JOURNAL OF
BIOLOGICAL
INSIGHTS

Vol. 1| Issue 1|pp:60-76| 2025 |
DOI: 10.4197/jbi.1-1.3

Sharing Knowledge, Shaping the Future

Published by Scientific Publishing Center, King Abdulaziz University

Research Article

Received: 2025/09/28| **Accepted**: 2025/11/22| **Published**: 2025/12/29|

Handling Editor: Irfan A. Rather | Department of Biological Sciences| King Abdulaziz University |Jeddah| Saudi Arabia

Hepatoprotective Potential of *Malus domestica* Seed Oil Against Thioacetamide-Induced Toxicity in Rats: Biochemical, Histopathological, and *In Silico* Study

Rayan A. Alghamdi[1], Atef M. Al-Attar[1, 3], Mohammed Y. Alomar[1], Yousef G. Alghamdi[4], Saleh A. Almuntashiri[1], Dhaifallah M. Alzahrani[1], Isam M. Abu Zeid[1, 2, 3,*]

[1]Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.
[2]Centre of Excellence in Bionanoscience, King Abdulaziz University, Jeddah, Saudi Arabia.
[3]Princess Dr. Najla Bint Saud Al-Saud Center for Excellence Research in Biotechnology, King Abdulaziz University, Jeddah, Saudi Arabia.
[4]Department of Chemistry Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia.

*Corresponding Author: ialmuan@kau.edu.sa

Abstract

Objective: To evaluate the hepatoprotective effects of *Malus domestica* against thioacetamide (TAA) induced liver injury in male rats and to explore a TNF-α–centered mechanism using molecular docking. **Methods:** Male Wistar rats (n = 40) were randomized into four equal groups (control, TAA, *Malus domestica* + TAA, *Malus domestica* only). TAA (300 mg/kg, twice weekly) and *Malus domestica* (800 mg/kg/day) were administered for 6 weeks according to the experimental protocol. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, albumin, and total protein were quantified, and liver histology was assessed. Molecular docking against TNF-α (PDB ID: 2AZ5) was performed using AutoDock-GPU, and the top-ranked poses and ligand–residue contacts were analyzed. **Results:** TAA administration significantly increased ALT, AST, ALP, and total bilirubin levels, while decreasing albumin and total protein relative to controls. *Malus domestica* co-treatment significantly reversed these perturbations, with values approaching control levels; *Malus domestica* alone remained comparable to normal. Histopathology corroborated biochemical protection, showing preserved hepatocyte morphology and intact sinusoids in *Malus domestica* treated groups versus necro-inflammatory lesions in rats injected with TAA. Docking analysis indicated that multiple *Malus domestica* phytochemicals occupy the TNF-α inhibitory pocket with favorable predicted affinities and recurrent contacts. **Conclusion:** *Malus domestica* confers robust hepatoprotection in the TAA-induced liver injury model, plausibly through combined antioxidant and anti-inflammatory mechanisms that may involve engagement of the TNF-α binding pocket.

Keywords: *Malus domestica*, thioacetamide, liver, biochemical parameters, *in silico*

1. Introduction

The liver is a unique organ with remarkable capabilities for repair and regeneration; processes associated with survival and recovery after diverse injuries. Previous research suggested that hepatic recovery depends not only on the regeneration of individual hepatocytes but also on other cellular interactions [1]. The liver, a complex organ, is essential for controlling physiological functions such as metabolism, detoxification, protein synthesis, and immunological response [2]. These functions are predominantly facilitated by hepatocytes, the principal parenchymal cells in the liver. The liver's non-parenchymal cells (NPCs), including liver sinusoidal endothelial cells (LSECs), hepatic stellate cells (HSCs), cholangiocytes, Kupffer cells (KCs), and many immune cell types, contribute to the maintenance of liver homeostasis [3].

Liver diseases encompass a diverse range of ailments marked by hepatocyte damage, infiltration of inflammatory cells, and activation of hepatic stellate cells, thereby compromising liver function and altering its structure [4]. Liver illnesses are associated with over 2 million fatalities each year, constituting 4% of worldwide mortality [5]. Acute liver illnesses frequently arise from infections by hepatotropic viruses, although drug-induced liver injury (DILI) is also becoming more common globally. Chronic liver diseases generally stem from causes such as alcohol intake, infections by the hepatitis B virus (HBV) and hepatitis C virus (HCV), as well as an increasing prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD) worldwide [6].

Thioacetamide, a sulfur-containing chemical commonly utilized as a prototype for producing liver fibrosis and cirrhosis, exhibits hepatotoxic effects *via* metabolic activation by cytochrome P450 enzymes in the liver. The toxic metabolite, TAA-S-oxide, initiates an intricate pathogenic sequence mainly by producing reactive oxygen species (ROS) and causing oxidative stress in liver tissue [7]. TAA oxidative damage induces increased translocation of nuclear factor erythroid 2-related factor-2 (Nrf2) to the nucleus, where it associates with antioxidant response elements to modulate essential antioxidant enzymes, such as glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT), which collaboratively neutralize ROS [8–10]. Simultaneously, TAA stimulates Kupffer cells to secrete pro-inflammatory cytokines such as interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α), thereby initiating an inflammatory cascade [11].

Medicinal plants (MPs) have historically contributed to human health and played a key role in combating a wide range of diseases [12–14]. Today, medicinal plants remain a cornerstone of primary healthcare, particularly in developing countries, where a large proportion of the global population still relies on them. Approximately 65% of pharmaceutical medications are produced from or derived from plant sources [15]. The apple (*Malus domestica* Borkh.) belongs to the genus *Malus*, which comprises approximately 30–55 interfertile species. It is believed to have originated in the region between the Caspian and Black Seas and is now one of the most widely cultivated fruit crops in temperate regions worldwide [16–18]. Archaeological evidence indicates that apples have been cultivated for at least 3,000 years and have held considerable cultural, spiritual, gastronomic, and economic significance for centuries [19,20]. Apples constitute 12.5% of global fruit consumption, ranking as the third most-produced fruit [21]. This fruit is well-known for its nutraceutical properties, offering vital nutrients and

bioactive substances including ascorbic acid, polyphenols, and pectin. It encompasses a broad spectrum of bioactive chemicals, including hydroxycinnamic acids, chlorogenic acid, epicatechin, phloridzin, flavan-3-ols, dihydrochalcones, dehydroascorbic acid, and carotenoids [22–26]. Moreover, it serves as a superior source of polyphenols and fibers, possessing antioxidant, anticancer, antibacterial, and anti-inflammatory attributes, while providing cardioprotective and immune-modulating benefits [27–29]. Apple polyphenols comprise flavonols (mostly catechin and proanthocyanidins), hydroxycinnamates, dihydrochalcones, anthocyanins, procyanidin dimers to pentadecamers, and phloretin glycosides, the latter being peculiar to red apples [30]. Considering these pharmacological features, the bioactive components in apple seed oil were chosen for assessment due to their potential antioxidant and hepatoprotective effects. This study seeks to assess the impact of active chemicals in apple seed oil on TAA-induced hepatitis in male rats.

