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Effect of the ethanolic extract of *Alhagi maurorum* Boiss. on serum liver function and oxidative stress markers in mice exposed to thioacetamide

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Abstract
Background: The liver is highly susceptible to injury caused by toxicants, with oxidative contributing to liver injury and dysfunction in liver injury and dysfunction.
Objectives: To assess the hepatoprotective and antioxidant effects of ethanolic root extract of *Alhagi maurorum* Boiss. in Thioacetamide (TAA) induced changes in liver-function and oxidative stress markers in mice.
Methodology: Swiss albino mice (n=30) were divided into control (n=10), TAA (n=10), and TAA + extract (n=10) groups. The extract was given by oral administration at 250 mg/kg/day for 7 days and TAA was injected intraperitoneally on days 6 and 7 at 100 mg/kg. Serum were measurement of ALT, AST, ALP, GGT, total bilirubin, MDA, SOD, CAT and GSH.
Results: TAA significantly increased ALT from 44.52±6.40 to 171.18±28.91 U/L, AST from 70.76±6.51 to 340.44±34.82 U/L, ALP from 117.03±13.67 to 243.74±27.71 U/L, GGT from 7.81±0.58 to 27.17±1.50 U/L, bilirubin from 0.41±0.07 to 1.41±0.24 mg/dL, and MDA from 2.15±0.21 to 6.20±0.49 nmol/mL (all $p<0.001$). Extract treatment reduced these values to 88.52±7.86, 181.99±17.98, 163.72±20.57, 13.82±1.40, 0.86±0.09, and 3.72±0.60, respectively. The antioxidant markers SOD, CAT and GSH which were reduced by TAA, were increased after treatment towards the control levels. Body weight remained comparable among groups ($F(2,27) = 1.866$, $p=0.174$).
Conclusion: Ethanolic extract of *A. maurorum* showed partial biochemical and antioxidant protection against the liver damage caused by TAA, suggesting the need to perform further dose-response, histological, and mechanistic studies.

Keywords: *Alhagi maurorum*, Thioacetamide, ALT, AST, MDA, SOD

Introduction

The liver is a central organ where nutrition metabolism, regulations of blood volume, immune function and detoxification are integrated [1]. The liver is extremely sensitive to injury caused by toxicants, since it is constantly metabolizing endogenous metabolites and foreign chemicals. Hepatic injury can occur through mechanisms involving generation of reactive oxygen species (ROS), lipid peroxidation, mitochondrial dysfunction, apoptosis/necrosis, and inflammatory signaling. A mechanism common to many liver diseases is oxidative stress which can worsen liver damage by altering lipids, proteins and nucleic acids [2]. Hepatic stellate cells can also be activated by persistent oxidative and inflammatory injury and lead to extracellular-matrix deposition, linking hepatocellular injury to fibrosis and cirrhosis [3]. The serum aminotransferases are general biochemical markers of hepatocellular injury. Alanine aminotransferase (ALT) is more liver specific than aspartate aminotransferase (AST) which is found in several tissues and is, therefore, interpreted together with histopathology and other biochemical markers [4]. In experimental toxicology, elevations of ALT and AST levels are typically associated with damage to hepatocyte membrane structure and leakage of hepatocyte enzymes into the blood. The histopathological examination complements these measurements by showing degeneration, necrosis, inflammatory infiltration, congestion and repair of the tissues. Therefore, a combination of biochemical, inflammatory and microscopic evaluation is more informative than any single one of these endpoints in assessing hepatic injury.

Thioacetamide (TAA) is a recognized hepatotoxin used to induce acute, subacute or chronic liver injury in laboratory animals. TAA is primarily bioactivated in the liver by cytochrome P450 2E1 to form TAA-S-oxide and TAA-S-dioxide [3] as reactive metabolites. TAA-S-dioxide has been shown to cause oxidative damage by lipid peroxidation of hepatocellular membranes and covalent binding of reactive metabolites to cellular macromolecules lead to centrilobular necrosis [5]. Species, dose, route, frequency and duration of exposure are all determinants of the injury phenotype. Single exposure causes acute hepatocellular necrosis, while prolonged exposure results in activation of transforming growth factor-β/Smad3 signaling and hepatic stellate cells, thereby promoting fibrosis and cirrhosis [3]. An inflammatory response also enhances the toxicity of TAA. DAMPs such as HMGB1, TLR4 and subsequent NF-κB signaling are activated in injured hepatocytes and trigger innate immunity. These pathways lead to the release of tumour necrosis factor-α, interleukin-1β and interleukin-6, which attract inflammatory cells and prolongs tissue injury [6]. Experimental modulation of HMGB1/RAGE/TLR4 signaling has been shown to be protective against hepatic abnormalities in the context of TAA [7]. Similarly, involvement of oxidative and inflammatory mechanisms was suggested in the model of TAA-induced acute liver failure, which can be reduced by regulation of redox-sensitive transcription factors [8].

