Original article

Comparative Effects of *Hibiscus rosa* Flower Extract and Silymarin on Lead Acetate Induced Hepatotoxicity in Male Albino Rats

Shazia Gul[1], Saima Abudul Waheed[2], Muhamamd Yasoob[3]

[1]Demonstrator Department of Anatomy, Postgraduate Medical Institute, Lahore-Pakistan, [2]Additional Principal Women Medical Officer, Department of Anatomy, Fatima Jinnah Medical University, Lahore-Pakistan, [3]Professor Department of Anatomy Postgraduate Medical Institute, Lahore-Pakistan

Correspondence to: Dr. Shazia Gul, Email: shzgul73@gmail.com

ABSTRACT

Background: Lead, a pervasive pollutant, induces liver toxicity through oxidative stress, inflammation, and enzyme disruption. *Hibiscus rosa-sinensis* and silymarin possess hepatoprotective properties, mitigating oxidative damage, stabilizing hepatocyte membranes, and promoting liver regeneration. Objective of this study is to examine the protective effects of a combination of *Hibiscus rosa-sinensis* flower extract and silymarin against lead acetate-induced liver toxicity in male albino rats.

Subjects and methods: An experimental animal study was conducted at PGMI and LGH, Lahore (July 2023–July 2024) after ethical approval. Fifty albino rats were divided into five groups: Group A (control) received distilled water, while Groups B–E were given 500 mg/kg lead acetate alone or with 300 mg/kg *Hibiscus rosa-sinensis* extract, 200 mg/kg silymarin, or both for 30 days. Body and liver weights, serum ALT, AST, and glutathione peroxidase levels were measured. Liver sections were analyzed using H&E and Masson's Trichrome staining. Histological toxicity indicators included hepatic architecture, portal triad inflammation, and fibrosis, assessed via the Histology Activity Index (HAI). Hepatic lobules were classified as normal (0) or abnormal (1), portal triad inflammation was scored from 0 to 4, and fibrosis ranged from 0 (none) to 4 (cirrhosis). Data were recorded, stratified according to toxicity indicators, and analysed using SPSS 25, with significance set at $p \leq 0.05$.Results: Lead exposure caused weight loss (180.7 ± 6.2 g), hepatomegaly (10.6 ± 0.7 g), and liver damage (ALT: 80.3 ± 12.3 U/L, AST: 164.4 ± 8.3 U/L, GPX: 76.3 ± 7.0 U/L, $p < 0.05$), indicating toxicity. *Hibiscus rosa-sinensis* and silymarin reduced these effects, with the combination (190.7 ± 8.1 g, 7.5 ± 0.5 g, ALT: 28.1 ± 3.9 U/L, AST: 60.9 ± 9.3 U/L, GPX: 124.4 ± 3.9 U/L) suggesting the hepatoprotective effect.

Conclusion: The combination of *Hibiscus rosa-sinensis* flower extract and silymarin exhibited the strongest hepatoprotective effects against lead acetate-induced liver damage in male albino rats, effectively preserving body and liver weight, reducing ALT and AST levels, and restoring GPX levels more effectively than either treatment alone.

Keywords:

Hibiscus Flower Extract, Silymarin, Lead Acetate-Induced Liver Toxicity, Albino Rats, *Hibiscus rosa-sinensis*

INTRODUCTION

Hepatotoxicity, caused by environmental toxins like lead acetate, is a global health concern due to its oxidative stress effects, leading to liver damage via lipid peroxidation, mitochondrial dysfunction, and antioxidants have shown promise due to their availability, inflammation.[1,2] The liver, central to detoxification, is vulnerable to oxidative damage, but plant-based safety, and free-radical scavenging propertie.[3] Antioxidants, including enzymatic (e.g., superoxide dismutase) and non-enzymatic (e.g., glutathione, vitamins C and E), neutralize reactive oxygen species (ROS) and prevent cellular damage.[4,5] Lead is a toxic heavy metal and environmental pollutant with six major sources of exposure worldwide.[6] In 2019, lead exposure caused over 902,000 deaths and 21.7 million DALYs globally, with South Asia, East Asia, and the Pacific most affected.[7] The CDC's 2021 guidelines set blood lead limits at 10 µg/dL for adults and 5 µg/dL for children. Lead is metabolized in the liver and eliminated through faeces, hair, nails, and sweat, with a blood half-life of 40 days, longer in children and pregnant women.[8] *Hibiscus rosa-sinensis*, also known as the Chinese hibiscus, is a therapeutic plant with hepatoprotective, antioxidant, and anti-inflammatory effects, attributed to its bioactive compounds like flavonoids, polyphenols, and anthocyanins.[9] Its flower extract is used to treat respiratory issues and appetite loss, and acts as a mild laxative and diuretic.[10] Silymarin, derived from Silybum marianum (milk thistle), is a well-established

ARTICLE INFO

Article History

Received: 26.03.2024, | Accepted: 10-01-2025

Conflict of Interest: The authors declared no conflict of interest exists.