2. Materials and Methods

2.1 Apple seed oil

Apple seed oil was obtained from Cocojojo (organic, Santa Ana, CA, USA; https://cocojojo.com).

2.1.1 Drugs and chemicals

Thioacetamide was obtained from Sigma-Aldrich (Cat. No. 172502; St. Louis, MO, USA).

2.2 Animals and experimental design

Male albino Wistar rats (*Rattus norvegicus*), weighing 100–120 g, were obtained from the Animal House, Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University. The experiments were performed in the Training Laboratory for Animal Experiments, Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University. Rats were accommodated in well-ventilated conventional plastic cages and kept under regulated laboratory conditions of humidity (65%), a consistent temperature (20 ± 1 °C), and a 12-hour light/12-hour dark cycle daily. Rats were provided with regular laboratory chow *ad* libitum and had unrestricted access to water. The experimental procedures adhered to the animal ethics rules established by the Animal Care and Use Committee (ACUC) of King Abdulaziz University. Furthermore, all studies were executed in adherence to the ARRIVE recommendations and in conformity with the EU Directive 2010/63/EU regarding animal experimentation.

Rats were randomly divided into four groups (n = 10/group), and the experiment was conducted for 6 weeks. Rats in the first group served as controls. Rats of the second group were given 300 mg/kg bw/day of TAA by intraperitoneal injection, twice weekly. Rats of the third group were orally supplemented with *Malus domestica* at a dose of 800 mg/kg bw/day and they were intraperitoneally injected with TAA at the same dose given to the second group. Rats of

the fourth group were orally supplemented with *Malus domestica* at the same dose given to the third group.

2.3 Blood collection and serum biochemical analyses

Animals were subjected to anaesthesia using diethyl ether. Blood specimens were collected from the ocular venous plexus into non-heparinized tubes, centrifuged at 2500 rpm for 15 minutes, after which the serum was collected and preserved at −80 °C for the subsequent examination of chosen biochemical parameters. Serum specimens were employed to ascertain the amounts of ALP, AST, ALT, total bilirubin, total protein, and albumin.

2.4 Histopathological examination

Liver specimens were preserved in 10% neutral buffered formalin and processed using an automated tissue processor. Initially, tissues were fixed in 10% buffered formalin for a duration of 48 hours, subsequent to washing in purified water for thirty minutes. Desiccation was performed through a graded series of ethanol solutions (70%, 90%, and 100%), consisting of immersion in 70% ethanol for two hours, 90% ethanol for a duration of 90 minutes, and two successive changes in absolute ethanol for 60 minutes each. Tissue clearing was performed in xylene, with samples immersed for 1 hour in a 50:50 alcohol–xylene solution and subsequently for 90 minutes in pure xylene. The tissues were thereafter infiltrated with molten paraffin wax, embedded, and sectioned using a manual rotary microtome (HistoCore BIOCUT; Leica Biosystems, USA). Paraffin sections (3–5 μm) were stained with hematoxylin and eosin (H&E) for histopathological evaluation [31]. Representative histological micrographs from the control and all treated groups were acquired using a light microscope (BX61; Olympus, USA) equipped with a motorized controller (BX-UCB; Olympus, USA) and a digital camera (DP72; Olympus, USA).

2.5 Bioinformatics Analysis

2.5.1 Ligand properties

The bioactive constituents of apple seed oil were obtained from reputable scientific databases, including PubChem (https://pubchem.ncbi.nlm.nih.gov) and others. The results supplied essential chemical information necessary for computer analyses and formed the foundation for further molecular docking and interaction research.

2.5.2 Protein selection

In this study, the key protein associated with liver disorders was selected: TNF-α (PDB ID: 2AZ5). This protein was chosen for its biological significance and its close association with hepatic pathological processes. The three-dimensional crystal structure was retrieved from the RCSB Protein Data Bank (https://www.rcsb.org), one of the largest and most reliable repositories of experimentally determined molecular structures (**Figure 1**).

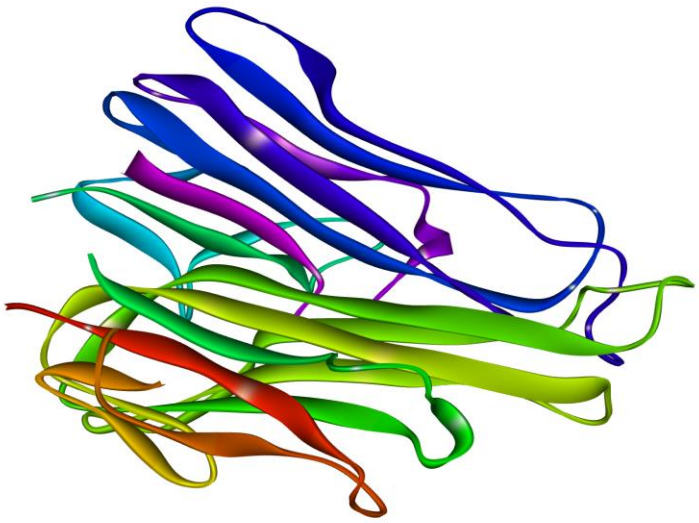

Figure 1. Three-dimensional crystal structure of TNF-α (PDB ID: 2AZ5). retrieved from the RCSB

2.5.3 Protein Preparation

Missing atoms in the protein were rebuilt and corrected using CHARMM-GUI (https://www.charmm-gui.org/). Polar hydrogen atoms were subsequently added, and appropriate partial charges were assigned. The protein structure was then prepared for molecular docking using AutoDockTools (version 1.5.7; https://autodock.scripps.edu), after which crystallographic waters, co-crystallized ligands, and other non-essential heteroatoms were removed. The final receptor model was saved in PDBQT format for subsequent docking studies.

2.5.4 Molecular docking procedure

The active site of the target protein was identified based on the position of the co-crystallized ligand present in the retrieved protein structure from the PDB. The ligand served as a reference to determine the binding pocket within the protein's three-dimensional structure. After defining the active site coordinates, the native ligand was removed, and the selected phytochemical compounds from the studied medicinal plants were docked into the same binding pocket using molecular docking techniques.

This strategy guaranteed that all ligands were assessed within identical binding site conditions, thereby ensuring consistent and comparable docking outcomes. Molecular docking was performed using AutoDock-GPU (https://github.com/ccsb-scripps/AutoDock-GPU). The grid box was designed to cover the full protein structure, enabling ligands to probe all possible binding regions without introducing bias. Standard docking parameters were employed, with two exceptions: the number of runs was increased to 300 (nrun = 300) and the population size was set at 500 (pop size = 500), thereby enhancing both the precision and variability of the predicted docking outcomes.

The Lamarckian Genetic Algorithm (LGA) implemented in AutoDock 4.2.6 was employed to explore the optimal conformations of the ligands. Following docking, the generated poses were ranked according to their binding energies (kcal/mol), and the top-ranked conformations were chosen for subsequent interaction analysis with the target protein.

