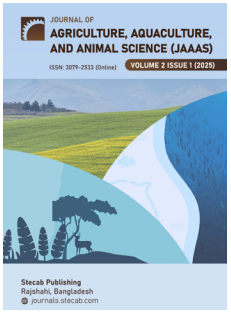

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Research Article

Ameliorative Effects of Mulberry Leaf Extract on Liver Enzymes and Antioxidant Status in Acetaminophen-Induced Liver Injury in Mice

*[1]Sama A. F. ALRubaie, [2]Rehab A. A. Alhamashi, [3]Noor Majeed Abdulhasan

About Article

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About Author

[1] Pathological Analysis Department, College of Science, University of Wasit, Wasit, Iraq

[2] Biology Department, College of Education for Pure Sciences, Wasit University, Iraq

[3] Forensic Evidence Department, College of Science, University of Wasit, Wasit, Iraq

Contact @ Sama A. F. ALRubaie
samaa@uowasit.edu.iq

ABSTRACT

Liver is a vital body's organ which performs various metabolic functions and exposed to multiple harmful conditions that might lead to acute or chronic injuries / disorders. Worldwide, several plants have been utilized in traditional medicine to treatment of different health problems in animals and humans, as they provided several bioactive beneficial compounds. Investigation the effect of acute liver injury in mice on liver enzymes [alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and total bilirubin (TB)], serum antioxidants [catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx)], and lipid peroxidation [malondialdehyde (MDA)], with estimation the ameliorative role of mulberry leave extract on these markers. After preparation acetaminophen for induction of acute liver injury and mulberry leaf extract for amelioration; a total of 32 male BALB/C mice were purchased, transported, acclimated for one week, fasted for 12 hours, and divided equally to four study groups including PCG (mice neither injected acetaminophen nor drenched the extract), PCG (mice injected acetaminophen but not drenched the extract), ELE (mice injected acetaminophen and drenched a low dose of extract), EHE (mice were injected acetaminophen and drenched a high dose of the extract). After 48 hours, all study animals were euthanized, blood sampled, and the obtained sera were examined by quantitative ELISA. For liver enzymes, values of ALP, ALT, AST, TB and LDH were reduced significantly in ELE and more obviously in EHE when compared to value of PCG. However, the findings of treated groups remain higher than the values of NCG. Regarding the antioxidants, the findings of CAT, GSH-Px and SOD were elevated significantly in mice of ELE and more obviously in those of EHE when compared to result of PCG. However, values of PCG and both treated groups were significantly lower than identified in NCG. Concerning the lipid peroxidation, the findings of MDA were reduced in mice of both treated groups; ELE and EHE when compared to values of PCG; however, values of both treated groups remain significantly higher than those of NCG. Since our study might represent the first Iraqi one insight hepatoprotective role of mulberry leaf extract in experimentally induced acute liver injury, suggesting the importance of furthermore studies for other parts of mulberry or extracts of other plants.

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1. INTRODUCTION

Acute liver injury is an important clinical syndrome since it creates rapid hepatic function decline and may transforms either to acute liver failure that remains a high-risk medical condition despite current developments in intensive care management methods or to coagulopathy, encephalopathy symptoms and chronic liver disease (Harrison, 2018; Bernal *et al*., 2021; Moreau *et al*., 2021). Liver is a vital organ that performing various metabolic tasks including protein synthesis together with detoxification and glucose regulation functions yet remains at risk from multiple triggers that cause acute harm ranged from viral infections and drug-induced hepatotoxicity to ischemic injury and autoimmune disorders (Alamri, 2018; Andrade *et al*., 2019; Al-Hetty *et al*., 2023; Mohajan, 2025).

2. LITERATURE REVIEW

Medicinal plants constitute a long-established field of traditional treatment options that provide accessible safe and effective care for a wide range of disorders due to their compounds that produced by these plants (Dar *et al*., 2023; Wahab *et al*., 2024). The therapeutic exploitation of plants for medicinal purposes spans across all recognized nations since humans started using medicines thousands of years ago (Banerjee, 2024). These plants keep their important value for healthcare delivery especially within developing nations because residents often lack access or cannot afford contemporary pharmaceutical medications (Srivastava, 2018). The population of these areas depends mainly on traditional medical systems for healthcare since medicinal plants stand as essential components against multiple diseases (Sofowora *et al*., 2013). Developed countries witness elevated demand for herbal medicine as an alternative or complementary medical solution because people seek natural and holistic healthcare methods (Enioutina *et al*., 2017). Numerous factors including anxiety over synthetic drug side effects combined with increasing health care expenses drive people to look for more personalized preventive healthcare solutions (Mathur & Sutton, 2017).

