PHARMACODYNAMIC EVALUATION OF DIETARY SUPPLEMENTATION OF *AVERRHOA CARAMBOLA* FRUIT EXTRACT AGAINST LEAD ACETATE-INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Lead is a toxicant that may induce a wide range of social, biochemical, and physiological changes in humans. This study is undertaken to evaluate the pharmacodynamic effects of dietary supplementation of *Averrhoa carambola,* fruit extract (ACF) against lead acetate-induced hepatotoxicity in rats. Six groups of rats were used in this study namely, control, lead acetate (20 mg kg^{-1}, ip), lead acetate (20 mg kg^{-1}, ip) + 200 mg kg^{-1} silymarin orally (reference drug), lead acetate + 100 mg kg^{-1} ACF orally, lead acetate + 150 mg kg^{-1} ACF orally and lead acetate + 200 mg kg^{-1} ACF extract orally. All experimental groups except the control received the lead acetate by intraperitoneal route for 5 days and normal saline or silymarin or ACF by oral route employing an orogastric cannula for seven days. Lead intoxication leads to a significant increase in ALT and AST activities, malondialdehyde (MDA), and a significant decline in liver homogenate, reduced glutathione (GSH) level, and superoxide dismutase (SOD) activity. Different doses of ACF supplement, as well as silymarin, led to improving biochemical parameters of serum and liver and prohibited the lead acetate-induced significant changes in plasma and antioxidant status of the liver. ACF or silymarin supplement exhibited more antioxidant activity. Conclusively, the present work results reveals that the treatment of lead-intoxicated rats with *A. carambola* fruit extract supplement revealed a significant increase in GSH level, CAT, SOD activity, and a decrease in TBARS levels as compared to lead-intoxicated rats, indicating its antioxidant activity.

Keywords: *Averrhoa carambola*, Lead acetate induced toxicity, Pharmacodynamics, Oxalidaceae, Kamrakh

INTRODUCTION

Lead, generally valuable for industrial production due to its specific physical features, is a dangerous environmental toxicant[1]. Lead is a harmful environmental toxicant in water, soil and the atmosphere. Raised lead concentrations in sediment and farmed fish may be profoundly dangerous to human health[2]. Lead is a naturally occurring bluish-grey metal found in small quantities in the soil's coating and can be found in all parts of our environment, such as food, water, air, and soil[3]. Low levels of lead have been related to behavioral abnormalities, decreased hearing, learning impairment, neuromuscular weakness, and reduced cerebral functions in humans and experimental animals[4]. High levels of lead cause injury to almost all organs and, most importantly, the central nervous system, kidneys, liver, and blood[5]. Problems associated with the liver are one of the primary causes of death worldwide. Liver injury is permanently related to cellular necrosis, increased tissue lipid peroxidation, and depletion in the tissue glutathione levels. In addition, serum levels of many biochemical markers like aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total bilirubin and total protein are elevated[6]. Hepatic cells are elaborate in a change of metabolic events, so establishing liver-protective agents is vital in the defense from liver damage[7].

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Extracts from traditional plants are realized as effective and harmless alternative treatments for hepatotoxicity[8], producing hepatoprotection against hepatotoxin-induced liver damage[9, 10]. *A. carambola* L. (family: Oxalidaceae), known as star-fruit or kamrakh, is commonly found in the world, mainly in tropical countries such as India, Philippines, Indonesia, and Malaysia[11]. *A. carambola* is widely cultivated on a commercial scale[12]. Kamrakh fruits are crispy, spicy, slightly bitter, acidic and sweet. This fruit is recognized to have antioxidant properties and is utilized in the preparation of pickles and salad[13]. Different parts of the plant of *A. carambola* are used in Indian traditional medicine for the treatment of many diseases such as inflammation, hypotensive, hepatotoxicity, fevers, mouth ulcer, toothache, cough, asthma, hiccups, indigestion, food poisoning, colic, diarrhoea, jaundice, malarial splenomegaly, hemorrhoids, skin rashes, pruritus, sunstroke and some eye-related problems and elimination of intestinal worms[14, 15].

Therefore, the present study evaluated the pharmacodynamics of dietary supplementation of *A. carambola* fruit extract against Lead acetate-induced hepatotoxicity in rats (Fig. 1).

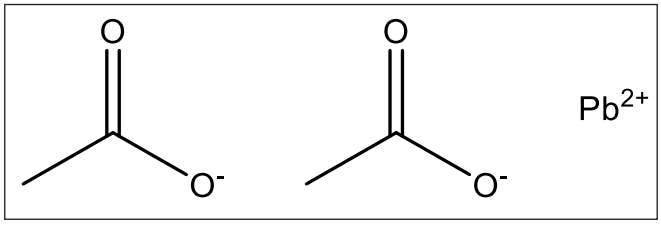

Fig. 1: Lead acetate

MATERIALS AND METHODS

Chemicals and instrumentation

All the chemicals used were of analytical grade: silymarin (Micro Labs Limited, Bangalore, India), LA (SD Fine Chemical Ltd., Mumbai, India), ALT test kits (AGD Biomedicals (P) Ltd., Maharashtra), AST test kits (AGD Biomedicals (P) Ltd., Maharashtra), albumin test kit (Span Diagnostics Ltd., Surat), bilirubin test kit (Span Diagnostics Ltd., Surat), alkaline phosphatase test kits (ARKRAY Healthcare Pvt. Ltd., Surat, Gujarat), total protein test kit (Span Diagnostics Ltd., Surat), bilirubin total test kit (Trivitron Healthcare Private Ltd., Chennai), mini centrifuge (Inspiration Biotech).