2.6 Statistical Analysis

Data were analysed using IBM SPSS Statistics, version 24 (IBM Corp., Armonk, NY, USA). To compare groups, a one-way analysis of variance (ANOVA) was applied. When significant differences were detected, pairwise group comparisons were performed using the Least Significant Difference (LSD) post-hoc test. A P-value of ≤ 0.05 was considered indicative of statistical significance.

3. Results

3.1 Biochemical Parameters

Serum levels of ALT, AST, ALP, and total bilirubin were markedly elevated in the TAA-intoxicated group compared with the normal control group 1 ($p < 0.001$). Treatment of rats with *Malus domestica* seed oil significantly reduced these elevations in both group 3 (TAA + *Malus domestica*) and group 4 (*Malus domestica* only) ($p < 0.001$), with levels in group 4 approaching normal values, while those in group 3 remained slightly higher than controls (Figure 2a–d).

TAA intoxication caused a significant reduction in serum albumin and total protein levels compared with the control group 1 ($p < 0.01$). Administration of *Malus domestica* seed oil significantly restored both parameters in group 3 and group 4 compared with group 2 ($p < 0.05$–0.01), with group 4 values approaching those of the control group (Figure 2e–f).

3.2 Histopathological Studies

Histopathological examination of liver tissues is presented in Figures 3a–f. The normal control group displayed preserved hepatic architecture with radially arranged cords of polygonal hepatocytes, centrally located nuclei, intact sinusoids, and no evidence of necrosis or inflammatory infiltration (Figure 3a). In contrast, the TAA-intoxicated group exhibited severe histopathological alterations, including hepatocellular degeneration, cytoplasmic vacuolation, nuclear pyknosis, vascular congestion, inflammatory infiltration, and focal necrosis, reflecting extensive hepatic injury (Figure 3b–d). Rats treated with *Malus domestica* oil in combination with TAA showed markedly improved hepatic architecture, with preserved hepatocyte morphology, intact sinusoids, and reduced vascular congestion, while necrosis and inflammation were largely absent, indicating a hepatoprotective effect of the oil (Figure 3e). Liver sections from the *Malus domestica* oil only demonstrated normal histoarchitecture comparable to the control group, with well-arranged hepatocytes and intact sinusoids, and no evidence of pathological alterations, confirming the non-toxic nature of the oil (Figure 3f).

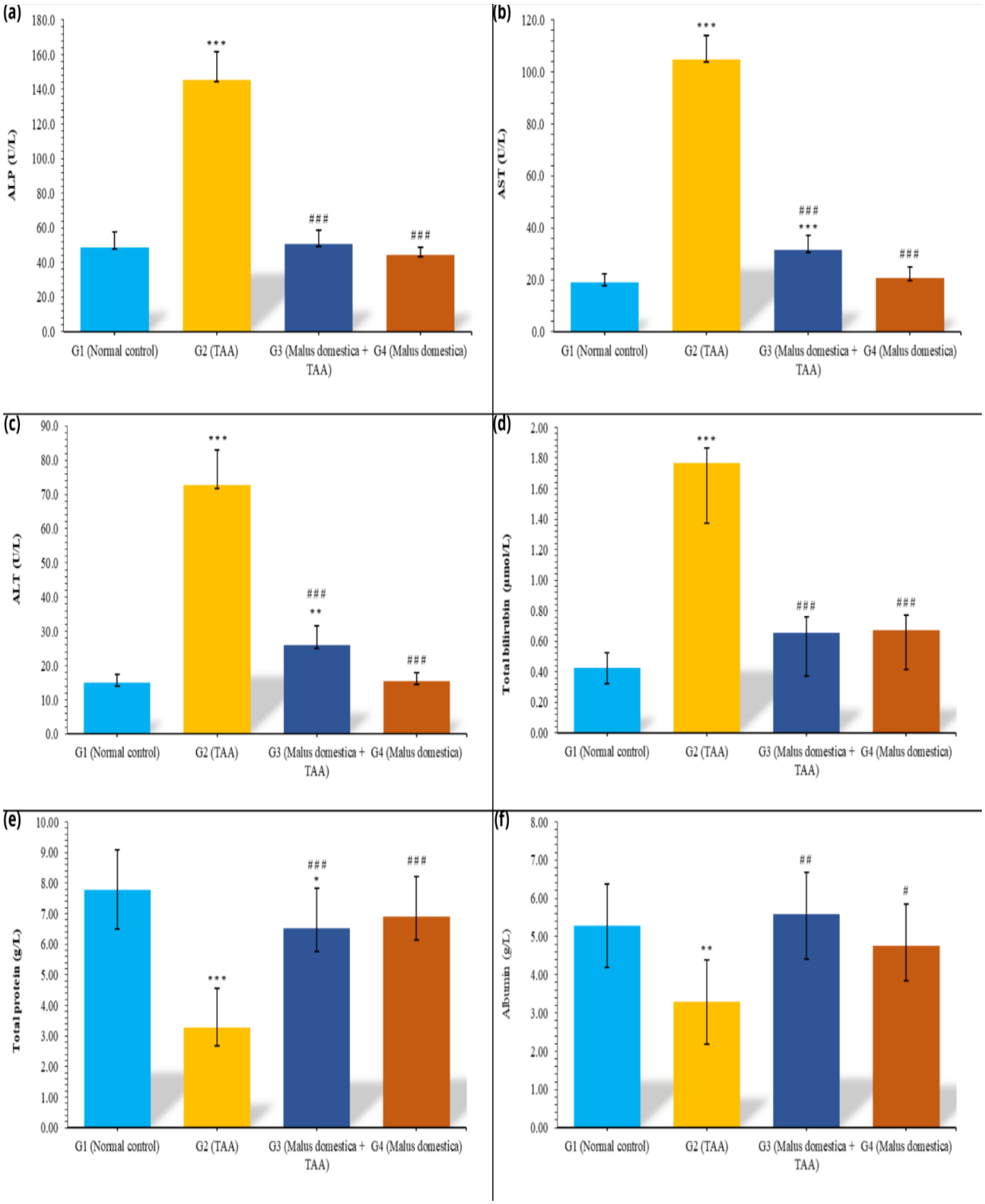

Figure 2. Serum levels of ALP (**a**), AST (**b**), ALT (**c**), total bilirubin (**d**), total protein (**e**), and albumin (**f**) in normal control, TAA-intoxicated, *Malus domestica* + TAA-treated, and *Malus domestica*-treated rats. Significant differences compared with the normal control group are denoted by $*P < 0.05$, $**P \leq 0.01$, and $***P \leq 0.001$. Significant differences compared with TAA-intoxicated rats are denoted by $\#P \leq 0.05$, $\#\#P \leq 0.01$, and $\#\#\#P \leq 0.001$. The number of symbols corresponds to the level of statistical significance, with more symbols indicating a higher level of significance.