Alhagi maurorum Boiss. (camelthorn) is a medicinal plant of the Fabaceae family, widely distributed throughout the

arid and semi-arid parts of Asia, Middle East and North Africa. Based on phytochemical investigations, the genus includes flavonoids, phenolic compounds, terpenoids, and other secondary that have an metabolites with antioxidant, anti-inflammatory, antispasmodic, anti-ulcer, and hepatoprotective effect [9]. This biological relevance of antioxidant and anti-inflammatory activity of plant preparations with high content of flavonoids is due to their ability to inhibit free-radical-mediated damage and modulate inflammatory processes [10]. *Alhagi maurorum (A. maurorum)* is pharmacologically relevant as demonstrated by experimental findings. The extracts have been shown to have anti-inflammatory and antiulcer effects in animal models and flavonoid constituents are suggested to be responsible for these effects [10]. In streptozotocin-induced diabetic rats, the aqueous and ethanolic extracts altered that altered the oxidative stress status and antioxidant enzyme activities, indicating the ability of the plant to reduce the sensitivity of cells to oxidative stress [11].

An ethanolic extract also reduced liver-function markers, histological lesions, oxidative stress and apoptosis in rats with lead-induced toxicity, which may be related to the restoration of the antioxidant defenses and reduction caspase-3- mediated cell death [12]. Likewise, an aqueous extract prevented the altered levels of liver enzymes, malondialdehyde, antioxidant enzymes and hepatic histological parameters caused by norfloxacin [13]. Another closely related species, Alhagi camelorum, has also been reported to have hepatoprotective properties by a hydroalcoholic extract of its roots against liver injury caused by valproic acid in rats [14]. The results suggest that the extracts of Alhagi act on various complementary molecular targets instead of a single one [15]. *A. maurorum* possesses some antioxidant and hepatoprotective activity but the biochemical effects of *A. maurorum* on liver damage caused by TAA are not well studied. Especially, it is unknown whether the ethanolic extract is capable of attenuating the changes in the liver-function markers and the markers of oxidative stress in the serum of the mice induced by TAA. The results of previous investigations have been limited to other toxicants, types of extracts or related Alhagi species, which makes direct interpretation of TAA exposure difficult. Therefore, this study aimed to investigate the effect of *A. maurorum* ethanolic extract against the hepatic injury induced by TAA in mice. We postulated that the ethanolic extract of the root of *A. maurorum* will reduce the changes in serum liver-function markers and oxidative-stress-related biochemical indices of mice induced by TAA.

Methodology

Collection and Authentication of Plant Material

In August 2025, fresh roots of *A. maurorum* Boiss. were collected from Al-Musayyib city, Babylon Governorate, Iraq. The roots were carefully dug out, the soil removed by washing, and loaded into clean, ventilated containers and brought to the laboratory. The plant material was authenticated by prof. from the College of Science, University of Babylon. Morphologically intact roots without visible signs of fungal growth, insects or mechanical decomposition were chosen. The authenticated material was used for the preparation of the experimental extract.

Preparation of the Ethanolic Root Extract

The roots were washed by running water, followed by distilled water and air-dried in the shade at room temperature for about two weeks, avoiding direct sunlight and high humidity. The dried roots were cut into small pieces and powdered using a clean laboratory mill. The extraction protocol was modified from that described for extraction of ethanolic roots of *A. maurorum* [16]. The extraction was carried out on 100 g of the powdered root material, which was extracted with 500 mL of absolute ethanol under reflux for 2 h. From the published method of ethanolic extraction of *A. maurorum* roots the ratio 1:5 (w/v) and reflux were chosen. The extract was filtered through qualitative filter paper and the plant residue was again extracted under the same conditions to improve extraction recovery. Filtrates were then concentrated together under reduced pressure in a water bath maintained at 40 °C or below in a rotary evaporator. The concentrated extract was then further dried under vacuum to a constant mass and the percentage yield was calculated as follows:

Extract yield (%) = [mass of dried extract (g)/mass of powdered roots (g)] × 100

The dried extract was stored in an amber-colored container, in airtight container at 4 °C until use. The necessary amount of extract was daily dispersed in 0.5% carboxymethylcellulose (CMC) in distilled water to prepare a new suspension. The suspension was vortex-mixed right before administration for uniformity. The extraction yield, look of each experimental batch, storage conditions and preparation date were recorded.