Funding: None

Copyright: © 2024 Gul et al. This article is licensed under the Creative Commons Attribution-Noncommercial 4.0 International License (CC BY-NC 4.0), which allows for unrestricted non-commercial use, sharing, and reproduction in any medium, as long as the original author and source are properly credited and acknowledged.

Citation: Gul S, Waheed SA, Yasoob M. Comparative Effects of *Hibiscus rosa-sinensis* flower extract and silymarin on lead acetate induced hepatotoxicity in male albino rats. J Fatima Jinnah Med Univ. 2024; 18(4): 205-211.

DOI: https://doi.org/10.37018/ASXC2354

hepatoprotective agent that stabilizes hepatocyte membranes, enhances antioxidant defences, and modulates inflammation.[11] Rat livers, with higher liver-to-body weight ratios and unique anatomy, serve as models for studying liver toxicity and antioxidant effects.[12]

Literature have underscored the impressive hepatoprotective properties of *Hibiscus rosa-sinensis* and silymarin when applied individually. Specifically, anthocyanins derived from *Hibiscus rosa-sinensis* have been shown to effectively inhibit lipid peroxidation and substantially improve antioxidant status in the livers of rats with diabetes induced by streptozotocin.[13] Concurrently, silymarin has been the subject of extensive research for its exceptional protective effects on the liver against a diverse range of toxins.[14] Together, these natural compounds provide strong evidence for their potential to enhance liver health and safeguard against damage. Lead poisoning causes serious oxidative stress induced liver damage due to its bioaccumulation, biomagnification, and toxicity.[15] *Hibiscus rosa-sinensis* flower extract is a natural antioxidant and Silymarin is well known for hepatoprotection. The objective of this study was to investigate the protective role of a combination of *Hibiscus rosa-sinensis* flower extract and silymarin against lead acetate induced toxicity in liver of male albino rats.

SUBJECTS AND METHODS

An animal experimental study involving male albino rats was carried out in the animal house and histology laboratory of the Anatomy Department at the Post Graduate Medical Institute (PGMI) and Lahore General Hospital (LGH), Lahore after the ethical approval. The study included adult male albino rats aged 6-8 weeks, with an average weight ranging from 180-220 gm. Female rats and those exhibiting any signs of illness were excluded from the study. Sample size was calculated by WHO formula of health studies for determination of minimum sample size keeping power of study equal to 90% and level of significance equal to 5%. Minimum sample size calculated was 10 rats. Total 50 rats were enrolled in current study equally divided into 5 groups.

Rosa sinensis flowers were obtained from the University of Punjab, Lahore, authenticated by a taxonomist, washed, and shadow-dried for 10 days. They were then transported to PCSIR laboratories for extraction, yielding 320 g of reddish-brown mucilage from 3 kg of dried flowers. The extract was prepared under the supervision of the chief research officer at PCSIR, Lahore. Fifty adult male albino rats (180–220 g) were obtained from the animal house at the University of Health Sciences, Lahore. The maximum liquid tolerated per rat is 20 mL/kg body weight. The dose of lead acetate powder was calculated based on 500 mg/kg, resulting in 100 mg per rat (0.2 kg), dissolved in 1 mL of distilled water. .[16] Similarly, the dose of silymarin powder was 200 mg/kg, amounting to 40 mg per rat, prepared in 1 mL of distilled water. .[17]Moreover,The dose of *Hibiscus rosa-sinensis* flower extract was 300 mg/kg, equating to 60 mg per rat, also dissolved in 1 mL of distilled water.[18]