Collection and authentication of the plant specimen

A. carambola fruits were collected from Gailua, of Sambhal district of U.P. and authenticated by Dr. Ashok Kumar, Head, Department of Botany, IFTM University, Moradabad (U.P.), India (Ref. no.: 2017/SOS/BOT/44). A voucher specimen was deposited to the authentication office for future reference.

Preparation of plant extract

The fresh fruits were subjected to size reduction to small pieces with the help of a stainless-steel knife, and ground with a grinder juice was collected. The extract was concentrated through a rotary vacuum evaporator at 45 °C for 10 mins (Yamato Scientific Co, Japan). It was kept in an air-tight container and stored in a desiccator for further pharmacological investigation[16].

Preliminary phytochemical screening

The preliminary qualitative phytochemical screening of the extract of *A. carambola* fruit was conducted by standard chemical tests for the presence and/or absence of alkaloids, glycosides, flavonoids tannins, and phenolic compounds, anthraquinones, saponins, volatile oils, cyanogenic glycosides, carbohydrates, steroids, amino acids, proteins and acidic compounds[17, 18].

Experimental animals

Wistar albino rats (160-200 g) were procured from Animal House, IFTM University Moradabad and kept in the departmental animal house, IFTM University, Moradabad. All the rats were housed separately in polypropylene cages at 50-60% relative humidity and temperature of 23 ± 2 °C with a 12 h light/dark cycle for seven days before and during the commencement implementation of the experiment. The rats were kept on a standard pellet diet and drinking water throughout the housing period. All the experiments were conducted in conformity with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) New Delhi[19]. Ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC), IFTM University, Moradabad (Reg. No.: 837/PO/Re/S//04/CPCSEA).

Experimental protocol

The animals were randomly divided into six groups of six rats (N=6). Group I (normal control) animals were treated with normal saline (1 mL kg^{-1} day^{-1}) for 7 days. Group II (experimental control) animals were treated with lead acetate (LA) (20 mg kg^{-1} day^{-1} ip) for 5 days. Group III (positive control) received silymarin (200 mg kg^{-1} day^{-1} po) and simultaneous administration of LA (20 mg kg^{-1} day^{-1} ip.) for 5 days. Group IV (treated group) received ACF (*A. carambola* fruit extract) (100mg kg^{-1} day^{-1} po)

along with LA (20 mg kg^{-1} day^{-1} ip) for 5 days. Group V (treated group) received ACF (150 mg kg^{-1} day^{-1} po) along with LA (20 mg kg^{-1} day^{-1} ip) for 5 days. Group VI (treated group) received ACF (200 mg kg^{-1} day^{-1} po) along with LA (20 mg kg^{-1} day^{-1} ip) for 5 days. On six- and seven days, normal dosing without LA was carried out. At the end of the experimental period (after 7 days), the animals were fasted overnight and sacrificed by decapitation. Blood samples were collected for haematological and biochemical analysis from retro-orbital in two tubes, i.e. one with anticoagulant ethylenediaminetetraacetic acid (EDTA) for plasma and another without anticoagulant for serum separation. The serum was separated by centrifuging at 2500 rpm for 15 min, and analysis was done for various biochemical parameters. The isolated liver was fixed in (10%) formalin for histopathological examination[20].

Blood parameters

Hematological parameters, such as HB, WBC, RBC, HCT, MCH, MCV, MCHC, and platelet count, were assessed by using semi auto-analyzer.

Biochemical analysis

Aspartate aminotransferase (AST), serum alanine transaminase (ALT), alkaline phosphatase (ALP), total bilirubin, (TB) and total protein (TP) were estimated by using standard kits from Span Diagnostic Ltd, India.

Assessment of kidney function test

Albumin, creatinine, uric acid and blood urea were estimated by using standard kits.

Measurement of hepatic antioxidants/markers of lipid peroxidation

Liver tissues from the experimental animals were perfused with ice-cold saline and transported from the laboratory to assay hepatic antioxidant enzymes. Liver pieces (100 mg) were diced and homogenized in 100 mL of 5 HCl buffer (pH 7.4), 1 mM EDTA and a complete mini protease inhibitor cocktail (Roche). The homogenates were centrifuged at 10,000 rpm for 10 min at 4 °C and the clear supernatant was used for the estimation of antioxidant parameters: glutathione (GSH)[21], catalase (CAT)[22], superoxide dismutase (SOD)[22], and thiobarbituric reacting substances (TBARS)[23].

Histopathological examination

Slices of the liver were stored in a 10 % neutral formalin solution to preserve them. The tissues were mounted by embedding in paraffin wax in the laboratory, and sections of the size of 6 mm were cut. The sections were stained with eosin and haemotoxylin dyes. The slides were observed under a light microscope and photomicrographs were captured by using the camera. These were observed for fibrosis, fatty infiltration, and centrilobular necrosis and lymphocyte infiltration.

Statistical analysis

The results are expressed as mean±SEM and analyzed using one-way variance analysis followed by Dunnett's test using GraphPad Prism 7 (Graph-Pad Software Inc., San Diego, California, USA). The value of $P<0.05$ was considered statistically significant[24].

RESULTS

Preliminary phytochemical screening

The qualitative phytochemical result shows the presence of carbohydrates, proteins, glycosides, phenolics, tannins, alkaloids and flavonoids (Table I).