Experimental Animals and Housing

Thirty adult healthy male Swiss albino mice (8–10 weeks old and weighting approximately (25-30) g were procured from an approved laboratory animal facility. To minimize the variability among female mice due to their estrous cycles, male mice were used. Under controlled environmental conditions - 12:12 h (light/dark) cycle, 22±2 °C and 50–60% relative humidity (RH), animals were kept in groups in clean polypropylene cages with autoclaved bedding. Commercial rodent commercial rodent pellets were provided and tap water was filtered and provided ad libitum. All animals had a 7-day acclimatization period prior to treatment. Animals were examined on a daily basis for appearance and activity, consumption of feed and water, respiratory patterns, diarrhoea, neurological symptoms and death. Animals experiencing severe and persistent distress were to be withdrawn from the study and treated as per prescribed veterinary guidelines.

Experimental Groups and Treatment Schedule

The 30 mice were randomly distributed into three groups: Group I (control, n=10), Group II (TAA control, n=10), Group III (TAA + *A. maurorum* extract, n=10) where the former underwent vehicle solutions, the latter TAA and the extract vehicle, and the latter both TAA and *A. maurorum* extract, respectively. The TAA control group was added due to the need to compare its data to the data from a vehicle-only control to examine whether the plant extract has a specific protective effect against TAA-induced injury. Oral gavage of either the control or the TAA-control groups was performed at 10 mL/kg BW that contained 0.5% CMC. Group III received the ethanolic root extract by oral through gavage at a dose of 250 mg/kg body weight (BW) for 7 days, daily. This dose was chosen as conservatively safe (lower bound of the range used in studies with Alhagi preparations and below is experimentally attainable and

where clinical effects due to its root extract in mice are observed). Groups II and III were injected intraperitoneally with TAA on day 6 and day 7, a minimum of 24 h apart, with a dose of 100 mg/kg body weight. The schedule was designed to create a time dependent model of toxic liver injury whilst minimising repeated chemical exposure. The extract was further given 60 min prior to the TAA injection, and continued daily on day 7 making the treatment a short course (preventative or concurrent) intervention. Two days later, animals received the second injection of TAA, and 24 h post injection, animals were sacrificed. All doses were adjusted to the latest body weight, and no volumes of injection exceeded 10 mL/kg.

Blood Collection and Preparation of Serum

The blood samples were collected in plain tube, allowed to clot at room temperature for 20–30 min. The serum samples were prepared by centrifugation at 3000 rpm for 5 min and stored at −20 °C till biochemical estimation. The serum was collected in polypropylene tubes, with care to avoid multiple freeze-thaw cycles.

Biochemical Evaluation of Liver Function

ALT, AST, ALP, GGT, and total bilirubin were measured in the serum by means of commercially available diagnostic kits according to the manufacturer's instructions. ALT and AST activities were measured by the standardized kinetic method, ALP by p-nitrophenyl-phosphate method, GGT activity by gamma-glutamyl substrate method, and total bilirubin by diazo colorimetric method. The absorbance was measured using a calibrated automated chemistry analyzer or microplate spectrophotometer at the specified wavelength. Calibrators, reagent blank, and two levels of quality control sera were included in each run. The results were reported in U/L for enzyme activities and mg/dL for bilirubin. Interpretation of aminotransferases activities was done according to the existing guidelines for biochemical evaluation of hepatocellular injury [17]. All samples were measured in duplicate, and mean value was taken for statistical analysis.

Evaluation of Oxidative Stress and Antioxidant Enzymes

The serum samples prepared as described above were used for determination of MDA, total GSH, SOD, and CAT. MDA was measured by thiobarbituric acid (TBA) method, and the reaction product was measured spectrophotometrically at 535 nm and expressed as nmol/mL. Total GSH was estimated using 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) as sulfhydryl reactive chromogen, and the absorbance of the yellow-colored reaction product was measured at 412 nm and expressed as μmol/mL. SOD activity was measured using modified photochemical nitroblue tetrazolium (NBT) method and expressed as U/mL. CAT activity was measured as the rate of H_2O_2 decomposition spectrophotometrically at 230 nm and expressed as U/mL [18]. All samples were measured in duplicate, and mean of duplicate measurements was taken for statistical analysis.