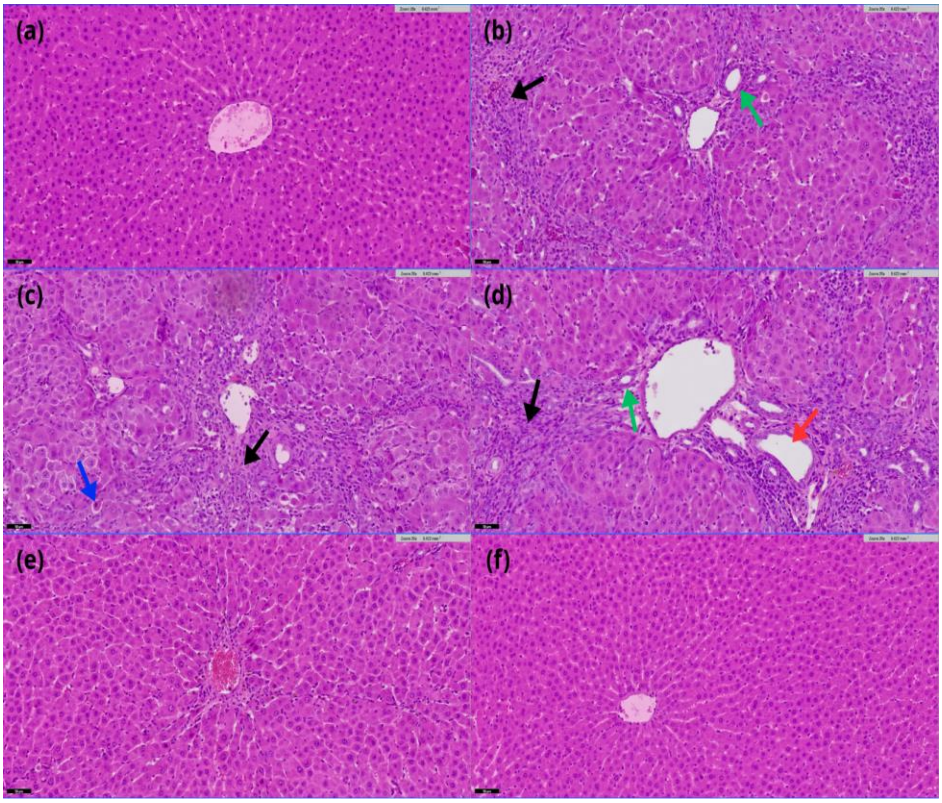

Figure 3. Representative photomicrographs of liver sections (a–f). (a) A normal hepatic architecture is observed, with hepatic cords neatly arranged and radiating from the central veins. (b–d) demonstrate pronounced architectural distortion with irregular ductular proliferations (black arrows) and thick-walled, remodelled portal vein branches (red arrows), together with hepatocellular degeneration accompanied by inflammatory cell infiltration and apoptosis (blue arrows) and an increased number of bile duct profiles (green arrows). (e) exhibits moderate architectural recovery, accompanied by partial inflammatory resolution and marked preservation of normal hepatic structure. (f) The *Malus domestica* treated group exhibits essentially normal hepatic architecture, comparable to the control, with well-organized hepatocyte plates and intact central veins. Original magnification: ×200.

3.3 Molecular docking analysis

Table 1 summarizes the binding affinities, interacting and non-interacting residues of the ligands with the target protein TNF-α. The ligand–protein complexes were further examined using BIOVIA Discovery Studio Visualizer, which revealed multiple types of interactions at the active sites (Figure 4a–j).

Table 1. Docking scores of receptor protein (2AZ5) with ligands (phytochemicals of *Malus domestica*) and interactions with amino acid residues.

Ligand	Binding affinity (Kcal/mol)	Amino acids involved	
		H-bond interaction	Non-H-bond interaction
Aglycone	-9.25	Leu120	Tyr59, Tyr119, Pro117
Procyanidin	-8.63	Tyr151, Ile58	Tyr119, Tyr59, Leu57
Corosolic acid	-8.03	Leu120, Gly121	Tyr119, Leu57, Ile155, Ser60
Caffeoylquinic acid	-7.75	Gly121, Tyr151 Leu120, Pro117, Lys98, Tyr119	-
Chlorogenic acid	-7.41	Tyr119, Pro117, Lys98, Tyr151, Gly121	Tyr59
Phlorizin	-6.91	Tyr151, Leu120 Gly121, Ser95, Tyr119, Lys98	Leu120
Epicatechin	-6.66	Tyr119, Ile118, Lys98, Tyr115	Pro117, Lys98, Glu116
Catechin	-6.47	Leu120, Tyr151, Gln61	Tyr119
Quercetin	-6.19	Leu120, Ser60, Gln61, Tyr151	Tyr119
Phloretin	-6.17	Tyr119, Lys98, Pro117, Tyr115, Glu116	Lys98