Fifty adult male albino rats, aged 6-8 weeks and weighing between 180-220 gm, were obtained from the animal house of the University of Veterinary and Animal Sciences, Lahore. The rats were housed in stainless-steel cages in a well-ventilated room with an ambient temperature of 28.0 ± 2.0°C and humidity of 60 ±10%, under a 12-hour light/dark cycle.[19] They had access to standard rat food and water ad-libitum, and hygienic conditions were strictly maintained. After a one-week acclimatization period, the experiment commenced. Each rat was weighed both before and after the experiment using an electronic scale. Group A (control, distilled water), Group B (lead acetate treatment), Group C (lead acetate and Hibiscus extract), Group D (lead acetate and silymarin), and Group E (lead acetate, Hibiscus extract, and silymarin). Each group received their respective treatments for 30 days via oral gavage. Liquid compounds were administered via oral gavage using a bulb-tipped needle or flexible cannula. The rat was gently restrained, and the needle was inserted along the mouth's side into the stomach. If resistance, coughing, or distress occurred, administration was stopped to prevent injury or aspiration.[20] The study followed ethical guidelines and aimed to evaluate the hepatoprotective effects of Hibiscus extract and silymarin against lead acetate toxicity. Blood samples were collected from rats under inhalant anesthesia using chloroform, followed by cardiac puncture at the beginning and end of the 30-day treatment period. To mitigate potential toxic effects of chloroform, control animals received only distilled water. Treatments were administered via oral gavage to ensure consistent exposure. After weighing, liver samples were fixed in 10% neutral-buffered formalin for histological assessment. Tissue sections (4–5 µm thick) were prepared, embedded in paraffin, and stained with Hematoxylin and Eosin (H&E) for microscopic examination. Serum was separated, centrifuged, and stored at -20°C for further analysis. Rats were euthanized 24 hours after the final dose, followed by a vertical incision for liver dissection. The liver was carefully washed, weighed, and examined for abnormalities. All animal remains were disposed of at PGMI, Lahore.Serum ALT, AST, and glutathione peroxidase levels were measured, and liver sections were analysed via H&E and Masson's Trichrome staining for histopathology and fibrosis. In histology, significant indicators of toxicity included Hepatic lobules, inflammation in the portal

triads, and hepatic fibrosis, assessed using the Histology Activity Index (HAI). Hepatic lobules were classified as normal (0) with a hexagonal structure, a central vein, and radiating hepatocyte cords, while architectural disruption was coded as abnormal (1). Portal triad inflammation was assessed under 40X magnification and scored using the Histology Activity Index (HAI) as follows: no inflammation (0), mild (<1/3 of portal triads, 1), moderate (1/3–2/3 of portal triads, 3), and marked (>2/3 of portal triads, 4). Hepatic fibrosis was evaluated using Masson's Trichrome stain under 10X magnification and scored based on HAI: no fibrosis (0), fibrous portal expansion (1), bridging fibrosis (3), and cirrhosis (4). All the data was collected on a predesign performa.

Data was analyzed through Statistical Package for the Social Sciences (SPSS) Version 25. Quantitative data was shown by mean ± standard deviation (SD). Qualitative data was expressed by percentage. Data was stratified according to hepatic lobules, portal traid inflammation and hepatic fibrosis A One-Way Analysis of Variance (ANOVA) was conducted to assess the mean differences in quantitative data. Statistical significance was considered at a p-value of ≤ 0.05.

RESULTS

The comparison of biochemical parameters among the study groups revealed significant differences ($p < 0.001$) across all measured variables, namely serum ALT, AST, and GPX levels. Group A, serving as the control (distilled water), demonstrated normal levels of these parameters, with serum ALT at 27.0 ± 6.1 U/L, AST at 61.5 ± 8.2 U/L, and GPX at 124.0 ± 7.2 U/L. In contrast, Group B (lead acetate treatment) exhibited significantly elevated serum ALT (80.3 ± 12.3 U/L) and AST (164.4 ± 8.3 U/L) levels, alongside a notable reduction in GPX levels (76.3 ± 7.0 U/L), reflecting the hepatotoxic and oxidative effects of lead acetate.(P-value<0.001)Groups C, D, and E, which involved treatments aimed at mitigating lead acetate toxicity, showed varying degrees of biochemical improvement. Group C (lead acetate and Hibiscus extract) displayed decreased ALT (58.3 ± 3.3 U/L) and AST (149.7 ± 5.0 U/L) levels compared to Group B, with a partial recovery of GPX levels (108.2 ± 3.5 U/L). Group D (lead acetate and silymarin) showed further improvement, with ALT, AST, and GPX levels recorded at 37.5 ± 4.4 U/L, 124.9 ± 12.5 U/L, and 117.6 ± 2.7 U/L, respectively. The most significant amelioration was observed in Group E (lead acetate, Hibiscus extract, and silymarin), where ALT and AST levels (28.1 ± 3.9 U/L and 60.9 ± 9.3 U/L) were near control values, and GPX levels (124.4 ± 3.9 U/L) were restored.

These findings suggest a synergistic protective effect of Hibiscus extract and silymarin in counteracting lead acetate-induced hepatotoxicity and oxidative stress (Table 1). Group A (control, distilled water), Group B (lead acetate treatment), Group C (lead acetate and Hibiscus extract), Group D (lead acetate and silymarin), and Group E (lead acetate, Hibiscus extract, and silymarin).

As for the histological parameters are concerned, Hepatic lobule architecture varied significantly ($p < 0.001$) across groups. While all control rats (Group A) had normal liver structure, lead acetate-treated rats (Group B) showed complete disruption. *Hibiscus* extract (Group C) and silymarin (Group D) provided partial protection, with 70% and 90% normal architecture, respectively. The combined treatment (Group E) fully preserved liver structure (100% normal), suggesting a synergistic protective effect. (Table 2) (Figure 1).