Quality Assurance and Data Management

The instruments were calibrated before use, reagents were used within their expiration date, and samples were assigned numeric codes unrelated to treatment code during biochemical measurement. The raw absorbance readings, calibration readings, duplicate measurements, rejected runs, and concentration values were recorded in a protected laboratory worksheet. Any duplicate coefficient of variation beyond laboratory's predefined limit of acceptable variability was repeated if sample quantity was sufficient. Outliers were not excluded only on the basis of being different from the group mean, but only due to documented technical errors or pre-specified animal exclusion criteria.

Statistical Analysis

The data are presented as mean ± standard deviation (SD). Difference between groups were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference test for pair wise comparisons. All tests were two-sided and $p<0.05$ was taken as statistically significant. The analyses were carried out using IBM SPSS Statistics version 26.0.

Results

Ethanol extraction of 100 g of powdered roots yielded 8.6 g of dried extract, which corresponds to 8.6% extraction yield. The extract had brownish-green color and was could be resuspended in 0.5% CMC. Recording the yield of extraction is essential for interpreting the results because it determines the amount of concentrated plant material, which was administered to the animals and helps reproduce nominal dose of 250 mg/kg in future studies. No death or any other clinical sign requiring animal exclusion occurred during follow up. As shown in (Table 1), final body weights were similar among the groups: 27.42 ± 1.10 g in control group, 26.90 ± 0.42 g in TAA group, and 27.66 ± 1.02 g in TAA + Extract group. One-way analysis of variance did not show statistically significant difference ($F(2,27) = 1.866$, $p = 0.174$). This result indicates that there was no statistically significant difference in final body weight among three groups during the experiment period. However, final body weight cannot be considered as evidence that of organ-specific toxicity or other adverse effect.

Table 1: Final body weight of mice in the control, TAA, and TAA + *A. maurorum* extract groups

Group	n	Final body weight (g, mean ± SD)
Control	10	27.42 ± 1.10
TAA	10	26.90 ± 0.42
TAA + Extract	10	27.66 ± 1.02

As shown in (Table 2), TAA exposure resulted in a notable change in the activity of the two main enzymes (ALT and AST) responsible for leakage of contents of liver cells in the bloodstream. While ALT was 44.52 ± 6.40 U/L in the control group, in the TAA group, it was increased to 171.18 ± 28.91 U/L, which means that approximately there was an increase of 284.5% as described in the (Table 2). The mean decreased to 88.52 ± 7.86 U/L when the extract was administered and thus was decreased by 48.3% compared to TAA but was still higher than the mean in the control group. Similarly, AST increased from 70.76 ± 6.51 to 340.44 ± 34.82 U/L and decreased to 181.99 ± 17.98 U/L in the extract group. The fact that there was a change in the parallel direction in both ALT and AST is depicted in (Figure 1) as well, and it shows that the extract diminished the effect of the TAA on the markers of hepatocellular damage without restoring them to their control levels. There is a change in ALP activity as shown in (Table 2). ALP

increased from 117.03 ± 13.67 U/L in the control group to 243.74 ± 27.71 U/L after TAA administration and to 163.72 ± 20.57 U/L after extract administration. ALP is not the specific marker of hepatocellular injury, but since it showed the same direction as ALT and AST, it can be supposed that there was a multivariate response in this model. (Figure 1) shows the means for each group, and the error bars represent standard deviations (SD). As can be seen in (Table 2), TAA increased GGT from 7.81 ± 0.58 to 27.17 ± 1.50 U/L and increased total bilirubin from 0.41 ± 0.07 to 1.41 ± 0.24 mg/dL. After treatment, these values decreased to 13.82 ± 1.40 U/L and 0.86 ± 0.09 mg/dL, respectively. The concurrent decrease of GGT, and bilirubin, together with aminotransferases, shows that there was partial restoration of the functional changes associated with the hepatocellular injury but the values remained higher than in the control group and thus cannot be interpreted as normal. All comparisons of biochemical markers showed statistically significant differences (one-way ANOVA, $F(2,27) = 33.970–647.933$; all $p < 0.001$). Reductions in the TAA + extract group as compared to the TAA group were 48.3% for ALT, 46.5% for AST, 32.8% for ALP, 49.1% for GGT, 39.0% for total bilirubin, and 40.0% for MDA.

