakar K, Mailafiya MM, Chiroma SM, Danmaigoro A, Zyoud TYT, Abdul Rahim E, *et al*. Ameliorative effect of curcumin on lead-induced hematological and hepatorenal toxicity in a rat model. J Bioch Mol Toxicol 2020;34(6):e22483.
3. Kucukler S, Benzer F, Yildirim S, Gur C, Kandemir FM, Bengu AS, *et al*. Protective effects of chrysin against oxidative stress and inflammation induced by lead acetate in rat kidneys: a biochemical and histopathological approach. Biol Trace Elem Res 2021;199(4):1501–14.
4. Renu K, Chakraborty R, Myakala H, Koti R, Famurewa AC, Madhyastha H, *et al*. Molecular mechanism of heavy metals (Lead, Chromium, Arsenic, Mercury, Nickel and Cadmium)-induced hepatotoxicity–A review. Chemosphere. 2021;271:129735.
5. Teschke R. Copper, iron, cadmium, and arsenic, all generated in the universe: Elucidating their environmental impact risk on human health including clinical liver injury. Int J Mol Sci 2024;25(12):6662.
6. Meghji KA, Memon TF, Raqeeb A, Pathan MF, Raqeeb U, Shaikh TZ. Vitamin D deficiency and chronic liver disease: Investigating predictive factors and their implications for patient care in Pakistan. Iranian J Public Health 2025;54(5):1054–63.
7. Teerasarntipan T, Chaiteerakij R, Prueksapanich P, Werawatganon D. Changes in inflammatory cytokines, antioxidants and liver stiffness after chelation therapy in individuals with chronic lead poisoning. BMC Gastroenterol 2020;20(1):263.
8. Offor SJ, Mbagwu HO, Orisakwe OE. Lead induced hepato-renal damage in male albino rats and effects of activated charcoal. Front Pharmacol 2017;8:107.
9. Patel H. Charcoal as an adsorbent for textile wastewater treatment. Sep Sci Technol 2018;53(17):2797–812.
10. Kumar P, Nashath Omer S, Reddy M, Saravanan P, Rajeshkannan R, Rajasimman M, *et al*. Exploring the role of activated charcoal from lignocellulosic biomass wastes for environmental pollution control. J Energy Inst 2024;114:101626.
11. Charan J, Biswas T. How to calculate sample size for different study designs in medical research? Indian J Psychol Med 2013;35(2):121–6.
12. Meghji KA, Memon TF, Ahmed I, Memon SG, Noor N, Abbas A. Nephroprotective effects of L-Arginine against chemotherapy induced acute kidney injury in wistar rats. J Islamabad Med Dent Coll 2020;9(4):249–55.
13. Hanif MS, Baloch MS, Meghji KA, Abbas A, Kashif S, Qureshi R. Histopathological changes in the gastric mucosa induced by carbaryl toxicity: an experimental rat model. Khyber Med Univ J 2020;12(2):137–42.
14. Meghji KA, Talpur RA, Uqaili AA, Nizammani YM, Kazi N, Nizammani GS. Resveratrol attenuates oxidative stress in chemotherapy induced acute kidney injury: An experimental rat model. Khyber Med Univ J 2019;11(2):85–9.
15. Uwikor FK, Nwachuku EO, Igwe F, Echonwere B, Bartimaeus ES. Evaluation of Haematological Changes In Lead-Acetate-Induced Albino Rats Treated With Aqueous Extract Of Hypoestes Rosea Leaf. Eur J Biomed 2020;7(2):509–17.
16. Meghji KA, Memon TF, Hanif MS, Baloch MS, Thalho AA, Noor N. Hepatoprotective role of unacylated ghrelin in different doses: an experimental study. Khyber Med Univ J 2024;16(3):249–54.
17. Poudel K, Ikeda A, Fukunaga H, Brune Drisse MN, Onyon LJ, Gorman J, *et al*. How does formal and informal industry contribute to lead exposure? A narrative review from Vietnam, Uruguay, and Malaysia. Rev Environ Health 2024;39(2):371–88.
18. Ohiagu FO, Chikezie PC, Ahaneku CC, Chikezie CM. Human exposure to heavy metals: toxicity mechanisms and health implications. Material Sci Eng 2022;6(2):78–87.
19. Samarghandian S, Shirazi FM, Saeedi F, Roshanravan B, Pourbagher-Shahri AM, Khorasani EY, *et al*. A systematic review of clinical and laboratory findings of lead poisoning: lessons from case reports. Toxicol Appl Pharmacol 2021;429:115681.
20. Twardy S. Therapeutic Approaches for Management of Lead Exposure from Embedded Lead Metal Fragments. (Master Thesis), University of California, Santa Cruz; 2021.
21. Abdel Fattah ME, Sobhy HM, Reda A, Abdelrazek HM. Hepatoprotective effect of Moringa oleifera leaves aquatic extract against lead acetate–induced liver injury in male Wistar rats. Environ Sci Pollut Res Int 2020;27:43028-43.
22. Abd Eldaim MA, Barakat ER, Alkafafy M, Elaziz SAA. Antioxidant and anti-apoptotic prophylactic effect of silymarin against lead-induced hepatorenal toxicity in rats. Environ Sci Pollut Res Int 2021;28(41):57997–8006.
23. Asiwe JN, Kolawole TA, Anachuna KK, Ebuwa EI, Nwogueze BC, Eruotor H, *et al*. Cabbage juice protect against Lead-induced liver and kidney damage in male Wistar rat. Biomarkers 2022;27(2):151–8.
24. Abdelhamid FM, Mahgoub HA, Ateya AI. Ameliorative effect