In group A, no inflammation of portal triads was seen in all the rats. In group B, 5 (50%) rats had moderate while 5 (50%) rats showed marked inflammation respectively. Mild inflammation of portal triads was present in all the rats of group C. It was absent in 8 (80%) and mild inflammation of portal triad was present in 2 (20%) rats respectively. 9 (90%) rats of group E showed no inflammation with presence of mild inflammation in 1 (10%) rat respectively (Table 3) (Figure 2).

A: Control group A liver shows no portal triad inflammation (green arrow). H&E, 40X.

B: Lead-treated group B shows moderate portal triad inflammation (green arrow). H&E, 40X.

C: Group C (lead + Hibiscus) shows mild portal triad inflammation (green arrow). H&E, 40X.

D: Group D (lead + Silymarin) shows no portal triad inflammation (green arrow). H&E, 40X.

E: Group E (lead + Hibiscus + Silymarin) shows no portal triad inflammation (green arrow). H&E, 40X.

In group A and E, no hepatic fibrosis was seen in all the rats. In group B, 3 (30%) rats had mild, and 7 (70%) rats showed moderate fibrosis respectively. Total 3 (30%) rats showed mild fibrosis of portal triads in rats of group C. It was mild in 1 (10%) rat and was absent in all (100 %) rats of group E (Table 4) (Figure 3).

Animals Body Weight (g): At the start of the experiment, there was no significant difference in body weight among the groups ($p = 0.873$), indicating comparable baseline values. By the end of the experiment, a significant difference was observed ($p = 0.045$), suggesting that lead acetate exposure (Group B) led to a decline in body weight, whereas groups receiving *Hibiscus rosa-sinensis* extract (Group C), silymarin (Group D), or both (Group E) showed weight maintenance or slight increases, similar to the control group. This indicates a potential protective effect of these treatments against lead-induced weight loss.

Table 11: Comparison of Biochemical parameters among study groups

Variable	Group A	Group B	Group C	Group D	Group E	p-value [#]
Serum ALT Levels	27.0 ± 6.1	80.3 ± 12.3	58.3 ± 3.3	37.5 ± 4.4	28.1 ± 3.9	< 0.001*
Serum AST Levels	61.5 ± 8.2	164.4 ± 8.3	149.7 ± 5.0	124.9 ± 12.5	60.9 ± 9.3	< 0.001*
Serum GPX Levels	124.0 ± 7.2	76.3 ± 7.0	108.2 ± 3.5	117.6 ± 2.7	124.4 ± 3.9	< 0.001*

Table 2: Comparison of architecture of hepatic lobule among groups

Group	Architecture of Hepatic Lobule		p-value
	Normal n (%)	Abnormal n (%)	
A	10 (100.0%)	0 (0.0%)	< 0.001*
B	0 (0.0%)	10 (100.0%)	
C	7 (70.0%)	3 (30.0%)	
D	9 (90.0%)	1 (10.0%)	
E	10 (100.0%)	0 (0.0%)	

Table 3: Comparison of inflammation of portal triads among groups

Group	Inflammation of Portal Triads				p-value
	No n (%)	Mild n (%)	Moderate n (%)	Marked n (%)	
A	10 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	< 0.001*
B	0 (0.0%)	0 (0.0%)	5 (50.0%)	5 (50.0%)	
C	0 (00.0%)	10 (100.0%)	0 (0.0%)	0 (0.0%)	
D	8 (80.0%)	2 (20.0%)	0 (0.0%)	0 (0.0%)	
E	9 (90.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	

Table 4: Comparison of hepatic fibrosis among groups

Group	Hepatic Fibrosis				p-value
	No n (%)	Mild n (%)	Moderate n (%)	Marked n (%)	
A	10 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	< 0.001*
B	0 (0.0%)	3 (30.0%)	7 (70.0%)	0 (0.0%)	
C	7 (70.0%)	3 (30.0%)	0 (0.0%)	0 (0.0%)	
D	9 (90.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	
E	10 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	

Table 5: Comparison of animal's body weight (g) of at start and end of experiment among study groups.