Table I: Preliminary phytochemical studies of ACF

Sr. No.	Phytoconstituent	Present or Absent
1	Carbohydrates	+
2	Proteins	+
3	Alkaloids	+
4	Glycosides	-
5	Amino acids	+
6	Steroids	-
7	Flavonoids	+
8	Acidic compounds	+
9	Tannins and phenolic compounds	+
10	Fixed oils and fats	-

+= Present; - = Absent

Acute toxicity

The ACF was found to be safe up to 2000 mg kg^{-1} with no signs of mortality or change in behavioral pattern. This result suggested that plant extract was not toxic and safe. Based on this result, it was optimized that dose levels 100, 150, and 200 mg kg^{-1} of *A. carambola* extract were selected for determining the pharmacodynamic effect on experimental induced hepatic injury (Table II).

Table II: Evaluation of acute oral toxicity test of ACF in rats

Sr. No.	Parameter	Observations (at)		
		24 h	72 h	14th day
1	Weight Loss	Nil	Nil	Nil
2	Death rate	Nil	Nil	Nil
3	CNS toxicity	Nil	Nil	Nil
4	Neurological disorder	Nil	Nil	Nil

Bodyweight

The changes in the total body weight of the experimental treated group of rats showed a significant reduction compared with the initial body weight (Table III). Combined treatments of LA along with ACF or LA along with silymarin induced significant differences in the total body weight.

Table III: Effects of *A. carambola* fruit extract on body weight of different group of rats

Treatment	Body weight of rats (g)			
	Initial	Final	Difference	% Change
Group I	184.47± 3.84	186.97± 3.97	2.50± 0.27	1.35± 0.15
Group II	183.83± 3.94	182.00± 4.14	1.83± 0.31[a]	0.99± 0.19
Group III	185.00± 4.32	187.00± 4.59	2.00± 0.37	1.05± 0.18
Group IV	183.33± 3.01	184.33± 3.06	1.00± 0.37	0.54± 0.20
Group V	184.17± 3.53	185.50± 3.74	1.33± 0.54	0.71± 0.29
Group VI	184.83± 3.36	186.67± 3.49	1.83± 0.28	0.98± 0.14

Values represent mean ± SEM, n = 6. Statistical analysis was performed using one-way ANOVA followed by Dunnet's test. a =P<0.05 against normal control rats, Group I received 1 mL kg^{-1} BW normal saline; Group II LA 20 mg kg^{-1} ip BW; Group III received Silymarin 200 mg kg^{-1} po BW and LA; Group IV received ACF 100 mg kg^{-1} po BW and LA; Group V received ACF 150 mg kg^{-1} po BW and LA; Group VI received ACF 200 mg kg^{-1} po BW and LA.

Blood parameters

The data in Table IV showed that LA treatment induced a significant (P<0.05) decrease of the RBCs count, HB content and HCT values compared to the control. Treatment with ACF in combination with LA-induced non-significant differences of the tested blood parameters against normal control and a significant increase with experimental control. Similar results were recorded for rats treated with silymarin along with LA. The calculated blood indices, MCH and MCHC, were non-significantly decreased in all treated groups, compared with the normal control group, whereas a significant reduction was recorded for an LA-treated group of rats. The erythrocytes MCV of LA-treated rats were significantly (P<0.05) reduced, whereas those treated with ACF or silymarin showed comparable to the normal control group and were statistically significant. The total WBCs count was non-significantly amplified in all the experimental groups as compared to the normal control group.

Biochemical parameters

LA treatment induced a significant increase in the level of AST, ALT, and ALP (Fig. 2). Also, the total bilirubin was significantly (P<0.05) raised. The concomitant administration of LA and ACF induced significant (P<0.05-P<0.001) changes of the levels of AST, ALT, ALP and total bilirubin against the experimental control group of rats. A similar result was recorded for rats with the concomitant administration of LA and silymarin.

Measurement of hepatic antioxidants/markers of lipid peroxidation

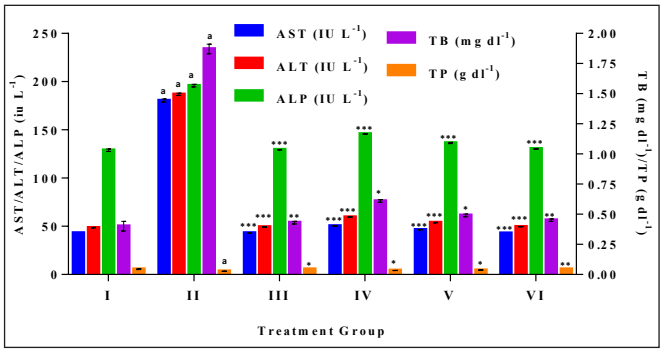

Fig. 2: Effect of *A. carambola* a fruits extracts on various biochemical parameters in LA-induced hepatotoxicity in rats

*The vertical bar represents mean ± SEM, n = 6. Statistical analysis was performed using one-way ANOVA followed by Dunnet's test. a =P<0.05 against normal control rats, *=P<0.05, **=P<0.01 and ***=P<0.001 against group II (Experimental control rats). Abbreviations: AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; TB: total bilirubin; TP: total protein. Group I received 1 mL kg^{-1} ip BW normal saline; Group II LA 20 mg kg^{-1} ip BW; Group III received silymarin 200 mg kg^{-1} po BW and LA; Group IV received ACF 100 mg kg^{-1} po BW and LA; Group V received ACF 150 mg kg^{-1} po BW and LA; Group VI received ACF 200 mg kg^{-1} po BW and LA.*

Table IV: Effect of *A. carambola* fruits extracts on various hematological parameters in LA induced hepatotoxicity in rats