Table 2: Serum ALT, AST, ALP, GGT, and total bilirubin in mice exposed to TAA and treated with *A. maurorum* extract

Biochemical parameter	Group	N	Mean ± SD
ALT (U/L)	Control	10	44.52 ± 6.40 [a]
	TAA	10	171.18 ± 28.91 [b]
	TAA + Extract	10	88.52 ± 7.86 [c]
	ANOVA	—	$p < 0.001$
AST (U/L)	Control	10	70.76 ± 6.51 [a]
	TAA	10	340.44 ± 34.82 [b]
	TAA + Extract	10	181.99 ± 17.98 [c]
	ANOVA	—	$p < 0.001$
ALP (U/L)	Control	10	117.03 ± 13.67 [a]
	TAA	10	243.74 ± 27.71 [b]
	TAA + Extract	10	163.72 ± 20.57 [c]
	ANOVA	—	$p < 0.001$
GGT (U/L)	Control	10	7.81 ± 0.58 [a]
	TAA	10	27.17 ± 1.50 [b]
	TAA + Extract	10	13.82 ± 1.40 [c]
	ANOVA	—	$p < 0.001$
Bilirubin (mg/dL)	Control	10	0.41 ± 0.07 [a]
	TAA	10	1.41 ± 0.24 [b]
	TAA + Extract	10	0.86 ± 0.09 [c]
	ANOVA	—	$p < 0.001$

[a, b, c] Different superscript letters indicate statistically significant pairwise differences according to Tukey's HSD test at $p < 0.05$.

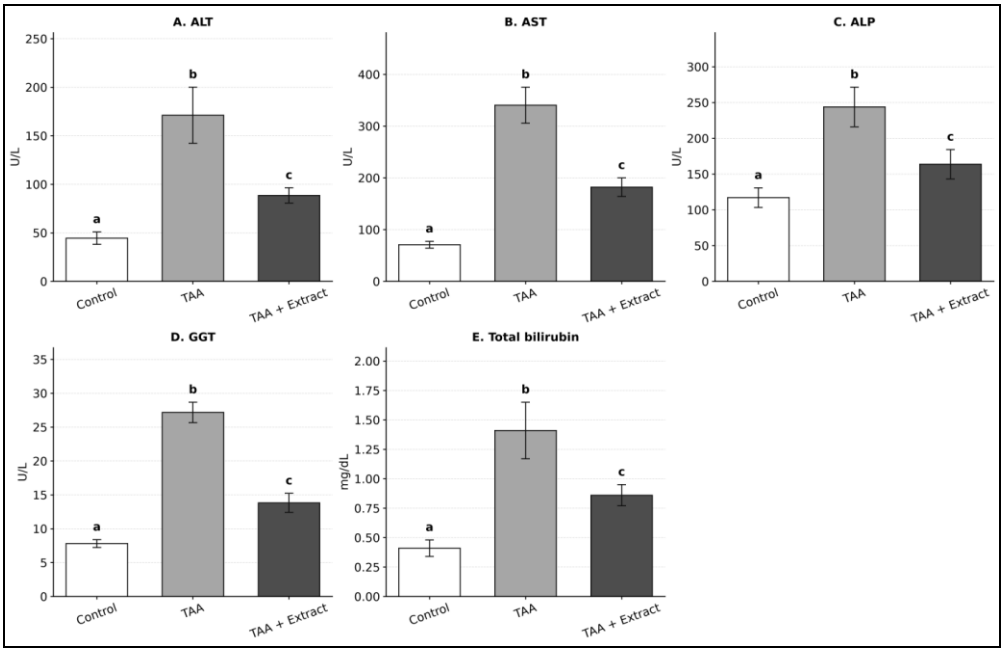

Fig 1: Group means ± SD for serum ALT, AST, ALP, GGT, and total bilirubin in the control, TAA, and TAA + extract groups