Group	Animal body weight (g)	
	At start of the experiment	At end of the experiment
Group A	187.5 ± 8.0	190.8 ± 8.0
Group B	184.1 ± 6.9	180.7 ± 6.2
Group C	186.9 ± 8.5	188.7 ± 8.7
Group D	186.9 ± 9.4	189.2 ± 9.4
Group E	187.6 ± 8.1	190.7 ± 8.1
p-value [#]	0.873	0.045*

ANOVA

Table 6: Comparison of animal liver weight (g) among study groups

Variable	Group A	Group B	Group C	Group D	Group E	p-value
Weight of Liver(g)	7.5 ± 0.7	10.6 ± 0.7	9.3 ± 0.9	8.5 ± 0.5	7.5 ± 0.5	< 0.001*

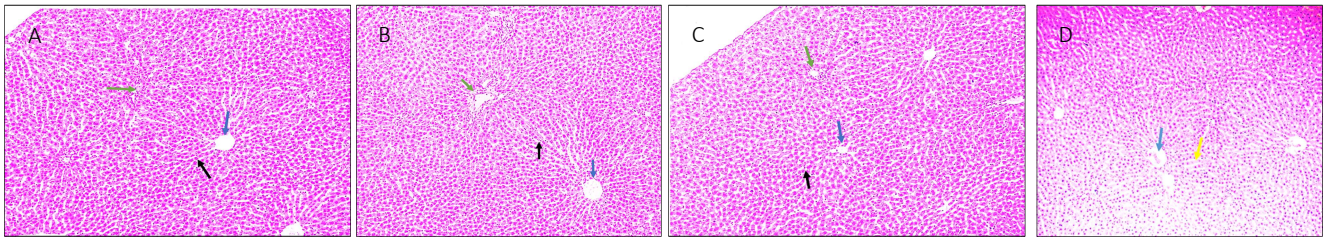

Figure 1: A: Photomicrograph of group C liver (lead + Hibiscus) shows normal hepatic architecture with a central vein (blue arrow), hepatocyte cords (black arrow), and portal triad (green arrow). H&E stain, 10X. **B:** Group D liver (lead + Silymarin) displays normal hepatic structure with a central vein, hepatocyte cords, and portal triad. H&E stain, 10X. **C:** Group E liver (lead + Hibiscus + Silymarin) exhibits normal hepatic architecture with a central vein, hepatocyte cords, and portal triad. H&E stain, 10X. **D:** Group A showing normal looking central vein (blue arrow) and sinusoids (yellow arrow). H&E stain, 10X.

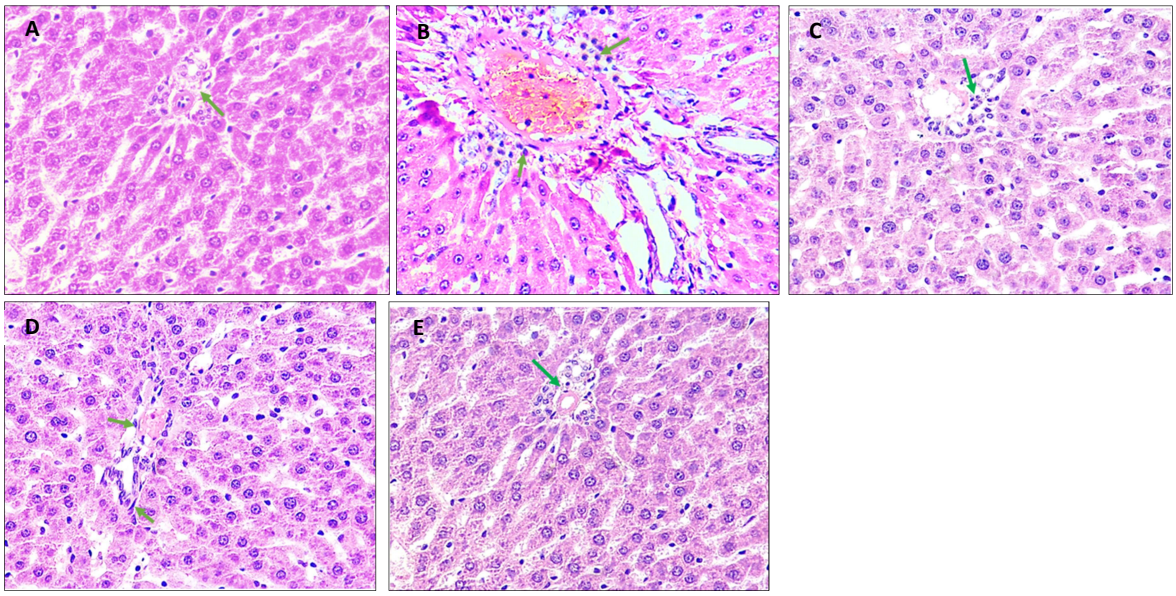

Figure 2: Histological analysis of liver sections stained with H&E (40X) showing portal triad inflammation across groups.