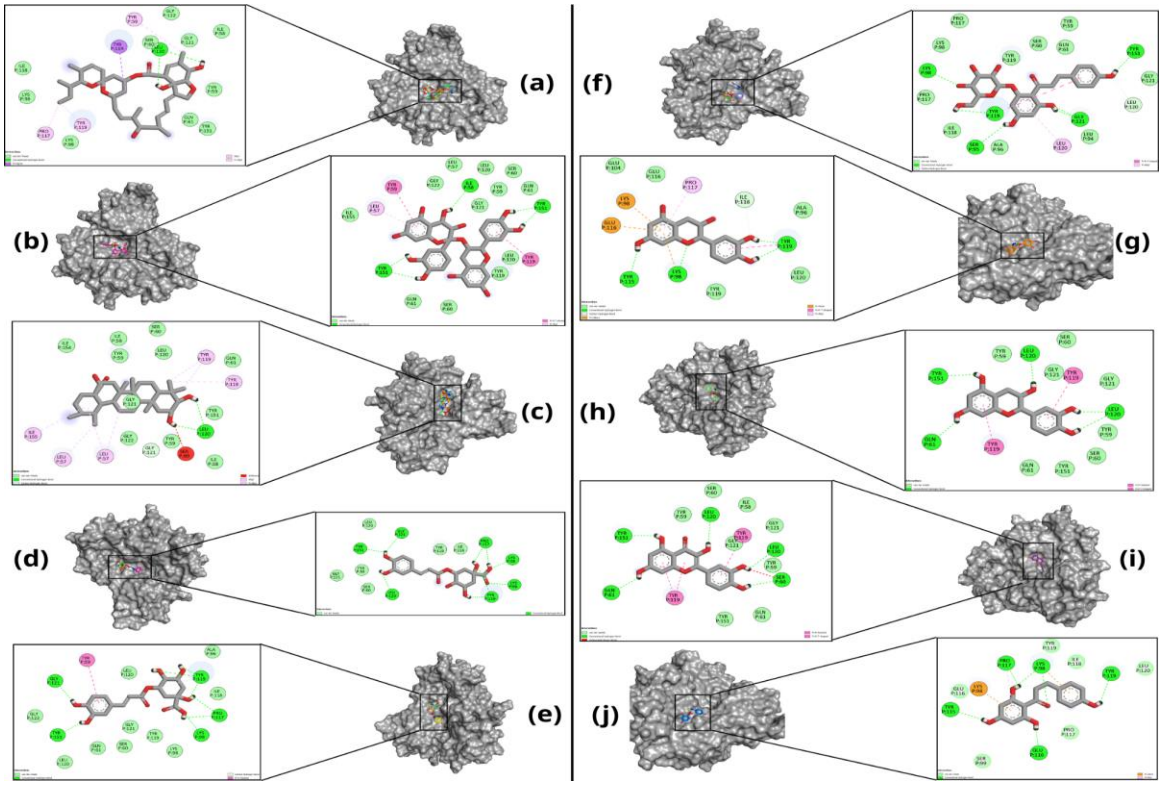

Figure 4. Docking of ligands in the active pocket of protein TNF-α a-j. Aglycone (a), Procyanidin (b), Corosolic acid (c), Caffeoylquinic acid (d), Chlorogenic acid (e), Phlorizin (f), Epicatechin (g), Catechin (h), Quercetin (i), and Phloretin (j).

4. Discussion

Chronic liver inflammation results in fibrosis, cirrhosis, and the onset of hepatocellular carcinoma (HCC) [32]. Hepatic fibrosis is the development of a fibrous scar due to the buildup of extracellular matrix (ECM) proteins, primarily crosslinked collagen types I and III, which substitute injured normal tissue [32].

Thioacetamide is one of the most significant hepatotoxicants frequently employed in rat models; upon metabolic activation it is highly reactive, leading to oxidative stress and liver fibrosis [33]. This study was designed to determine whether treatment with *Malus domestica* confers protective effects against TAA-induced hepatic injury in male rats. In the present study, serum ALT, AST, ALP, and total bilirubin were significantly elevated in TAA-treated rats compared with controls, indicating hepatocellular necrosis and membrane disruption that permit leakage of these enzymes into the circulation. These findings are consistent with previous reports [34]. Moreover, histopathological examination confirmed that TAA induces marked degenerative alterations; similar histopathological lesions were observed in the TAA-treated experimental animals [35]. The current study demonstrates that TAA compromises the structural and functional integrity of the hepatocyte plasma membrane, as evidenced by significant elevations in serum ALT, AST, and ALP, alongside an increase in total bilirubin. It is

well established that substantial disruption of the hepatocyte plasma membrane resulting in cellular leakage underlies the observed increases in serum liver enzyme activities [36]. The improvement in serum albumin and total protein further supports the hepatoprotective profile of *Malus domestica*.

Thioacetamide classically depresses hepatic protein synthesis, leading to reduced albumin, a liver-specific product and negative acute-phase reactant, and lower total protein. In this study, *Malus domestica* co-treatment mitigated these decrements, with albumin and total protein values approaching the control group, while *Malus domestica* alone remained comparable to normal. Moreover, the findings of Radwan *et al.* [37] help contextualize these biochemical results. In the present study, administration of *Malus domestica* seed oil significantly restored serum AST, ALT, and ALP toward control levels and improved total bilirubin relative to the TAA-intoxicated group. Histological examination showed preservation of hepatocyte plasma-membrane integrity and hepatic architecture, thereby mitigating TAA-induced injury and substantiating its hepatoprotective efficacy. In a similar study, it is indicated that polyphenol-rich phytochemicals lower serum ALT, AST, ALP, and total bilirubin and ameliorate histopathological fibrosis, thereby supporting a class-effect of hepatoprotection [38].

TNF-α is a cytokine that triggers tissue inflammation and is released by blood cells and damaged liver cells when an injury occurs [39]. This substance increases the permeability of blood vessels to help more immune cells reach the affected area and combat infections or injuries [40]. However, although this reaction offers some protection، heightened levels of TNF-α can result in inflammation and permanent harm to tissues, adversely impacting liver functions [41].

The molecular docking results provide a mechanistic rationale for the hepatoprotective signals observed *In vivo* by showing that multiple apple seed oil–derived phytochemicals can occupy a conserved pocket on TNF-α with favorable predicted binding energies and complementary interaction patterns. Among the tested ligands, the aglycone, procyanidin, and corosolic acid exhibited the highest predicted affinities (≈ −9.25, −8.63, and −8.03 kcal/mol, respectively). Key hydrogen-bond contacts involved residues Ile58, Tyr151, Leu120, and Gly121, whereas non-hydrogen-bond interactions were observed with Tyr59, Tyr119, Pro117, Leu57, Ile155, and Ser60. Moreover, prior studies on TNF-α signaling have reported similar docking poses and anti-inflammatory activity for these bioactive compounds, supporting multi-ligand engagement at the TNF-α small-molecule pocket and consistent with the observed *in vivo* hepatoprotection [42–44]. In contrast, other polyphenols caffeoylquinic/chlorogenic acids, phlorizin, epicatechin, catechin, quercetin, and phloretin exhibited moderately strong predicted binding energies (≈ −7.75 to −6.17 kcal/mol). Prominent hydrogen-bond contacts were mapped to residues Gly121, Tyr151, Leu120, Pro117, Lys98, Tyr119, Ser95, Ile118, Tyr115, Gln61, Ser60, and Glu116, whereas non-hydrogen-bond interactions were observed with Tyr59, Leu120, Pro117, Lys98, Glu116, and Tyr119. Moreover, previous studies have investigated structurally related plant-derived ligands targeting the TNF-α small-molecule pocket and consistently reported comparable docking affinities with recurrent contacts particularly H-bonds to Gly121, Tyr151, Leu120, Pro117, Lys98, Tyr119, Ser95, Ile118, Tyr115, Gln61, Ser60, and Glu116, and hydrophobic interactions with Tyr59, Leu120, Pro117, Glu116, and Tyr119 thereby corroborating the interaction map described herein [45–52]. However, this study has several limitations,

including the use of a single sex and strain of rats and a single TAA-induced hepatotoxicity model, the evaluation of only one treatment dose and time point, the lack of molecular dynamics simulations to assess the stability of the predicted ligand–protein complexes over time, and the absence of direct antioxidant assays to characterize oxidative stress status and endogenous antioxidant defenses. These constraints may limit the generalizability of these findings and should be addressed in future studies.

5. Conclusions

Malus domestica markedly mitigated TAA induced hepatotoxicity, evidenced by normalization of serum ALT, AST, ALP, and total bilirubin, recovery of serum albumin and total protein, and preservation of hepatic architecture. *In silico* findings provide a mechanistic basis for these effects, as multiple *Malus domestica* phytochemicals engaged the small-molecule inhibitory pocket of TNF-α with favorable predicted affinities and recurrent stabilizing contacts. Collectively, these data indicate that *Malus domestica* exerts hepatoprotection plausibly through concerted antioxidant and anti-inflammatory actions that include modulation of TNF-α signaling. Future studies should isolate and standardize active constituents, establish dose–response and pharmacokinetic profiles, and verify target engagement using biophysical and cell-based assays.