Scientific researchers extensively investigate mulberry leaf extract derived from Morus alba and other Morus species because its rich phytochemical profile demonstrates numerous possible therapeutic uses (Kadam *et al*., 2019; Fatima *et al*., 2024). The historical use of mulberries in traditional medicine, especially within the framework of Ayurveda and traditional Chinese medicine, underscores its long-standing recognition as a valuable medicinal resource (Afsharmanesh *et al*., 2024; Sharma *et al*., 2024). Modern scientific methods are currently investigating the medicinal benefits of mulberry plant leaves while silkworm larvae have received these leaves as their primary silvicultural nourishment for centuries (Saini *et al*., 2023).

Medical practitioners have conducted several studies to understand the components of mulberry leaf extract which contributed effectively in treatment of different health problems because this knowledge enables better clinical outcome predictions and development of specific therapeutic approaches (Cui *et al*., 2023; Zhang *et al*., 2014; Lin *et al*., 2025; Mao *et al*., 2025). Therefore, the current study aims to investigate the effect of acute liver injury on liver enzymes (ALT, AST, ALP, and TB), serum antioxidants (CAT, SOD, and GPx), and lipid peroxidation (MDA), with estimate the ameliorative role of mulberry leaf extract on these markers

3. METHODOLOGY

3.1. Preparation of mulberry leaf extract

Approximately 100 grams of freshly dried mulberry leaves were collected locally, powdered, and subjected to extraction by the Soxhlet apparatus using of ethanol alcohol (70%) at 45°C. Then, the extract was filtered, concentrated under vacuum conditions (150rpm, 40°C, 20 hours), collected into dark glass containers and kept cooled until be used (Liu *et al*., 2021; Wahab *et al*., 2024). The administered doses were calculated based on the body weight of study animal as either the low dose (100mg/kg.BW) or high dose (200mg/kg.BW) and given orally.

3.2. Preparation of acetaminophen

At the time of utilization, acetaminophen solution was prepared by adding a tablet of 500mg of acetaminophen into 16.7ml of 0.9% normal saline. The administered dose was calculated based on the body weight of study animal as BW x 16.7 (Muhammad-Azam *et al*., 2019). Therefore, the administered dose to each study mouse was ranged 0.37-0.45ml and given intraperitoneally for once time.

3.3. Animals and study designs

Totally, 32 male BALB/C mice, 22-27 grams of weight and 7-8 weeks of age, were purchased from a private animal house in Al-Qadisiyah province (Iraq), transported and subjected for one week as an acclimation period; during which, the study mice were received the ready to use pellet and tap water, and exposed to 12/12 light/dark conditions (Hussen *et al*., 2024). Then, the study mice were fasted for 12 hours, divided equally and randomly into four groups as following:

1. Negative control (PCG): Study mice neither injected acetaminophen nor treated mulberry leaf extract, but they received pellets and tap water.
2. Positive control (PCG): Study mice were injected acetaminophen but not treated mulberry leaf extract, and received pellets and tap water.
3. Experimental low-dose extract (ELE): Study mice were injected acetaminophen, treated the low dose of mulberry leaf extract, and received pellets and tap water.
4. Experimental high-dose extract (EHE): Study mice were injected with acetaminophen, treated with the high dose of mulberry leaf extract, and received pellets and tap water.

After 48 hours, all study animals were euthanized and subjected for direct sampling of blood from the heart into free-anticoagulant glass gel tubes. The obtained sera were kept frozen until be examined by ELISA.

3.4. Quantitative serology

According to manufacturer's instructions of ELISAs' kits provided by the SunLong Biotech Company (China), the serum samples and contents of each kit were prepared at room temperature, processed, and the absorbance was read at an optical density (OD) of 450nm. The concentrations of each marker in serum samples were measured by plotting the concentrations and ODs of the Standard Solution as well as the ODs of serum samples (Almaliky *et al*., 2024).