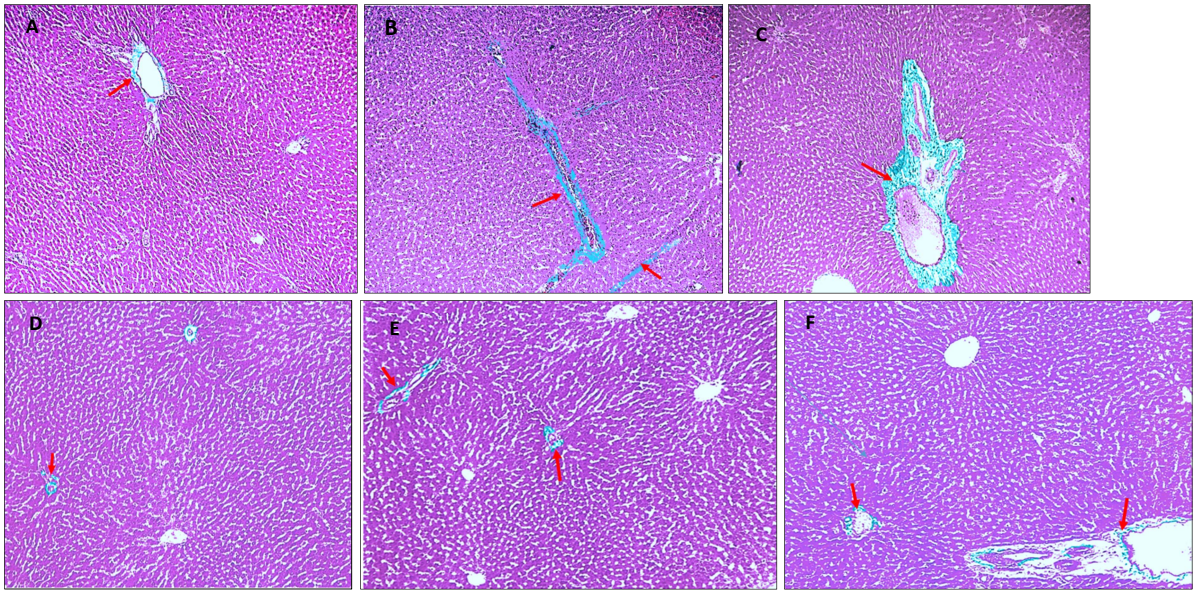

Figure 3: A: Control group A shows no hepatic fibrosis (red arrow). B: Lead-treated group B shows moderate fibrosis (red arrow). C: Lead-treated group B shows mild hepatic fibrosis (red arrow). D: Group C (lead + Hibiscus) shows no hepatic fibrosis (red arrow). E: Group D (lead + Silymarin) shows no hepatic fibrosis (red arrow). Masson's trichrome, 10X.

Animal Liver Weight (g): Liver weight varied significantly among the study groups ($p < 0.001$). The control group (Group A) and the combination treatment group (Group E) had the lowest liver weights (7.5 ± 0.7 g and 7.5 ± 0.5 g, respectively), while the lead acetate group (Group B) showed the highest liver weight (10.6 ± 0.7 g), indicating possible hepatomegaly due to toxicity. Groups receiving *Hibiscus rosa-sinensis* extract (Group C, 9.3 ± 0.9 g) or silymarin (Group D, 8.5 ± 0.5 g) exhibited reduced liver weights compared to Group B, suggesting a protective effect. The results indicate that *Hibiscus rosa-sinensis* and silymarin mitigated lead-induced liver enlargement, with the combination treatment (Group E) offering the most protection.

DISCUSSION

Lead can have a wide range of toxicological consequences and is a contaminant in both the environment and industry. Lead has a significant impact on a number of organs in both humans and animals, particularly the