The patterns of the oxidative-stress and antioxidant-defense biomarkers are opposite for MDA and antioxidant-defense markers. The MDA levels were elevated from 2.15 ± 0.21 nmol/mL in the control group to 6.20 ± 0.49 nmol/mL in the TAA group and then decreased to 3.72 ± 0.60 nmol/mL after treatment (Table 3). (Figure 2) shows the MDA peak in the TAA group was lower in the treatment group, yet it still did not reach the control level. On the contrary, SOD level

decreased from 8.28 ± 0.76 U/mL in the control group to 4.52 ± 1.43 U/mL in the TAA group and then increased to 6.74 ± 0.73 U/mL in the treatment group (Table 3 & Figure 2). CAT exhibited similar trends, decreasing from 31.71 ± 3.89 to 15.04 ± 2.33 U/mL and then increasing to 24.33 ± 2.24 U/mL. These results indicate that the extract partially preserved the efficiency of the free-radical clearance system; however, the relative variability in the TAA group is significant due to small number of observations. GSH also demonstrated the similar tendency, decreasing from 7.91 ± 0.45 to 3.58 ± 0.55 µmol/mL after TAA treatment and then increasing to 5.80 ± 0.53 µmol/mL after extract treatment (Table 3 & Figure 2). The trend was observable but the improvement was insufficient to bring MDA to the control mean level. Taking into account the levels of MDA, SOD, CAT, and GSH, the data show that relatively consistent pattern was revealed: the exposure to TAA-induced the increased oxidative stress and impaired the functioning of antioxidants, while treatment helped to restore at least partially. The decrease in MDA in the TAA + extract group relative to the TAA group was 40.0%. In case of antioxidant measures, the extract group exhibited approximately 59.0% of the difference between the means in the TAA and control groups in terms of SOD, 55.7% of the difference in terms of CAT, and 51.3% of the difference in terms of GSH. These are only descriptive measures of recovery and cannot replace the estimation of effect size and confidence interval. The measurements of the oxidative-stress markers were made using serum samples; thus, the results cannot be directly generalized to the tissue level.

Table 3: Serum MDA, SOD, CAT, and GSH in mice exposed to TAA and treated with *A. maurorum* extract

Biochemical parameter	Group	N	Mean ± SD
MDA (nmol/mL)	Control	10	2.15 ± 0.21 [a]
	TAA	10	6.20 ± 0.49 [b]
	TAA + Extract	10	3.72 ± 0.60 [c]
	ANOVA	—	p < 0.001
SOD (U/mL)	Control	10	8.28 ± 0.76 [a]
	TAA	10	4.52 ± 1.43 [b]
	TAA + Extract	10	6.74 ± 0.73 [c]
	ANOVA	—	p < 0.001
CAT (U/mL)	Control	10	31.71 ± 3.89 [a]
	TAA	10	15.04 ± 2.33 [b]
	TAA + Extract	10	24.33 ± 2.24 [c]
	ANOVA	—	p < 0.001
GSH (µmol/mL)	Control	10	7.91 ± 0.45 [a]
	TAA	10	3.58 ± 0.55 [b]
	TAA + Extract	10	5.80 ± 0.53 [c]
	ANOVA	—	p < 0.001

[a, b, c] Different superscript letters indicate statistically significant pairwise differences according to Tukey's HSD test at $p < 0.05$.