Parameter	Group I	Group II	Group III	Group IV	Group V	Group VI
HB (g dL^{-1})	14.60±0.11	11.79±0.04[a]	14.49±0.04*	13.52±0.09*	14.01±0.08*	14.48±0.28*
WBC (10^3 μL^{-1})	4.75±0.05	3.16±0.02[a]	4.75±0.04*	4.28±0.04*	4.43±0.05*	4.70±0.05*
RBC (10^6 μL^{-1})	9.27±0.24	8.40±0.08[a]	9.22±0.07*	8.68±0.07*	8.91±0.05*	9.20±0.02*
HCT (%)	43.83±0.04	36.13±0.13[a]	43.74±0.05**	40.45±0.06*	41.96±0.06*	43.38±0.12**
MCH (pg)	15.81±0.37	14.04±0.10[a]	15.73±0.13*	15.58±0.21*	15.73±0.10*	15.74±0.31*
MCV (%)	47.48±1.32	43.03±0.39[a]	47.47±0.34*	46.60±0.36*	47.12±0.25	47.16±0.12*
MCHC (g dL^{-1})	33.31±0.25	32.64±0.21[a]	33.13±0.09	33.42±0.26*	33.38±0.16	33.37±0.64*
PLT ($(10^3$ μL^{-1})	952.83±2.93	327.50±3.82[a]	944.17±5.83***	929.17±4.36***	922.00±2.08***	940.83±2.39***

*Values represent mean ± SEM, n = 6. Statistical analysis was performed using one-way ANOVA followed by Dunnet's test. [a]=P<0.05 against normal control rats, *=P<0.05, **=P<0.01 and ***=P<0.001 against group II (experimental control rats). Abbreviations: HB: haemoglobin, WBC: white blood cells, RBC: red blood cells, HCT: Hematocrit, MCH: mean corpuscular haemoglobin, MCV: mean corpuscular volume, MCHC: mean corpuscular haemoglobin concentration, PLT: platelets count. Group I received 1 mL kg^{-1} ip BW normal saline (normal control); Group II LA 20 mg kg^{-1} ip BW (experimental control); Group III received silymarin 200 mg kg^{-1} po BW (positive control) and LA; Group IV received ACF 100 mg kg^{-1} po BW and LA; Group V received ACF 150 mg kg^{-1} po BW and LA; Group VI received ACF 200 mg kg^{-1} po BW and LA.*

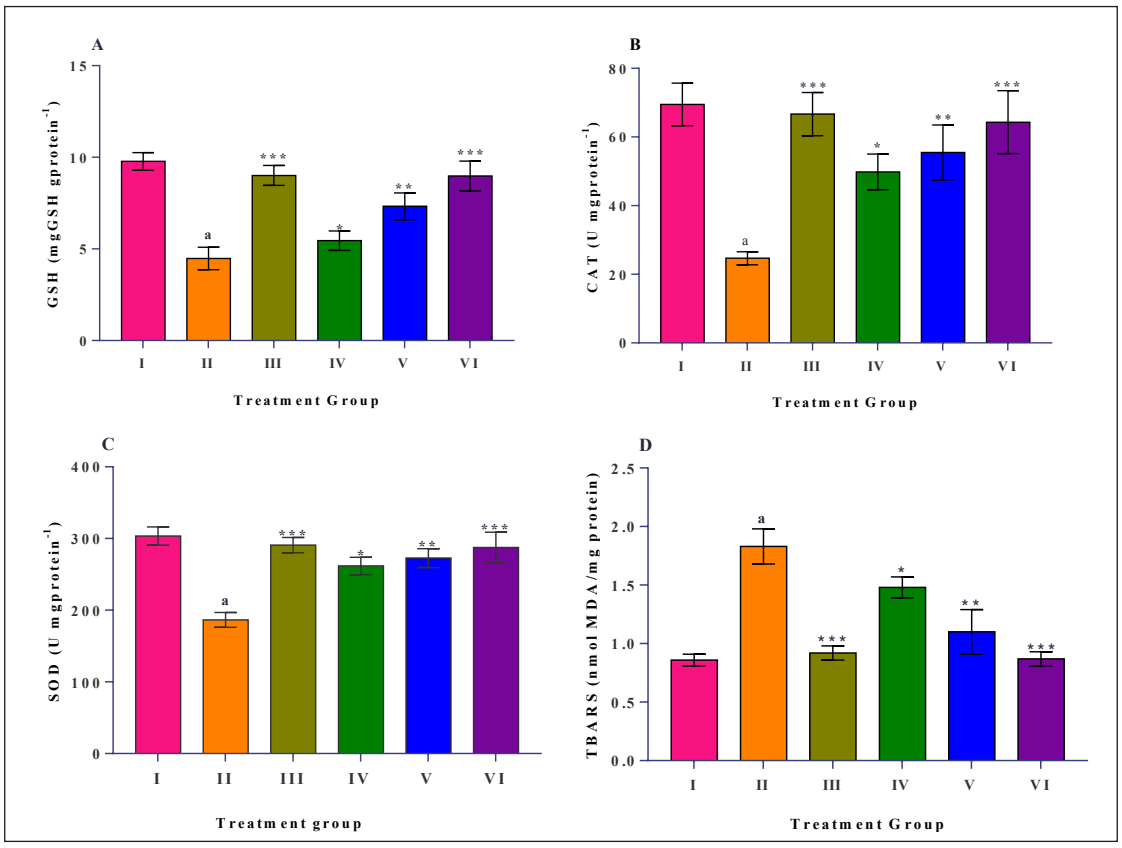

Fig. 3: Effect of ACF and silymarin during lead acetate induced hepatotoxicity in rats