Author Contributions

The authors contributed equally.

Funding

No funding.

Institutional Review Board Statement

The study was executed in adherence to the ARRIVE recommendations and in conformity with the EU Directive 2010/63/EU regarding animal experimentation.

Informed Consent Statement

Not applicable.

Data Availability Statement

The data available are cited within the manuscript.

Acknowledgments

I would like to express my sincere gratitude to King Abdulaziz University for its outstanding services and excellent research facilities. I am also deeply thankful to the reviewers for their constructive comments and valuable feedback.

Conflicts of Interest

Author Isam M. Abu Zeid serves as Editor-in-Chief of Journal of Biological Insights. This manuscript was handled by an independent editor, and Author Isam M. Abu Zeid had no involvement in the peer-review process or editorial decision.

References

[1] Michalopoulos GK, Bhushan B. Liver regeneration: biological and pathological mechanisms and implications. *Nat Rev Gastroenterol Hepatol*. 2021;18(1):40–55. https://doi.org/10.1038/S41575-020-0342-4;SUBJMETA.

[2] Santos AA, Delgado TC, Marques V, Ramirez-Moncayo C, Alonso C, Vidal-Puig A, Hall Z, Martínez-Chantar ML, Rodrigues CMP. Spatial metabolomics and its application in the liver. *Hepatology*. 2024;79(5):1158–79. https://doi.org/10.1097/HEP.0000000000000341.

[3] Schulze RJ, Schott MB, Casey CA, Tuma PL, McNiven MA. The cell biology of the hepatocyte: a membrane trafficking machine. *J Cell Biol*. 2019;218(7):2096–112. https://doi.org/10.1083/JCB.201903090.

[4] Han H, Desert R, Das S, Song Z, Athavale D, Ge X, Nieto N. Danger signals in liver injury and restoration of homeostasis. *J Hepatol*. 2020;73(4):933–51. https://doi.org/10.1016/J.JHEP.2020.04.033.

[5] Devarbhavi H, Asrani SK, Arab JP, Nartey YA, Pose E, Kamath PS. Global burden of liver disease: 2023 update. *J Hepatol*. 2023;79(2):516–37. https://doi.org/10.1016/J.JHEP.2023.03.017.

[6] Wong MCS, Huang JLW, George J, Huang J, Leung C, Eslam M, Chan HLY, Ng SC. The changing epidemiology of liver diseases in the Asia–Pacific region. *Nat Rev Gastroenterol Hepatol. 2018 16:1*. 2018;16(1):57–73. https://doi.org/10.1038/s41575-018-0055-0.

[7] Ezhilarasan D. Molecular mechanisms in thioacetamide-induced acute and chronic liver injury models. *Environ Toxicol Pharmacol*. 2023; 99:104093. https://doi.org/10.1016/J.ETAP.2023.104093.

[8] Jomova K, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, Valko M. Several lines of antioxidant defense against oxidative stress: antioxidant enzymes, nanomaterials with multiple enzyme-mimicking activities, and low-molecular-weight antioxidants. *Arch Toxicol. 2024 98:5*. 2024;98(5):1323–67. https://doi.org/10.1007/S00204-024-03696-4.

[9] G. Bardallo R, Panisello-Roselló A, Sanchez-Nuno S, Alva N, Roselló-Catafau J, Carbonell T. Nrf2 and oxidative stress in liver ischemia/reperfusion injury. *FEBS J*. 2022;289(18):5463–79. https://doi.org/10.1111/FEBS.16336.

[10] Carmo de Carvalho e Martins M do, da Silva Santos Oliveira AS, da Silva LAA, Primo MGS, de Carvalho Lira VB. Biological indicators of oxidative stress [malondialdehyde, catalase, glutathione peroxidase, and superoxide dismutase] and their application in nutrition. *Springer*; 2022;, p. 833–56.

[11] Dwivedi DK, Jena GB. Simultaneous modulation of NLRP3 inflammasome and Nrf2/ARE pathway rescues thioacetamide-induced hepatic damage in mice: role of oxidative stress and inflammation. *Inflammation 2021 45:2*. 2021;45(2):610–26. https://doi.org/10.1007/S10753-021-01571-3.

[12] Lobato Gómez M, Huang X, Alvarez D, He W, Baysal C, Zhu C, Armario-Najera V, Blanco Perera A, Cerda Bennasser P, Saba-Mayoral A, Sobrino-Mengual G, Vargheese A, Abranches R, Abreu IA, Balamurugan S, Bock R, Buyel JF, da Cunha NB, Daniell H, Faller R, Folgado A, Gowtham I, Häkkinen ST, Kumar S, Ramalingam SK, Lacorte C, Lomonossoff GP, Luís IM, Ma JKC, McDonald KA, Murad A, Nandi S, O'Keefe B, Oksman-Caldentey KM, Parthiban S, Paul MJ, Ponndorf D, Rech E, Rodrigues JCM, Ruf S, Schillberg S, Schwestka J, Shah PS, Singh R, Stoger E, Twyman RM, Varghese IP, Vianna GR, Webster G, Wilbers RHP, Capell T, Christou P. Contributions of the international plant science community to the fight against human infectious diseases – part 1: epidemic and pandemic diseases. *Plant Biotechnol J*. 2021;19(10):1901–20. https://doi.org/10.1111/PBI.13657;JOURNAL:JOURNAL:14677652;ISSUE:ISSUE:DOI.

[13] He W, Baysal C, Lobato Gómez M, Huang X, Alvarez D, Zhu C, Armario-Najera V, Blanco Perera A, Cerda Bennaser P, Saba-Mayoral A, Sobrino-Mengual G, Vargheese A, Abranches R, Alexandra Abreu I, Balamurugan S, Bock R, Buyel JF, da Cunha NB, Daniell H, Faller R, Folgado A, Gowtham I, Häkkinen ST, Kumar S, Sathish Kumar R, Lacorte C, Lomonossoff GP, Luís IM, K.-C. Ma J, McDonald KA, Murad A, Nandi S, O'Keef B, Parthiban S, Paul MJ, Ponndorf D, Rech E, Rodrigues JCM, Ruf S, Schillberg S, Schwestka J, Shah PS, Singh R, Stoger E, Twyman RM, Varghese IP, Vianna

GR, Webster G, Wilbers RHP, Christou P, Oksman-Caldentey KM, Capell T. Contributions of the international plant science community to the fight against infectious diseases in humans—part 2: affordable drugs in edible plants for endemic and re-emerging diseases. *Plant Biotechnol J*. 2021;19(10):1921–36. https://doi.org/10.1111/PBI.13658;PAGE:STRING:ARTICLE/CHAPTER.