3.5. Statistical analysis

One-sample t-test in GraphPad Prism Software was applied to detect significant differences at p<0.05 between the obtained values. Statistically, the findings of mean ± standard error (SEM) in addition to standard deviation (SD), 95% Confidence Interval and R-squared (partial or squared) were calculated between the findings of study groups (Mohammad *et al.*, 2022).

4. RESULTS AND DISCUSSION

4.1. Liver enzymes

In comparison with the value of PCG (1283.875 ± 87.64 pg/ml), the findings of ALP were reduced significantly (p<0.05) in ELE (639.625 ± 68.21 pg/ml) and more obviously in EHE (517.875 ± 62.02 pg/ml), (Table 1, Figure 1). For ALT, although no significant differences (p>0.05) were detected between values of ELE (332.875 ± 16.87 pg/ml) and EHE (333.375 ± 17.82 pg/ml), the findings of both treated groups were reduced significantly (p<0.05) when compared to those of PCG (480.5 ± 38.25 pg/ml), (Table 2, Figure 2). Significantly (p<0.05), values of AST in ELE (1.52375 ± 0.1004 ng/ml) and more markedly in EHE (1.26 ± 0.0745 ng/ml) were lower than observed in PCG (2.40375 ± 0.08168 ng/ml), (Table 3, Figure 3).

Table 1. Statistical analysis of ALP among the mice of four study groups

Value	NCG	PCG	ELE	EHE
Mean	232.75	1283.875	639.625	517.875
SD	131.9	247.9	192.9	175.4
SE	46.62	87.64	68.21	62.02
t, df	t=4.992, df=7	t=14.65, df=7	t=9.377, df=7	t=8.350, df=7
p-value	0.0016	<0.0001	<0.0001	<0.0001
p-value summary	**	****	****	****
Significance	Yes	Yes	Yes	Yes
95% CI	122.5 to 343.0	1077 to 1491	478.3 to 800.9	371.2 to 664.5
R squared	0.7807	0.9684	0.9263	0.9088

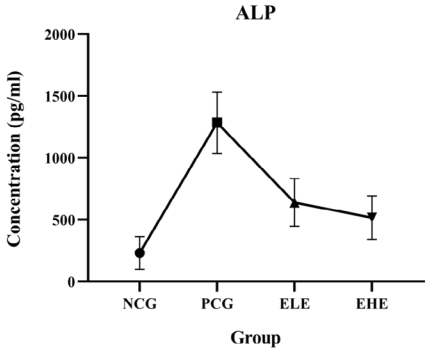

Figure 1. Concentrations of ALP (pg/ml) among the mice of various study groups

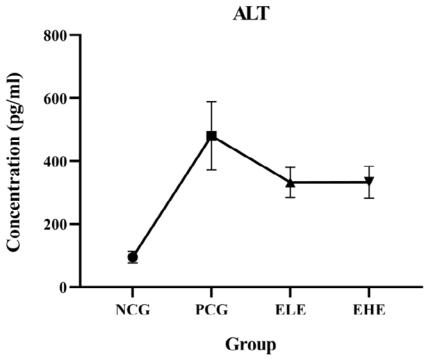

Figure 2. Concentrations of ALT (pg/ml) among the mice of various study groups

Table 2. Statistical analysis of ALT among the mice of four study groups

Value	NCG	PCG	ELE	EHE
Mean	95.5	480.5	332.875	333.375
SD	19.08	108.2	47.7	50.4
SE	6.745	38.25	16.87	17.82
t, df	t=14.16, df=7	t=12.56, df=7	t=19.74, df=7	t=18.71, df=7
p-value	<0.0001	<0.0001	<0.0001	<0.0001
p-value summary	****	****	****	****
Significance	Yes	Yes	Yes	Yes
95% CI	79.55 to 111.5	390.1 to 570.9	293.0 to 372.8	291.2 to 375.5
R squared	0.9663	0.9575	0.9823	0.9804

Table 3. Statistical analysis of AST among the mice of four study groups

Value	NCG	PCG	ELE	EHE
Mean	0.5775	2.40375	1.52375	1.26
SD	0.2081	0.231	0.2839	0.2107
SE	0.07358	0.08168	0.1004	0.0745
t, df	t=7.849, df=7	t=29.43, df=7	t=15.18, df=7	t=16.91, df=7
p-value	0.0001	<0.0001	<0.0001	<0.0001
p-value summary	***	****	****	****
Significance	Yes	Yes	Yes	Yes
95% CI	0.4035 to 0.7515	2.211 to 2.597	1.286 to 1.761	1.084 to 1.436
R squared	0.898	0.992	0.9705	0.9761