*A: GSH Level B: CAT level; C: SOD Level and D: TBARS level. The vertical bar represents mean ± SEM, n = 6. Statistical analysis was performed using one-way ANOVA followed by Dunnet's test. a =P<0.05 against normal control rats, *=P<0.05, **=P<0.01 and ***=P<0.001 against group II (experimental control rats). Group A received 10 mL kg^{-1} po BW normal saline; Group B received Group II LA 20 mg kg^{-1} ip BW; Group C received silymarin 50 mg kg^{-1} po BW and LA; Group D received ACF 100 mg kg^{-1} po BW and LA; Group E received ACF 150 mg kg^{-1} po BW and LA; Group F received ACF 200 mg kg^{-1} po BW and LA.*

The data reported in Fig. 3 showed that LA treatment produced a significant decrease in glutathione (GSH); catalase (CAT) and superoxide dismutase (SOD) values, whereas TBARS value increases for the experimental control group of rats when compared to the normal control group of rats. Concomitantly administering ACF in combination with LA replenishes the GSH, CAT, and SOD significantly ($P<0.05$) against an experimental control group of rats. Similar results were found for rats concomitantly administration of silymarin along with LA. The values of glutathione (GSH); catalase (CAT) and superoxide dismutase (SOD), were significantly decreased in all treated groups of ACF, against the experimental control group of rats.

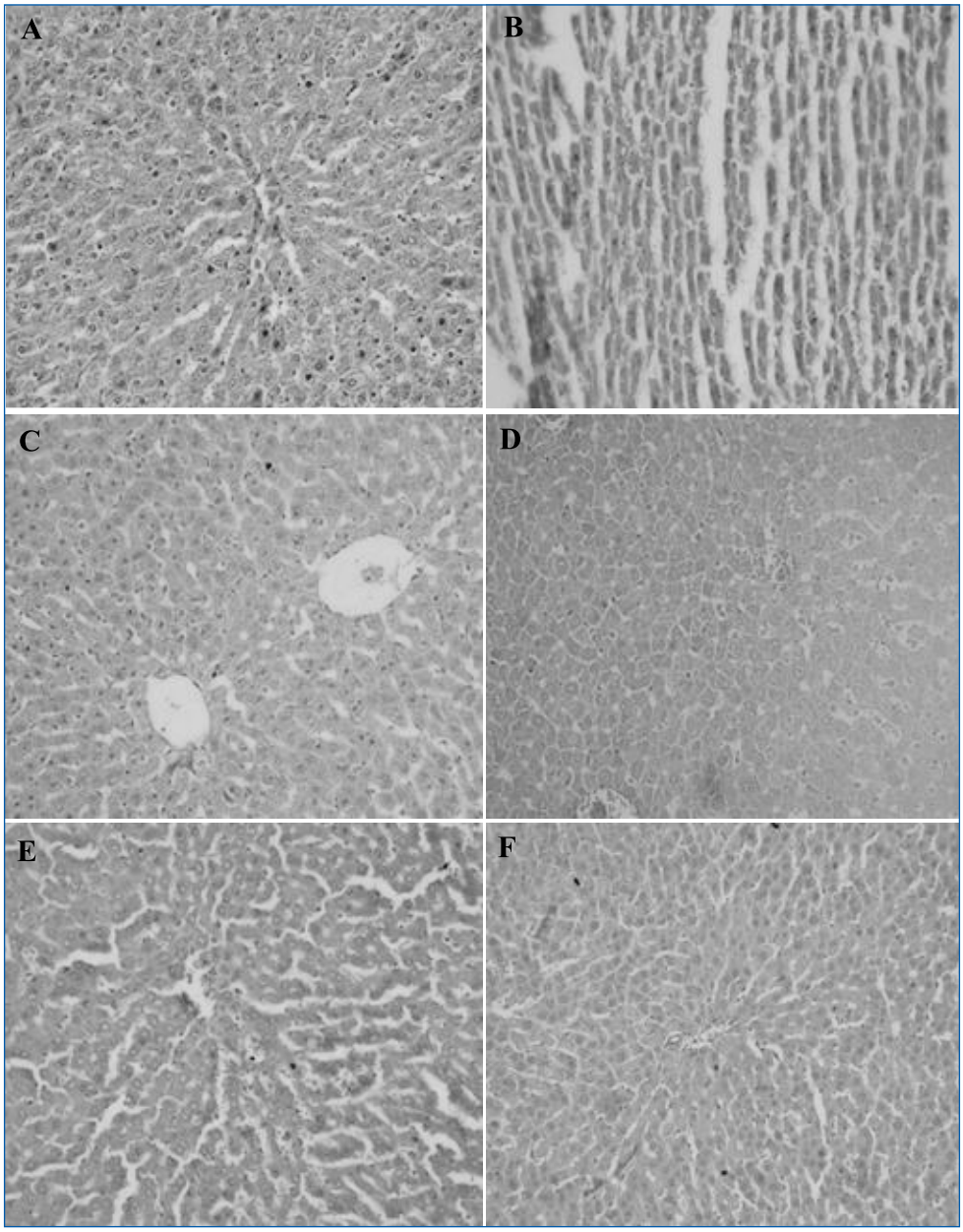

Fig. 4: Representative of liver sections for histopathological evaluations (100X)