of curcumin against lead acetate–induced hemato-biochemical alterations, hepatotoxicity, and testicular oxidative damage in rats. Environ Sci Pollut Res Int 2020;27:10950–65.
25. Zellner T, Prasa D, Färber E, Hoffmann-Walbeck P, Genser D, Eyer F. The use of activated charcoal to treat intoxications. Dtsch Aerztebl Int 2019;116(18):311–7.
26. Zhao Y, Zhang Y, Kong H, Zhang M, Cheng J, Wu J, *et al*. Carbon Dots from *Paeoniae Radix Alba* Carbonisata: Hepatoprotective Effect. Int J Nanomedicine 2020;15:9049–59.
27. Albasher G, Al Kahtani S, Alwahibi MS, Almeer R. Effect of Moringa oleifera Lam. methanolic extract on lead-induced oxidative stress-mediated hepatic damage and inflammation in rats. Environ Sci Pollut Res Int 2020;27:19877–87.
28. Hassan M, Wang Y, Rajput SA, Shaukat A, Yang P, Farooq MZ, *et al*. Ameliorative effects of luteolin and activated charcoal on growth performance, immunity function, and antioxidant capacity in broiler chickens exposed to deoxynivalenol. Toxins (Basel) 2023;15(8):478. https://doi.org/10.3390/toxins15080478
29. Zou H, Sun J, Wu B, Yuan Y, Gu J, Bian J, *et al*. Effects of cadmium and/or lead on autophagy and liver injury in rats. Biol Trace Elem Res 2020;198(1):206–15.

Address for Correspondence:

Dr Shahnaz Bano Memon, Department of Pharmacology, Isra University, Hyderabad, Pakistan. **Cell:** +92-332-2714042
Email: drshahmemon@gmail.com

Received: 20 Sep 2024 **Reviewed:** 13 May 2025 **Accepted:** 19 Jun 2025

Contribution of Authors:

MT: Concept, design, drafting of article, final approval
RM: Drafting, Statistical expertise
TT: Collection and assembly of data
SBM: Drafting of article, Critical review
AA: Drafting, Collection and assembly of data
NL: Collection and assembly of data

Conflict of Interest: None
Funding: None