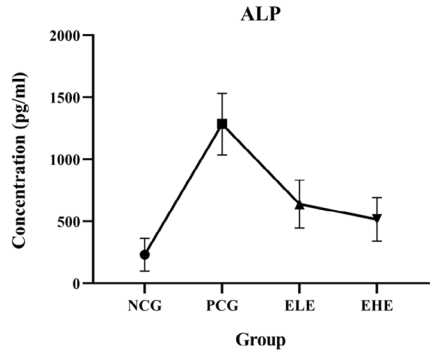

Figure 3. Concentrations of AST (ng/ml) among the mice of various study groups

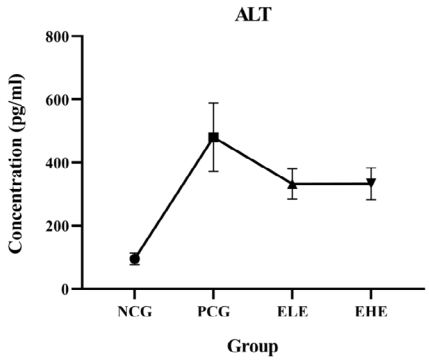

Figure 4. Concentrations of TB (µmol/L) among the mice of various study groups

Concerning the TB, although the findings of both treated groups were reduced significantly ($p<0.05$) when compared to PCG (26.64375 ± 2.088 µmol/L), values of EHE (20.51 ± 1.039 µmol/L) were significantly ($p<0.05$) higher than ELE (18.99875 ± 1.406 µmol/L), (Table 4, Figure 4).

Table 4. Statistical analysis of TB among the mice of four study groups

Value	NCG	PCG	ELE	EHE
Mean	7.50125	26.64375	18.99875	20.51
SD	1.754	5.907	3.977	2.94
SE	0.6201	2.088	1.406	1.039
t, df	t=12.10, df=7	t=12.76, df=7	t=13.51, df=7	t=19.73, df=7
p-value	<0.0001	<0.0001	<0.0001	<0.0001
p-value summary	****	****	****	****
Significance	Yes	Yes	Yes	Yes
95% CI	6.035 to 8.967	21.71 to 31.58	15.67 to 22.32	18.05 to 22.97
R squared	0.9544	0.9588	0.9631	0.9823

Regarding LDH, there were significant decreases ($p<0.05$) in values of ELE (562.75 ± 40.52 pg/ml) and more significantly in mice of EHE (362.5 ± 16.09 pg/ml) in comparison with those of PCG (1112.875 ± 47.75 pg/ml), (Table 5, Figure 5).

Table 5. Statistical analysis of LDH among the mice of four study groups

Value	NCG	PCG	ELE	EHE
Mean	223.3	1112.875	562.75	362.5
SD	56	135	114.6	45.51
SE	19.8	47.75	40.52	16.09
t, df	t=11.28, df=7	t=23.31, df=7	t=13.89, df=7	t=22.53, df=7
p-value	<0.0001	<0.0001	<0.0001	<0.0001
p-value summary	****	****	****	****
Significance	Yes	Yes	Yes	Yes
95% CI	176.4 to 270.1	1000 to 1226	466.9 to 658.6	324.5 to 400.5
R squared	0.9478	0.9873	0.965	0.9864