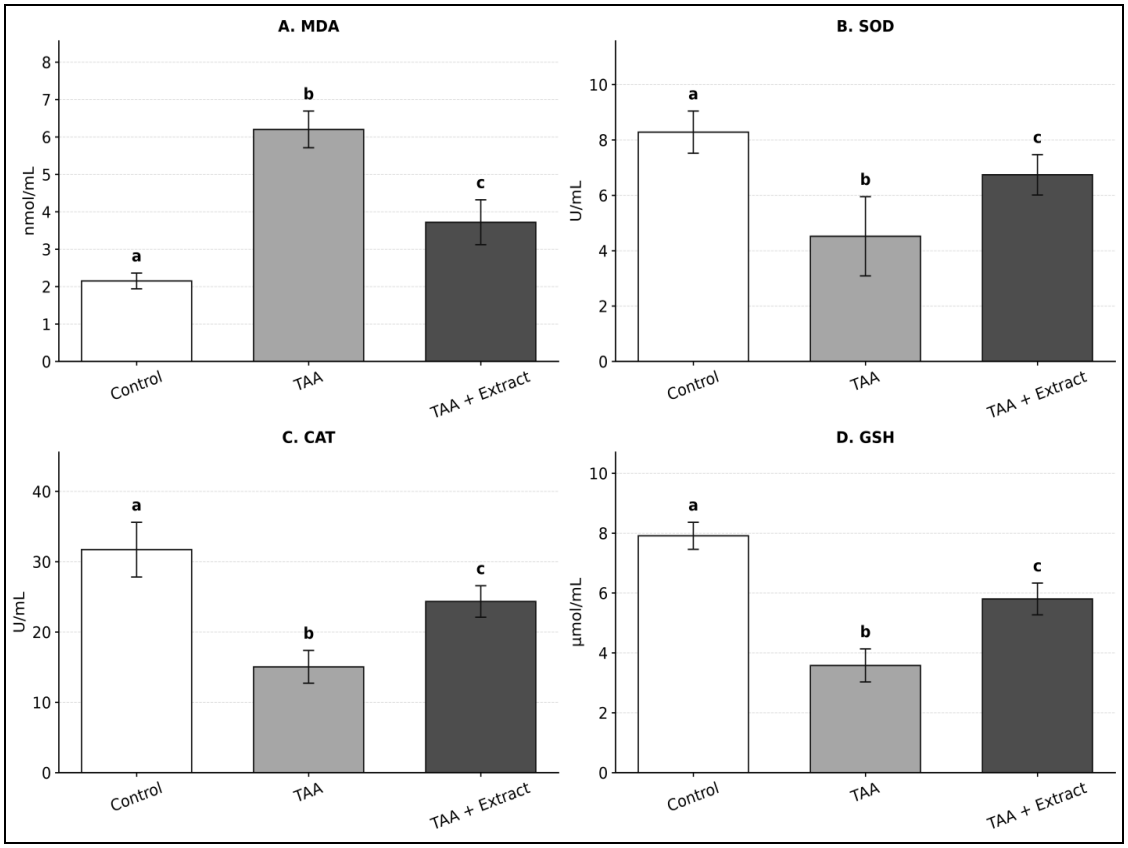

Fig 2: Group means ± SD for serum MDA, SOD, CAT, and GSH in the control, TAA, and TAA + extract groups

Discussion

In the present study, the present study showed that a short-term exposure to thioacetamide (TAA) caused a consistent pattern of biochemical changes consistent with hepatic injury in mice; the use of ethanolic extract of roots of *A. maurorum* partially attenuated these biochemical alterations. TAA caused significant elevation in levels of ALT, AST, ALP, GGT and total bilirubin; all of these changes were reduced significantly by the administration of the extract. Partial restoration of these parameters was an important finding because it indicated attenuation of injury, and not protection of hepatocytes. The concomitant elevation of aminotransferases and cholestasis indices also pointed to the presence of functional changes, and not isolated injury of one enzyme. The magnitude and direction of the response to TAA in the extract-administration group corresponded to those described in the literature in experiments with other hepatoprotectors. For example, Wong *et al*. have reported increased ALT, AST, ALP, bilirubin and oxidative stress indices in chronic TAA-treated rats, whereas consumption of a mushroom preparation restored the values towards control values [19]. Also, green tea extract caused decreased TAA-induced elevation of serum enzyme activity and bilirubin as well as increased antioxidant status [20]. Thus, the findings of the present study extend pharmacological observations reported previously to *A. maurorum* and a short-term (preventive/concurrent) exposure regimen. However, it was biologically justified that there was a non-complete normalization in the extract group. The treatment was initiated shortly prior to the second phase of TAA administration and lasted for only seven days; in contrast, the comparator studies usually involved prolonged drug administration.

The oxidative stress findings provided the mechanistic rationale behind the hepatoprotective activity of the extract. TAA increased MDA from 2.15 to 6.20 nmol/mL, indicating elevated lipid peroxidation, while SOD, CAT and GSH were substantially decreased. The use of the extract decreased MDA level to 3.72 nmol/mL and normalized the values of SOD, CAT and GSH to intermediate levels. The coordinated change served as more convincing evidence of the improvement in redox balance than isolated changes in a single antioxidant. Similar restoration of elevated MDA and depletion of antioxidant enzymes was reported in experiments with rutin [21] and fractions of Cassia fistula [22]. Additionally, green tea treatment in a chronic TAA model has restored SOD and CAT levels and reduced MDA, indicating that preservation of antioxidant status was an important component of hepatoprotection. The results of the present study are also consistent with the findings on redox-sensitive signaling as a common target for therapeutic effects. For instance, saikosaponin-d increased hepatic antioxidant enzymes and decreased ALT, AST, inflammatory mediators and NF-κB expression in TAA-exposed mice [23]. Also, gardeniae fructus has been reported to attenuate TAA-induced oxidation and fibrosis through AMPK/SIRT1/Nrf2-associated mechanisms [24]. Recently, shikimic acid has increased liver enzymes and antioxidant status and suppressed MDA, inflammatory cytokines and fibrogenic markers through Nrf2/NF-κB modulation [25]. Although the present experiment did not measure the levels of Nrf2, NF-κB, cytokines and tissue proteins, the concomitant normalization of MDA, GSH, SOD and CAT can be interpreted as an increase in endogenous antioxidant capacity. However, it would be premature to make any conclusions regarding pathway activation without molecular confirmation.