[14] Raskin I, Ribnicky DM, Komarnytsky S, Ilic N, Poulev A, Borisjuk N, Brinker A, Moreno DA, Ripoll C, Yakoby N, O'Neal JM, Cornwell T, Pastor I, Fridlender B. Plants and human health in the twenty-first century. *Trends Biotechnol*. 2002;20(12):522–31. https://doi.org/10.1016/S0167-7799(02)02080-2.

[15] Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *J Nat Prod*. 2020;83(3):770–803. https://doi.org/10.1021/ACS.JNATPROD.9B01285.

[16] Tian Z, Wu B, Liu J, Zhang L, Wu T, Wang Y, Han Z, Zhang X. Genetic variations in MdSAUR36 participate in the negative regulation of mesocarp cell division and fruit size in Malus species. *Molecular Breeding 2024 44:1*. 2024;44(1):1–22. https://doi.org/10.1007/S11032-024-01441-4.

[17] Manzoor M, Singh J, Gani A. Assessment of physical, microstructural, thermal, techno-functional and rheological characteristics of apple (*Malus domestica*) seeds of Northern Himalayas. *Sci. Rep. 2021 11:1*. 2021;11(1):1–10. https://doi.org/10.1038/s41598-021-02143-z.

[18] Geană EI, Ciucure CT, Ionete RE, Ciocârlan A, Aricu A, Ficai A, Andronescu E. Profiling of phenolic compounds and triterpene acids of twelve apple (*Malus domestica Borkh*.) cultivars. *Foods* 2021, Vol 10, Page 267. 2021;10(2):267. https://doi.org/10.3390/FOODS10020267.

[19] Rode PA, Bizarro GL, Spohr GA, Malmann D, Schussler M, Ferla NJ. Mite fauna on apples in southern Brazil - Review and perspectives. *An Acad Bras Cienc*. 2023;95(3):e20231113. https://doi.org/10.1590/0001-3765202320221113.

[20] Cornille A, Gladieux P, Smulders MJM, Roldán-Ruiz I, Laurens F, Le Cam B, Nersesyan A, Clavel J, Olonova M, Feugey L, Gabrielyan I, Zhang XG, Tenaillon MI, Giraud T. New insight into the history of domesticated apple: secondary contribution of the European wild apple to the genome of cultivated varieties. *PLoS Genet*. 2012;8(5):e1002703. https://doi.org/10.1371/JOURNAL.PGEN.1002703.

[21] Spengler RN. Origins of the apple: the role of megafaunal mutualism in the domestication of *Malus* and rosaceous trees. *Front Plant Sci*. 2019;10:452853. https://doi.org/10.3389/FPLS.2019.00617/FULL.

[22] Orozco-Flores LA, Salas E, Rocha-Gutiérrez B, Peralta-Pérez MDR, González-Sánchez G, Ballinas-Casarrubias L. Determination of polyphenolic profile of apple pomace (*Malus domestica* golden delicious variety) by HPLC-MS. *ACS Omega*. 2024;9(1):196–203. https://doi.org/10.1021/ACSOMEGA.3C03218/ASSET/IMAGES/LARGE/AO3C03218_0001.JPEG.

[23] Shelke V, Kale A, Kulkarni YA, Gaikwad AB. Phloretin: a comprehensive review of its potential against diabetes and associated complications. *J Pharm Pharmacol*. 2024;76(3):201–12. https://doi.org/10.1093/JPP/RGAE010.

[24] Salant LC, Socaci SA, Nour V. Quality characteristics, anthocyanin stability and antioxidant activity of apple (*Malus domestica*) and Black Chokeberry (*Aronia melanocarpa*) juice blends. *Plants* 2022, Vol 11, Page 2027. 2022;11(15):2027. https://doi.org/10.3390/PLANTS11152027.

[25] Ponder A, Jariené E, Hallmann E. The effect of storage conditions on the content of molecules in *Malus domestica* 'chopin' cv. and Their *in Vitro* antioxidant activity. *Molecules*. 2022;27(20):6979. https://doi.org/10.3390/MOLECULES27206979/S1.

[26] Macià A, Romero MP, Yuste S, Ludwig I, Pedret A, Valls RM, Salamanca P, Solà R, José Motilva M, Rubió L. Phenol metabolic fingerprint and selection of intake biomarkers after acute and sustained consumption of red-fleshed apple versus common apple in humans. The AppleCOR study. *Food Chem*. 2022;384:132612. https://doi.org/10.1016/J.FOODCHEM.2022.132612.

[27] Dadkhah Tehrani S, Keshani M, Rouhani MH, Moallem SA, Bagherniya M, Sahebkar A. The effects of apple cider vinegar on cardiometabolic risk factors: a systematic review and meta-analysis of clinical trials. *Curr Med Chem*. 2023;31. https://doi.org/10.2174/0929867331666230822102021.

[28] Sanchez-Tamayo M, Ruiz-Llacsahuanga B, Raad R, Kerr W, Critzer F. Inactivation of foodborne pathogens on gala apples by application of antimicrobial waxes. *Food Control*. 2024;155:110049. https://doi.org/10.1016/J.FOODCONT.2023.110049.

[29] Navarro-Hoyos M, Arnáez-Serrano E, Quesada-Mora S, Azofeifa-Cordero G, Wilhelm-Romero K, Quirós-Fallas MI, Alvarado-Corella D, Vargas-Huertas F, Sánchez-Kopper A. HRMS characterization, antioxidant and cytotoxic activities of polyphenols in *Malus domestica* cultivars from Costa Rica. *Molecules.* 2021, Vol 26, Page 7367. 2021;26(23):7367. https://doi.org/10.3390/MOLECULES26237367.

[30] Koutsos A, Riccadonna S, Ulaszewska MM, Franceschi P, Trošt K, Galvin A, Braune T, Fava F, Perenzoni D, Mattivi F, Tuohy KM, Lovegrove JA. Two apples a day lower serum cholesterol and improve cardiometabolic biomarkers in mildly hypercholesterolemic adults: a randomized, controlled, crossover trial. *Am J Clin Nutr*. 2020;111(2):307–18. https://doi.org/10.1093/AJCN/NQZ282.

[31] Suvarna KS, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques E-Book. *Elsevier health sciences*; 2018;

[32] Friedman SL. Liver fibrosis – from bench to bedside. *J Hepatol*. 2003;38(1):38–53. https://doi.org/10.1016/S0168-8278(02)00429-4.

[33] Ebaid H, Bashandy SAE, Morsy FA, Al-Tamimi J, Hassan I, Alhazza IM. Protective effect of gallic acid against thioacetamide-induced metabolic dysfunction of lipids in hepatic and renal toxicity. *J King Saud Univ Sci*. 2023;35(3):102531. https://doi.org/10.1016/J.JKSUS.2022.102531.

[34] Ahmed RF, Elgamal AM, El-Nashar HAS, Mowaad NA, Elgohary R, El-Saied MA, Farag MA, Imagawa H, Elshamy AI, Abd-ElGawad AM. Phenolic-rich extracts of *Teucrium oliverianum* confer protection against thioacetamide-induced liver fibrosis in rats: insights from metabolomics, biochemical and histopathological analysis. *PLoS One*. 2025;20(9):e0330595. https://doi.org/10.1371/JOURNAL.PONE.0330595.