liver.[21] Protective role of Silymarin in liver intoxication is proven. *Hibiscus rosa-sinensis* flower in the form of hibiscus tea is a popular beverage used in the world. Consumption of hibiscus flower extract has many beneficial effects on health in general and on liver in particular. In current study the lead treated group displayed increased mean serum levels of ALT & aspartate AST among groups that was correlated with study by Gaskill et al. (2019) who conveyed that it damaged the K+-Ca2+ channels which resulted in cell lysis leading to increased serum levels of ALT and AST. *Hibiscus rosa-sinensis* treated group expressed decrease in serum enzyme levels that coincided with findings of a previous study which demonstrated that it reduced lipid peroxidation of hepatocyte cell membrane and regulated cellular permeability leading to decrease in serum levels of ALT and AST.[22] Silymarin treated group indicated decrease in serum enzyme levels that was identical with finding of a previous study which highlighted that it has cytoprotective and free radical scavenging properties that lead to stability of hepatocellular membrane resulting in decrease in hepatic functanality.[23] Moreover, the mean serum level of enzyme glutathione peroxidase (GPX) among groups. Lead treated group showed decrease in serum enzyme levels that was also reported by an earlier study which intimated that it produced electrophilic metabolites resulting in consumption of antioxidant enzyme glutathione peroxidase in liver leading to decreased serum levels of serum GPX.[19] *Hibiscus rosa-sinensis* treated group noticed increase in serum levels of glutathione peroxidase.[24] Similar findings were seen by Raza and coworkers, who told that it had very high concentration of phenols and flavonoids resulting into redox activities against free radicals and increase in serum level of GPX.[25] Silymarin treated group conveyed increase in serum level of glutathione peroxidase that correlated with findings of Jalali and coresearchers who manifested that it quenched singlet and triplet oxygen molecules and increased serum level of GPX.[26] The study demonstrated that the combination of *Hibiscus rosa-sinensis* flower extract and silymarin (Group E) had a superior hepatoprotective effect compared to their individual effects (Groups C and D) on lead acetate-induced hepatotoxicity in male albino rats, aligning with findings of a previous study on combined antioxidant therapies.[27] Histological analysis showed significant restoration of hepatic architecture in treated groups, with Hibiscus improving oxidative stress-induced damage through its flavonoids, polyphenols, and anthocyanins and silymarin aiding hepatocyte repair. These findings are consistent with previous studies.[23,24] Portal triad inflammation, significantly reduced in treated groups ($p < 0.001$), was mitigated by Hibiscus through anti-inflammatory pathways and by silymarin's inhibition of inflammatory metabolites.[29] Hepatic fibrosis, prominent in the lead-treated group ($p < 0.0001$), was alleviated by Hibiscus via suppression of CYP2E1 and preservation of mitochondrial function and by silymarin through the inhibition of hepatic stellate cells.[30]

There are certain limitations of present study. It is a small-scale study, a larger sample size and a study of longer duration might have produced more accurate results, but it was not economically viable. It is a rat study, studies on higher mammals are required to reach the goal. It is a protective study. Therapeutic trials are needed.

CONCLUSION

Hibiscus rosa-sinensis flower extract as well as silymarin can protect against lead acetate induced hepatotoxicity but a combination of hepatoprotective effect can be better than their individual effect. The current study opens doors for further where a combination of *Hibiscus rosa-sinensis* and silymarin can be tried for hepatoprotection against other oxidative stress induced liver injuries. The study may provide a preliminary evidence for possible human therapeutic role in future.

Author Contributions

SG: Conceptualization, manuscript drafting, data Collection and manuscript editing, editing and revision of manuscript.
SAW: Conceptualization, manuscript drafting, final review.
MY: Data Analysis, drafting the manuscript, editing and revision of manuscript.

REFERENCES

1. Flora G, Gupta D, Tiwari A. Toxicity of lead: a review with recent updates. Interdisciplinary Toxicology. 2012;5(2):47–58.
2. Wang Z, Zhao H, Xu Y, et al. Early-life lead exposure induces long-term toxicity in the central nervous system: From zebrafish larvae to juveniles and adults. Science of the Total Environment. 2022;804:150185.
3. Chaudhary P, Janmeda P, Docea AO, et al. Oxidative stress, free radicals and antioxidants: Potential crosstalk in the pathophysiology of human diseases. Frontiers in Chemistry. 2023;11:1158198.
4. Murphy MP, Bayir H, Belousov V, et al. Guidelines for measuring reactive oxygen species and oxidative damage in cells and in vivo. Nature Metabolism. 2022;4(5):651–62.
5. Irato P, Santovito G. Enzymatic and non-enzymatic molecules with antioxidant function. Antioxidants. 2021;10(4):579.
6. Obeng-Gyasi E. Sources of lead exposure in various countries. Reviews on Environmental Health. 2019;34(1):25–34.
7. Malihe R, Zahra E, Aria N, et al. Estimating the burden of diseases attributable to lead exposure in the North Africa and Middle East region, 1990–2019: a systematic analysis for the Global Burden of Disease study 2019. Environmental Health. 2022;21(1):1–14.
8. Balali-Mood M, Naseri K, Tahergorabi Z, Khazdair MR, Sadeghi M. Toxic mechanisms of five heavy metals: mercury, lead, chromium, cadmium, and arsenic. Frontiers in Pharmacology. 2021;12:643972.
9. Bala R, Kaur R, Kaur B, Kaur P. Hibiscus rosa-sinensis Linn.: A phytochemical and pharmacological review. International Journal