Histopathological liver section A: Group I received 1 mL kg^{-1} ip BW normal saline (normal control); B: Group II LA 20 mg kg^{-1} ip BW (experimental control); C: Group III received silymarin 200 mg kg^{-1} po BW (positive control) and LA; D: Group IV received ACF 100 mg kg^{-1} po BW and LA; E: Group V received ACF 150 mg kg^{-1} po BW and LA; F: Group VI received ACF 200 mg kg^{-1} po BW and LA

Histopathology

Healthy control rats showed no histopathological changes in the liver in Fig. 4 (A), the treatment with lead acetate (LA) induced hepatic necrosis, hepatocellular damage and infiltration of inflammatory cells in Fig. 4 (B). The liver histopathology of rats treated with silymarin along with LA or ACF along with LA showed few degenerated cells in Fig. 4 (C and D). By examining the histological activity index (HAI) of the liver, it was found that rats treated with LA showed moderate pathological changes (necrosis and cell degeneration) and mild inflammation. The combined treatment of LA and ACF showed few degenerated cells, whereas, silymarin, along with LA mixed treatment, showed insignificant central necrosis, hepatocyte degeneration and inflammation, respectively, which is significantly different from the control group. Thus, the mutual treatment of ACF and LA significantly reduces the histopathological effects as represented in Fig. 4 (E and F). Histological observations are presented in Table V.

Table V: Effect of *A. carambola* fruits extracts on histopathology

Treatment	Vascular changes	Necrosis	Infiltration	Dilation of sinusoids
Group I	-	-	-	-
Group II	+++	+++	+++	+++
Group III	+	++	+	+
Group IV	++	+++	+	+
Group V	++	+++	+	+
Group VI	+	++	+	+

- Nil; + Mild; ++ Moderate; +++ Severe

DISCUSSION

Lead is widely used in different industries such as paint, ceramic, plastics, etc. Lead toxicity is considered a significant health problem as it induces deleterious effects on various body organs, including the liver[1]. The present study showed that ingestion of lead acetate for 5 days resulted in severe hepatotoxicity in the dose 20 mg kg^{-1}; ip. A significant increase in serum AST, ALT and ALP with lead acetate treatment confirmed the severe hepatic damage. Liver enzymes such as AST, ALT, and ALP are marker enzymes for liver function and hepatic integrity. These enzymes are the markers for hepatotoxicity; damage to the hepatocyte membrane will cause the release of many of these enzymes into circulation. Releasing of ALT and AST from the cell cytosol can occur as secondary changes to cellular necrosis[25]. The high AST and ALT activities are accompanied by high liver microsomal membrane fluidity, free radical generation, and alteration in the liver tissue[26]. A raised level of ALP suggests biliary destruction or obstruction of the biliary tree, which interrupts blood flow to the liver[27]. The dose-dependent decrease in serum levels of these enzymes may be due to the prevention of their leakage from the liver cytosol by supplementing *A. carambola* fruit extracts, probably due to a reduction in blood lead level. Our histological evaluation further held that disturbances in the hepatic structural integrity prompted by lead acetate, where severe hepatocyte damage, vascular congestion, and necrosis were perceived after 5 days of lead ingestion. The observed increase in ALP, AST, and ALT confirms the earlier observations[28, 29]. Similar changes were also reported earlier[30, 31].

Administration of rats with lead acetate in this study caused a significant ($P<0.05$) reduction in PCV, Hb concentration, RBC, and WBC count. Our results were in agreement with those of other scientists around the globe[26, 32]. Lead directly affects the hematopoietic system by restraining the synthesis of Hb by inhibiting various vital enzymes in the heme synthesis pathway. It also reduces the lifetime of circulating erythrocytes by increasing the cell membrane's fragility. The outcome of these two processes leads to anemia[33, 34]. Supplementation of *A. carambola* fruit extracts reversed these outcomes significantly in a dose-dependent manner.

The increase of bilirubin values in rats treated with lead acetate in this study may be due to excessive hem destruction and blockage of the biliary tract resulting in inhibition of the conjugation reaction and release of unconjugated bilirubin from damaged hepatocytes[35]. Bilirubin is protective against oxidative damage of cell membranes induced by metal[36]. In our experiment, *A. carambola* fruit extracts showed a significant decrease in bilirubin level, thereby augmenting the repair of damage done by the lead acetate.

Lead toxicity leads to free radical damage either by the generation of reactive oxygen species (ROS), including hydroperoxides, singlet oxygen, and hydrogen peroxides, evaluated by TBARS, which measures MDA levels as end products of lipid peroxidation, or the direct exhaustion of antioxidant reserves[37]. During the evaluation, there was a significant increase in MDA levels and a significant decrease in GSH levels in the lead acetate-ingested group of rats. Our results are similar to another previous research[38, 39]. Also, there was a significant decrease in SOD levels in lead acetate-intoxicated rats. These results were in agreement with another scientist[40]. The

increased MDA level registered in the study was also due to diminished SOD activity, a pointer to oxidative stress. The probable description could be related to the proposed role of GSH in the active elimination of lead through bile by attaching to the thiol group of GSH and then excreting. A reduction in GSH levels could affect oxidative stress and increase MDA[41]. One of the therapeutics for liver diseases, protective drugs such as antioxidants have fascinated more and more attention, and proton radical-scavenging action is well known as a distinctive mechanism of antioxidation. In our study, the treatment of lead-intoxicated rats with *A. carambola* fruits extracts supplement reveals a significant increase in GSH level, CAT level, SOD activity and decrease in TBARS levels as related to lead acetate induced toxicity in rats indicating its antioxidant activity.