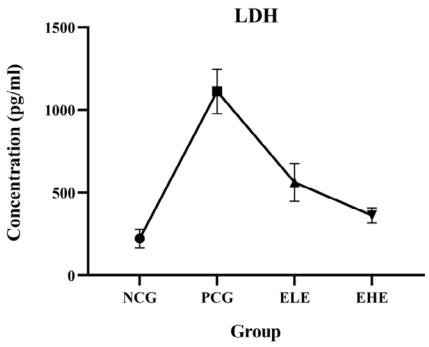

Figure 5. Concentrations of LDH (pg/ml) among the mice of various study groups

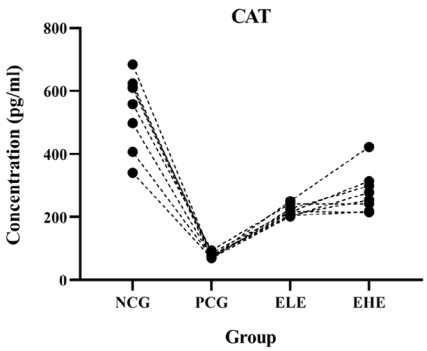

Figure 6. Concentrations of CAT (pg/ml) among the mice of various study groups

4.2. Antioxidants and lipid peroxidation

Significantly ($p<0.05$), CAT was elevated in ELE (220.625 ± 6.347 pg/ml) and more obviously in EHE (280.375 ± 23.96 pg/ml) when compared to result of PCG (77.61125 ± 3.038 pg/ml). However, values of PCG and both treated groups were significantly ($p<0.05$) lower than identified in NCG (542.0125 ± 41.75 pg/ml), (Table 6, Figure 6).

Table 6. Statistical analysis of CAT among the mice of four study groups.

Value	NCG	PCG	ELE	EHE
Mean	542.0125	77.61125	220.625	280.375
SD	118.1	8.594	17.95	67.78
SE	41.75	3.038	6.347	23.96
t, df	t=12.98, df=7	t=25.54, df=7	t=34.76, df=7	t=11.70, df=7
p-value	<0.0001	<0.0001	<0.0001	<0.0001
p-value summary	****	****	****	****
Significance	Yes	Yes	Yes	Yes
95% CI	443.3 to 640.7	70.43 to 84.80	205.6 to 235.6	223.7 to 337.0
R squared	0.9601	0.9894	0.9942	0.9514

In comparison to values of PCG (1.70625 ± 0.1592 ng/ml), there was a significant increase ($p<0.05$) in values of GSH-Px among the mice of ELE (3.315 ± 0.1471 ng/ml) and more significantly in mice of EHE (3.87125 ± 0.2481 ng/ml). However, values of EHE were significantly higher than the values of NCG (3.64375 ± 0.1463 ng/ml), (Table 7, Figure 7).

Table 7. Statistical analysis of GSH-Px among the mice of four study groups.

Value	NCG	PCG	ELE	EHE
Mean	3.64375	1.70625	3.315	3.87125
SD	0.4138	0.4503	0.416	0.7019
SE	0.1463	0.1592	0.1471	0.2481
t, df	t=24.91, df=7	t=10.72, df=7	t=22.54, df=7	t=15.60, df=7
p-value	<0.0001	<0.0001	<0.0001	<0.0001
p-value summary	****	****	****	****
Significance	Yes	Yes	Yes	Yes
95% CI	3.298 to 3.990	1.330 to 2.083	2.967 to 3.663	3.284 to 4.458
R squared	0.9888	0.9425	0.9864	0.972

For SOD, mice of ELE (2.97 ± 0.1745 ng/ml) and EHE (4.8525 ± 0.2773 ng/ml) were shown a significant increase ($p<0.05$) in their values in comparison with those of PCG (1.63125 ± 0.1541 ng/ml); however, values of these study groups were significantly ($p<0.05$) lower than values of NCG (6.59125 ± 0.55 ng/ml), (Table 8, Figure 8).

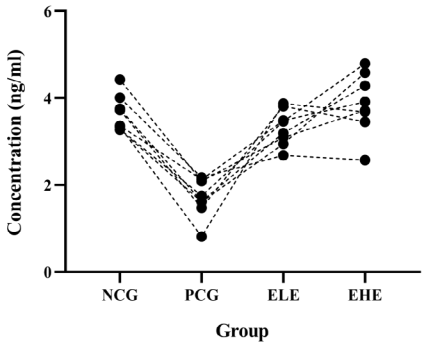

Figure 7. Concentrations of GSH-Px (ng/ml) among the mice of various study groups

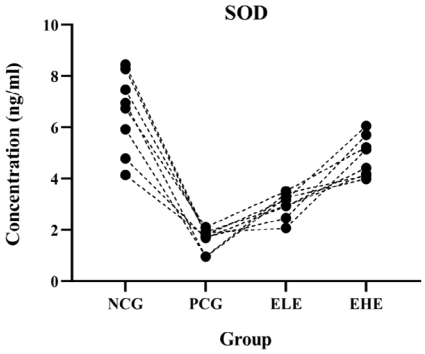

Figure 8. Concentrations of SOD (ng/ml) among the mice of various study groups

Table 8. Statistical analysis of SOD among the mice of four study groups.