of Health Sciences (Qassim). 2022;6(S4):5165–93.
10. Preciado-Saldaña AM, Abraham Domínguez-Avila J, Fernando Ayala-Zavala J, et al. Formulation and characterization of an optimized functional beverage from hibiscus (Hibiscus sabdariffa L.) and green tea (Camellia sinensis L.). Food Science and Technology International. 2019;25(6):547–61.
11. Gillessen A, Schmidt HH-J. Silymarin as supportive treatment in liver diseases: a narrative review. Advances in Therapy. 2020;37(4):1279–301.
12. Nešić I, Krstić N, Djelić N, et al. Liver anatomy, intrahepatic vascular and biliary branching system of the mole rat (Spalax leucodon). Folia Morphologica. 2021;80(4):888–94.
13. Kalpana VNS, Mary J, Mini S, Soumya NPP, Mondal S. Hibiscus rosa-sinensis L. anthocyanins prevent lipid peroxidation and improve antioxidant status in the liver of streptozotocin-induced diabetic rats. Bioactive Compounds in Health and Disease. 2021;4(11):240–55.
14. Vargas-Mendoza N, Madrigal-Santillán E, Morales-González Á, et al. Hepatoprotective effect of silymarin. World Journal of Hepatology. 2014;6(3):144–9.
15. Renu K, Chakraborty R, Myakala H, et al. Molecular mechanism of heavy metals (Lead, Chromium, Arsenic, Mercury, Nickel and Cadmium)-induced hepatotoxicity–A review. Chemosphere. 2021;271:129735.
16. Azab AE-S. Hepatoprotective effect of sesame oil against lead induced liver damage in albino mice: Histological and biochemical studies. American Journal of Bioscience. 2014;9(1):1–11.
17. Javed S, Kohli K, Ali M. Reassessing bioavailability of silymarin. Alternative Medicine Review. 2011;16(3):239–49.
18. Jana TK, Das S, Ray A, Mandal D, Giri Jana S, Bhattacharya J. Study of the effects of hibiscus rosa-sinensis flower extract on the spermatogenesis of male albino rats. Journal of Physiology and Pharmacology Advances. 2013;3(6):167–71.
19. Institute of Laboratory Animal Resources (US), Committee on Care and Use of Laboratory Animals, National Research Council (US). Guide for the care and use of laboratory animals. Washington (DC): US Department of Health and Human Services, Public Health Service, National Institutes of Health; 1986.
20. Rodriguez-Palacios A, Khoretonenko MV, Ilic S. Institutional protocols for the oral administration (gavage) of chemicals and microscopic microbial communities to mice: Analytical consensus. Experimental Biology and Medicine. 2019;244(5):459–70.
21. Mitra S, Chakraborty AJ, Tareq AM, et al. Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. Journal of King Saud University - Science. 2022;34(1):101865.
22. Zaki LH, Mohamed SM, Bashandy SAE, Morsy FA, Tawfik KM, Shahat AA. Hypoglycemic and antioxidant effects of Hibiscus rosa-sinensis L. leaves extract on liver and kidney damage in streptozotocin induced diabetic rats. African Journal of Pharmacy and Pharmacology. 2017;11(7):161–9.
23. Mirzaei E, Sabetian G, Masjedi M, et al. The effect of silymarin on liver enzymes and antioxidant status in trauma patients in the intensive care unit: a randomized double blinded placebo-controlled clinical trial. Clinical and Experimental Hepatology. 2021;7(2):149–55.
24. Vacchi-Suzzi C, Viens L, Harrington JM, Levine K, Karimi R, Meliker JR. Low levels of lead and glutathione markers of redox status in human blood. Environmental Geochemistry and Health. 2018;40(3):1175–85.
25. Raza B, Javed M, Ambreen F, Latif F. Toxic effect of lead chloride on antioxidant enzyme in the liver and kidney of fish. Journal of Bioresource Management. 2016;3(2):1–5.
26. Jalali SM, Najafzadeh H, Bahmei S. Protective role of silymarin and D-penicillamine against lead-induced liver toxicity and oxidative stress. Toxicology and Industrial Health. 2017;33(6):512–8.
27. Hasan Khudhair D, Al-Gareeb AI, Al-Kuraishy HM, et al. Combination of vitamin C and curcumin safeguards against methotrexate-induced acute liver injury in mice by synergistic antioxidant effects. Frontiers in Medicine. 2022;9:866343.
28. Mukhtar S, Xiaoxiong Z, Qamer S, et al. Hepatoprotective activity of silymarin encapsulation against hepatic damage in albino rats. Saudi Journal of Biological Sciences. 2021;28(2):717–23.
29. Doğan D, Meydan I, Kömüroğlu AU. Protective effect of silymarin and gallic acid against cisplatin-induced nephrotoxicity and hepatotoxicity. International Journal of Clinical Practice. 2022;2022:6541026.
30. Sattar Ali Z. Hepatic impact of different concentrations of hibiscus rosa zinc oxide nanoparticles on rats. Archives of Razi Institute. 2022;77(4):1199–206.