The hepatoprotective profile of the extract resembles findings in experiments with other botanical preparations. Moringa oleifera ethanolic extract was reported to normalize antioxidant, apoptotic and inflammatory markers in TAA-treated rats [26], while Cichorium glandulosum extracts were shown to decrease TAA-related hepatic fibrosis and alter TGF-β/Smad signaling [27]. Amaranthus hybridus leaf extract was reported to improve TAA-related liver function, oxidative stress and tissue damage [28]. There are similar studies of Syzygium aromaticum [29], apigenin [30] and dendropanoxide [31]; all these studies indicate the regularity that decreases in transaminases are usually accompanied by improvement in antioxidant status. These comparisons demonstrate that the results of the present experiment are consistent with the experimental literature in which attenuation of TAA-associated biochemical disturbances has been frequently associated with the improvement in antioxidant indices. However, it is important to consider the limitations due to the differences in species, TAA exposure schedule, composition of the extract and the duration of treatment.

It is worth mentioning that, along with biological limitations, there were some statistical limitations. The sample size was relatively small, with 10 animals per group, and several biochemical endpoints have been analyzed. Therefore, the issue of false-positive resulting across several endpoints should be considered, especially if there were no adjustments for multiplicity of tests. The absence of the extract-only group limited the ability to differentiate between the protection against TAA-induced injuries and the independent biochemical effect of the extract.

There were relatively few animals (10 per group), one extract dose and one short-term observation period were used. The measurement of oxidative markers was carried out for serum but not for the hepatic tissue; no histopathology, inflammatory cytokines, markers of apoptosis or assay of molecular pathways were done. Therefore, the results provide evidence of biochemical attenuation but not of structural protection of the liver, its anti-inflammatory activity or a specific molecular mechanism. This difference is important because the tissue effects and pathway effects cannot be extrapolated from serum results. It is necessary to carry out further research involving the analysis of dose-response and recovery groups, a standard comparator (silymarin) and phytochemical fingerprinting, analysis of liver tissue and histopathology.

Overall, the results of the experiment confirm the hypothesis about the attenuating action of the ethanolic extract of *A. maurorum* roots on the TAA-related liver dysfunction and oxidative disturbance in mice. The action was partial but internally consistent across five liver function parameters and four redox-related parameters, without any significant difference in body weight at the short experimental period. Therefore, it seems to be reasonable to investigate further

the potential of *A. maurorum* as a candidate hepatoprotector, while claiming the clinical effectiveness requires additional research. Comparison with other plant preparations (resveratrol [32] and Daucus carota [33]) demonstrating comparable protective effects supports the validity of this approach.

Conclusion

Ethanol root extract of *A. maurorum* has been identified to show a partial, though significant, attenuation of TAA-induced biochemical and oxidative stress-related alterations in serum of mice. The administration of TAA resulted in a significant increase in serum ALT, AST, ALP, GGT, bilirubin, and MDA levels, and decreased levels of SOD, CAT, and GSH. The treatment with the extract led to an approach of the indicators towards normal values, thus reflecting partial amelioration of TAA-induced biochemical alterations. The lack of a significant difference in body weight and the lack of any recorded severe clinical signs indicate that there were no signs of significant impairment of general state in the experimentally short period.

Ethical Approval

All procedures performed in these experiments have been examined and approved by the Animal Care and Use Committee at Al-Furat Al-Awsat Technical University with protocol number EC435, issued on April 7, 2025. This study has been carried out according to the relevant national laws and guidelines on animal experimentation.

Conflict of Interest

There is no conflict of interest in this article.

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