Value	NCG	PCG	ELE	EHE
Mean	6.59125	1.63125	2.97	4.8525
SD	1.556	0.4359	0.4936	0.7843
SE	0.55	0.1541	0.1745	0.2773
t, df	t=11.98, df=7	t=10.59, df=7	t=17.02, df=7	t=17.50, df=7
p-value	<0.0001	<0.0001	<0.0001	<0.0001
p-value summary	****	****	****	****
Significance	Yes	Yes	Yes	Yes
95% CI	5.291 to 7.892	1.267 to 1.996	2.557 to 3.383	4.197 to 5.508
R squared	0.9535	0.9412	0.9764	0.9777

Significantly ($p<0.05$), the findings of MDA were reduced in mice of both treated groups; ELE (157.875 ± 15.66 ng/ml) and EHE (141 ± 13.26 ng/ml) when compared to values of PCG (211.25 ± 9.584 ng/ml); however, values of both treated groups remain significantly ($p<0.05$) higher than those of NCG (50.25 ± 8.163ng/ml), (Table 9, Figure 9).

Table 9. Statistical analysis of MDA among the mice of four study groups.

Value	NCG	PCG	ELE	EHE
Mean	50.25	211.25	157.875	141
SD	23.09	27.11	44.29	37.5
SE	8.163	9.584	15.66	13.26
t, df	t=6.156, df=7	t=22.04, df=7	t=10.08, df=7	t=10.63, df=7
p-value	0.0005	<0.0001	<0.0001	<0.0001
p-value summary	***	****	****	****
Significance	Yes	Yes	Yes	Yes
95% CI	30.95 to 69.55	188.6 to 233.9	120.8 to 194.9	109.6 to 172.4
R squared	0.8441	0.9858	0.9356	0.9417

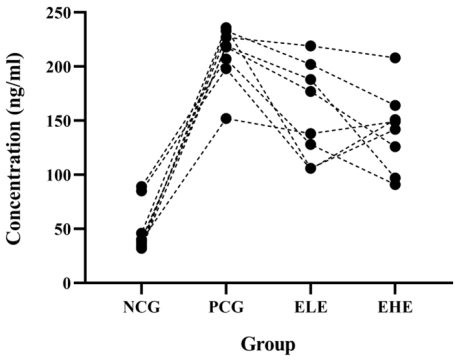

Figure 9. Concentrations of MDA (ng/ml) among the mice of various study groups

4.2. Discussion

As observed in this study, acute liver injury is characterized by a rapid decline in liver function, often indicated by a significant increase in serum levels of liver enzymes, most notably ALP, ALT, AST, TB and LDH due to leaking of these intracellular enzymes that existed within the hepatocytes into bloodstreams. However, the extent of enzyme elevation is typically correlates with the severity of liver damage, although the specific pattern of enzyme elevation can provide clues about the nature and location of the injury (Andrade *et al.*, 2019; Kalas *et al.*, 2021). Inflammatory responses that result from liver injury serve as a major factor that harms hepatocytes while releasing enzymes. Macrophages as well as neutrophils invade the injured liver tissue where they activate numerous inflammatory mediators which intensify hepatocellular damage (Liu *et al.*, 2021; Gong *et al.*, 2023). Prolonged adaptations to injury might occasionally create an environment that makes liver cells more sensitive to subsequent external trauma (Chen *et al.*, 2024). The inflammatory response outcome and liver damage result from how pro-inflammatory signals and anti-inflammatory signals maintain equilibrium between each other (Hammerich & Tacke, 2023). The hepatocyte death processes including apoptosis, necrosis and necroptosis contributed to releasing liver enzymes (Shojaie *et al.*, 2020). The mechanisms leading to enzyme release differ according to the kind and extent of liver damage. The programmed cell death known as apoptosis results in controlled cell shrinkage with fragmentation while memory enzymes might escape during this process but unverified research indicates that necrosis produces a greater release of liver enzymes because of its association with uncontrolled cell lysis and inflammation (D'arcy, 2019; Obeng, 2020). When necrosis occurs the body releases intracellular substances which start inflammatory responses that worsen liver damage (Lemasters & Jaeschke, 2020). Hypoxic hepatic tissue provokes reactive oxygen species (ROS) production most prominently in mitochondria and then stimulates endoplasmic reticulum stress and ultimately leads to cell necrosis (Tang *et al.*, 2022). The process of necroptosis leads to programmed necrosis which plays a role in liver damage in situations involving ischemia-reperfusion injury and drug-induced liver injury (Baidya *et al.*, 2020). The combined effect of all these processes creates a trophic increase of liver enzymes throughout circulation which indicates acute liver injury (Ma *et al.*, 2024).