CONCLUSION

This study recognized the regulatory feature of metabolism by *A. carambola* fruits extracts in the liver with lead acetate poisoning and suggested that *A. carambola* fruits extract ameliorated metabolic dysfunction impaired by lead acetate, at least in part, through an integrated mechanism associated with oxidative stress. Dietary supplements of *A. carambola* fruit extracts could supply a novel method with a common side effect to maintain metabolic homeostasis and protect human health against lead exposure.

REFERENCES

1. Bojdi M. K., Mashhadizadeh M. H., Behbahani M., Farahani A., Davarani S. S. H. and Bagheri A.: Synthesis, characterization and application of novel lead imprinted polymer nanoparticles as a high selective electrochemical sensor for ultra-trace determination of lead ions in complex matrixes, **Electrochim. Acta,** 2014, 136, 59-65.
2. Qiu Y.-W., Lin D., Liu J.-Q. and Zeng E. Y.: Bioaccumulation of trace metals in farmed fish from South China and potential risk assessment, **Ecotoxicol. Environ. Saf.,** 2011, 74(3), 284-293.
3. Aldahmash B. A. and El-Nagar D. M.: Antioxidant effects of captopril against lead acetate-induced hepatic and splenic tissue toxicity in Swiss albino mice, **Saudi J. Biol. Sci.,** 2016, 23(6), 667-673.
4. Verina T., Rohde C. A. and Guilarte T. R.: Environmental lead exposure during early life alters granule cell neurogenesis and morphology in the hippocampus of young adult rats, **Neurosci.,** 2007, 145(3), 1037-1047.
5. Abdel-Moneim A. E., Dkhil M. A. and Al-Quraishy S.: The redox status in rats treated with flaxseed oil and lead-induced hepatotoxicity, **Biol. Trace Elem. Res.,** 2011, 143(1), 457-467.
6. Ramachandra Setty S., Quereshi A. A., Viswanath Swamy A. H. M., Patil T., Prakash T., Prabhu K. and Veeran Gouda A.: Hepatoprotective activity of *Calotropis procera* flowers against paracetamol-induced hepatic injury in rats, **Fitoterapia,** 2007, 78(7), 451-454.
7. Huang B., Ban X., He J., Tong J., Tian J. and Wang Y.: Hepatoprotective and antioxidant activity of ethanolic extracts of edible lotus (*Nelumbo nucifera* Gaertn.) leaves, **Food Chem.,** 2010, 120(3), 873-878.
8. Sabir S. M. and Rocha J. B. T.: Water-extractable phytochemicals from Phyllanthus niruri exhibit distinct *in vitro* antioxidant and *in vivo* hepatoprotective activity against paracetamol-induced liver damage in mice, **Food Chem.,** 2008, 111(4), 845-851.
9. Yousef M. I., Omar S. A., El-Guendi M. I. and Abdelmegid L. A.: Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat, **Food Chem. Toxicol.,** 2010, 48(11), 3246-3261.
10. Choi J. H., Choi C. Y., Lee K. J., Hwang Y. P., Chung Y. C. and Jeong H. G.: Hepatoprotective effects of an anthocyanin fraction from purple-fleshed sweet potato against acetaminophen-induced liver damage in mice, **J. Med. Food,** 2009, 12(2), 320-326.
11. Kadir M. F., Bin Sayeed M. S. and Mia M. M. K.: Ethnopharmacological survey of medicinal plants used by traditional healers in Bangladesh for gastrointestinal disorders, **J. Ethnopharmacol.,** 2013, 147(1), 148-156.
12. Khoo H. E., Azlan A., Kong K. W. and Ismail A.: Phytochemicals and Medicinal Properties of Indigenous Tropical Fruits with Potential for Commercial Development, **Evid.-based Complement. Altern. Med.,** 2016, 2016, 7591951.
13. Chang C. T., Chen Y. C., Fang J. T. and Huang C. C.: Star fruit (*Averrhoa carambola*) intoxication: an important cause of consciousness disturbance in patients with renal failure, **Ren. Fail.,** 2002, 24(3), 379-382.
14. Cabrini D. A., Moresco H. H., Imazu P., da Silva C. D., Pietrovski E. F., Mendes D. A., da Silveira Prudente A., Pizzolatti M. G., Brighente I. M. and Otuki M. F.: Analysis of the Potential Topical Anti-Inflammatory Activity of *Averrhoa carambola* L. in Mice, **Evid.-based Complement. Altern. Med.,** 2011, 2011, 908059.
15. Sheth A., Mitaliya K. D. and Joshi S., The herbs of ayurveda. Gujarat: Ashok Sheth; 2005.
16. Azeem A. K., Mathew M., C D. and Nair C.: Hepatoprotective effect of *Averrhoea carambola* fruit extract on carbon tetrachloride induced hepatotoxicity in mice, **Asian Pac. J. Trop. Med.,** 2010, 3(8), 610-613.
17. Sachan N., Patel A. V. and Chandra P., Antioxidant and Antidiabetic Potential of *Curcuma zedoaria* Rosc *In vitro*, *In vivo* Antioxidant and Antidiabetic Activity of *Curcuma zedoaria* Rosc. Ist ed: Lap Lambert Academy Publishing, Heinrich-Bocking-Str., Saarbraeucken, Germany; 2016.
18. Mukherjee P. K., Chapter 7 - Bioactive Phytocomponents and Their Analysis. In: Mukherjee P. K., ed. Quality Control and Evaluation of Herbal Drugs. Elsevier; 2019, 237-328.