In current study, mulberry leaf extract revealed a significant efficacy in ameliorating of acute liver injury through decreasing of liver enzymes in animals injected with acetaminophen. Worldwide, several studies demonstrated that mulberry leaf extract has multiple pharmacological properties that consist of antidiabetic activities as well as anti-inflammatory and neuroprotective abilities and antioxidant functions and anticancer potential (Faisal & Al-Saadi, 2024; Fatima *et al.*, 2024; Lin *et al.*, 2025). The research field has shown growing interest in mulberry leaf extract as a hepatoprotective compounds by demonstrating how it enhances glucose metabolism and decreases blood sugar in vitro and in vivo experiments (Zheng *et al.*, 2024; Mao *et al.*, 2025). Hepatoprotective mechanisms in mulberry leaf extract stem from multiple facets involved removal of ROS and suppression of inflammatory pathway with controlling hepatic cells. The antioxidant compounds of mulberry leaf extract containing flavonoids and phenolic acids theoretically act to neutralize free radicals that form during hepatic damage in a way that prevents hepatocyte oxidative stress and subsequent enzyme excretion (Liang *et al.*, 2021; Yu *et al.*, 2022; Abbas *et al.*, 2024). Anti-inflammatory elements within mulberry leaf extract aid liver enzyme elevation control through their suppression of pro-inflammatory cytokines such as tumor necrosis factor-alpha and interleukin-6 that contribute

to liver injury pathogenesis (Yang *et al.*, 2022; Saxena *et al.*, 2023). Studies showed that mulberry leaf extract has the ability to control hepatic cell activation which is essential for the prevention of chronic liver disease progression (Lee *et al.*, 2020; James *et al.*, 2024).

Our finding demonstrated that the concentrations of serum antioxidants (CAT, GSH-Px, and SOD) were decreased significantly; while, the lipid peroxidation of serum MDA was elevated significantly. This decrease in antioxidants plays a critical role in pathogenesis and progression of liver damage since the stress becomes oxidative when ROS production exceeds the ability of antioxidant defense mechanisms to inactivate these substances (Sachdev *et al.*, 2021; Allameh *et al.*, 2023). Glutathione operates as a vital component within the defense system because it maintains action as a major antioxidant and redox regulator in cells (Brigelius-Flohé & Flohé, 2020). Acute liver injury creates an intense pressure on glutathione consumption because the liver works to detoxify harmful agents while controlling oxidative damage progression which depletes glutathione levels and reduces its total liver presence (Vairetti *et al.*, 2023). This usually leads to ischemia-reperfusion injury when performing liver resection or transplantation along with cardiac arrest and hemorrhagic shock scenarios (Zhang *et al.*, 2019). The restricted blood supply during ischemia results in hypoxia of liver tissue that leads mitochondria to produce ROS. When ROS production reaches high levels, it exceeds hepatic antioxidant capacity, which causes cell components, including lipids, proteins, and DNA to become damaged (Juan *et al.*, 2021; Tang *et al.*, 2022). After blood flow returns to the injured tissue, it surprisingly produces worse damage through the delivery of oxygen-containing fluids and inflammatory agents (Li *et al.*, 2022). The influx of oxygen into injured hepatic tissue facilitates more ROS production and results in discharge from activated inflammatory cells both ROS and pro-inflammatory cytokines (Tang *et al.*, 2022). The elevated free radicals in the body trigger lipid peroxidation MDA and protein cross-linking as well as DNA fragmentation (Valgimigli, 2023). The prolonged existence of lipotoxicity and inflammation creates an ongoing pattern of augmented ROS production combined with inflammation and cellular destruction that ultimately activates hepatic cells as they generate a fibrogenic extracellular matrix (Zisser *et al.*, 2021).

5. CONCLUSION

Since our study might represent the first Iraqi one insight hepatoprotective role of mulberry leaf extract in experimentally induced acute liver injury, suggesting the importance of furthermore studies for other parts of mulberry or extracts of other plants. The high levels of liver enzymes and MDA with low levels of antioxidants clearly reflect liver damage progression which enables healthcare providers to use it both for diagnostic purposes and prognosis estimation and therapeutic response assessment.

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