19. CPCSEA. Compendium of CPCSEA 2018. Committee for the Purpose of Control and Supervision of Experiments on Animals. In: Ministery of Environment, Forest and Climate Change, Government of India; 2018, 1-213.
20. Abdel Moneim A. E.: Flaxseed oil as a neuroprotective agent on lead acetate-induced monoamineric alterations and neurotoxicity in rats, **Biol. Trace Elem. Res.,** 2012, 148(3), 363-370.
21. Ellman G. L.: Tissue sulfhydryl groups, **Arch. Biochem. Biophys.,** 1959, 82(1), 70-77.
22. Kakkar P., Das B. and Viswanathan P. N.: A modified spectrophotometric assay of superoxide dismutase, **Indian J. Biochem. Biophys.,** 1984, 21(2), 130-132.
23. Draper H. H. and Hadley M.: Malondialdehyde determination as index of lipid peroxidation, **Methods Enzymol.,** 1990, 186, 421-431.
24. Agarwal V. K., Amresh G. and Chandra P.: Pharmacodynamic evaluation of self micro-emulsifying formulation of standardized extract of L *Agerstroemia speciosa* for antidiabetic activity, **J. Ayurveda Integr. Med.,** 2018, 9(1), 38-44.
25. Gaskill C. L., Miller L. M., Mattoon J. S., Hoffmann W. E., Burton S. A., Gelens H. C., Ihle S. L., Miller J. B., Shaw D. H. and Cribb A. E.: Liver histopathology and liver and serum alanine aminotransferase and alkaline phosphatase activities in epileptic dogs receiving phenobarbital, **Vet. Pathol.,** 2005, 42(2), 147-160.
26. Ibrahim N. M., Eweis E. A., El-Beltagi H. S. and Abdel-Mobdy Y. E.: Effect of lead acetate toxicity on experimental male albino rat, **Asian Pac. J. Trop. Biomed.,** 2012, 2(1), 41-46.
27. Farida T., Salawu O. A., Tijani A. Y. and Ejiofor J. I.: Pharmacological evaluation of *Ipomoea asarifolia* (Desr.) against carbon tetrachloride-induced hepatotoxicity in rats, **J. Ethnopharmacol.,** 2012, 142(3), 642-646.
28. Sharma A., Sharma V. and Kansal L.: Therapeutic effects of *Allium sativum* on lead-induced biochemical changes in soft tissues of swiss albino mice, **Pharmacogn. Mag.,** 2009, 5(20), 364-371.
29. Sharma A., Sharma V. and Kansal L.: Amelioration of lead-induced hepatotoxicity by *Allium sativum* extracts in Swiss albino mice, **Libyan J. Med.,** 2010, 5(1), 4621.
30. Daggett D. A., Oberley T. D., Nelson S. A., Wright L. S., Kornguth S. E. and Siegel F. L.: Effects of lead on rat kidney and liver: GST expression and oxidative stress, **Toxicology.,** 1998, 128(3), 191-206.
31. Mudipalli A.: Lead hepatotoxicity & potential health effects, **Indian J. Med. Res.,** 2007, 126(6), 518-527.
32. Offor S. J., Mbagwu H. O. C. and Orisakwe O. E.: Lead Induced Hepato-renal Damage in Male Albino Rats and Effects of Activated Charcoal, **Front. Pharmacol.,** 2017, 8, 107.
33. Guidotti T. L., McNamara J. and Moses M. S.: The interpretation of trace element analysis in body fluids, **Indian J. Med. Res.,** 2008, 128(4), 524-532.
34. Flora G., Gupta D. and Tiwari A.: Toxicity of lead: A review with recent updates, **Interdiscip. Toxicol.,** 2012, 5(2), 47-58.
35. Ali Z. Y., Atia H. A. and Ibrahim N. H.: Possible hepatoprotective potential of *Cynara scolymus*, *Cupressus sempervirens* and *Eugenia jambolana* against paracetamol-induced liver injury: *In vitro* and *in vivo* evidence, **Nature Sci.,** 2012, 10(1), 75-86.
36. Noriega G. O., Tomaro M. L. and del Batlle A. M.: Bilirubin is highly effective in preventing *in vivo* delta-aminolevulinic acid-induced oxidative cell damage, **Biochim. Biophys. Acta,** 2003, 1638(2), 173-178.
37. El-Nekeety A. A., El-Kady A. A., Soliman M. S., Hassan N. S. and Abdel-Wahhab M. A.: Protective effect of *Aquilegia vulgaris* (L.) against lead acetate-induced oxidative stress in rats, **Food Chem. Toxicol.,** 2009, 47(9), 2209-2215.
38. Newairy A.-S. A. and Abdou H. M.: Protective role of flax lignans against lead acetate induced oxidative damage and hyperlipidemia in rats, **Food Chem. Toxicol.,** 2009, 47(4), 813-818.
39. Reglero M. M., Taggart M. A., Monsalve-González L. and Mateo R.: Heavy metal exposure in large game from a lead mining area: Effects on oxidative stress and fatty acid composition in liver, **Environmen. Pollut.,** 2009, 157(4), 1388-1395.
40. El-Tantawy W. H.: Antioxidant effects of Spirulina supplement against lead acetate-induced hepatic injury in rats, **J. Tradit. Complement. Med.,** 2016, 6(4), 327-331.
41. Manoj Kumar V., Henley A. K., Nelson C. J., Indumati O., Prabhakara Rao Y., Rajanna S. and Rajanna B.: Protective effect of *Allium sativum* (garlic) aqueous extract against lead-induced oxidative stress in the rat brain, liver, and kidney, **Environ. Sci. Pollut. Res.,** 2017, 24(2), 1544-1552.