[35] Jantararussamee C, Rodniem S, Taweechotipatr M, Showpittapornchai U, Pradidarcheep W. Hepatoprotective effect of probiotic lactic acid bacteria on thioacetamide-induced liver fibrosis in rats. *Probiot antimicrob proteins.* 2020 13:1. 2020;13(1):40–50. https://doi.org/10.1007/S12602-020-09663-6.

[36] Baraka SM, Mowaad NA, Ibrahim S, Korany RMS, El-Sayed AF, Hassan AA, Mansour DA. Green synthesized cerium oxide nanoparticles ameliorate hepatic and cognitive dysfunctions in thioacetamide-induced hepatic encephalopathy in rats: modulation of TLR-4/NF-κB/Caspase-3 signaling pathways. *J Drug Deliv Sci Technol*. 2023;87:104846. https://doi.org/10.1016/J.JDDST.2023.104846.

[37] Radwan AM, Fatoh SA, Massoud A, Tousson E. Effectiveness of curcumin nanoparticles in rat liver fibrosis caused by thioacetamide. *Environ Toxicol*. 2024;39(1):388–97. https://doi.org/10.1002/TOX.23984;WGROUP:STRING:PUBLICATION.

[38] Ahmed RF, Elgamal AM, El-Nashar HAS, Mowaad NA, Elgohary R, El-Saied MA, Farag MA, Imagawa H, Elshamy AI, Abd-ElGawad AM. Phenolic-rich extracts of *Teucrium oliverianum* confer protection against thioacetamide-induced liver fibrosis in rats: insights from metabolomics, biochemical and histopathological analysis. *PLoS One*. 2025;20(9):e0330595. https://doi.org/10.1371/JOURNAL.PONE.0330595.

[39] Wang H, Xi Z, Deng L, Pan Y, He K, Xia Q. Macrophage polarization and liver ischemia-reperfusion injury. *Int J Med Sci*. 2021;18(5):1104–13. https://doi.org/10.7150/IJMS.52691.

[40] Kim KK, Mahajan A, Patel K, Syed S, Acevedo-Jake AM, Kumar VA. Materials and cytokines in the healing of diabetic foot ulcers. *Adv Ther (Weinh)*. 2021;4(9):2100075. https://doi.org/10.1002/ADTP.202100075.

[41] Rostami M, Farahani P, Esmaelian S, Bahman Z, Fadel Hussein A, A. Alrikabi H, Hosseini Hooshiar M, Yasamineh S. The role of dental-derived stem cell-based therapy and their derived extracellular vesicles in post-covid-19 syndrome-induced tissue damage. *Stem Cell Rev Rep. 2024 20:8*. 2024;20(8):2062–103. https://doi.org/10.1007/S12015-024-10770-Y.

[42] Sinaga E, Ilyas S, Hutahaean S, Sitorus P. Hepatoprotective activity of pirdot leaves (*Saurauia vulcani* korth) ethanol extract in laboratory rats (*rattus norvegicus*) and characterization of bioactive compounds using a molecular docking approach. *Open Access Maced J Med Sci*. 2021;9:1265–70. https://doi.org/10.3889/OAMJMS.2021.7624.

[43] Endo K, Matsui R, Sugiyama M, Asami T, Inaba C, Kobayashi S, Makabe H, Tanaka S. Procyanidin B2 gallate regulates TNF-α production from T cells through inhibiting glycolytic activity via mTOR-HIF-1 pathway. *Biochem Pharmacol*. 2020;177:113952. https://doi.org/10.1016/J.BCP.2020.113952.

[44] Rivera RG, Regidor PJS, Ruamero EC, Delos Santos CDR, Gomez CB, Allanigue EJ V., Salinas M V. Applying network pharmacology and molecular docking in the screening for molecular mechanisms of ampalaya (*Momordica charantia L.*) and Banaba (*Lagerstroemia speciosa L.*) against type 2 diabetes mellitus. *Acta Med Philipp*. 2024;58(8):108. https://doi.org/10.47895/AMP.VI0.7351.

[45] Cao Z, Wang X, Zeng Z, Yang Z, Lin Y, Sun L, Lu Q, Fan G. The improvement of modified Si-Miao granule on hepatic insulin resistance and glycogen synthesis in type 2 diabetes mellitus involves the inhibition of TNF-α/JNK1/IRS-2 pathway: network pharmacology, molecular docking, and experimental validation. *Chin Med. 2024 19:1*. 2024;19(1):1–20. https://doi.org/10.1186/S13020-024-00997-9.

[46] Lakshmi MA, Prasanth D, Manjusha KG, Rao BG. *In vivo* and *In silico* anti-arthritic studies of chlorogenic acid from the rhizome of *Lasia spinosa (L.)* thwaites (*Araceae*). *Thai J Pharm Sci*. 2021;45(4):284–94.

[47] Kumar S, Chhimwal J, Kumar S, Singh R, Patial V, Purohit R, Padwad YS. Phloretin and phlorizin mitigates inflammatory stress and alleviate adipose and hepatic insulin resistance by abrogating PPARγ S273-Cdk5 interaction in type 2 diabetic mice. *Life Sci*. 2023;322:121668. https://doi.org/10.1016/J.LFS.2023.121668.

[48] Liao ZY, Hung CY, Hsu YJ, Liang IC, Chen YC, Sung CH, Hung CF. Phlorizin protects against oxidative stress and inflammation in age-related macular degeneration model. *Biomolecules*. 2025;15(4):523. https://doi.org/10.3390/BIOM15040523/S1.

[49] Joshi KR, Devkota HP, Al-Mutairi KA, Sugimura K, Yahara S, Khadka R, Thapa S, Shekh MU, Poudel S, Watanabe T. Therapeutic potential of *Leea asiatica*: chemical isolation and validation of ethnomedicinal claims through *In vitro* and *In silico* assessment of antioxidant and anti-inflammatory properties. *Heliyon*. 2024;10(19):e38074. https://doi.org/10.1016/j.heliyon.2024.e38074.

[50] Ganeshpurkar A, Saluja A. *In silico* interaction of catechin with some immunomodulatory targets: a docking analysis. *Indian J Biotechnol*. 2018;17:626–31.

[51] Costea L, Chițescu CL, Boscencu R, Ghica M, Lupuliasa D, Mihai DP, Deculescu-ioniță T, Duțu LE, Popescu ML, Luță EA, Nițulescu GM, Olaru OT, Gîrd CE. The polyphenolic profile and antioxidant activity of five vegetal extracts with hepatoprotective potential. *Plants*. 2022;11(13):1680. https://doi.org/10.3390/PLANTS11131680/S1.

[52] Chen S, Xue W, Wu Z, Lu D, Zheng L, Zhou M, Li Y, Wang Y, Liu T. Quercetin, a compound of the total flavonoids of periploca forrestii schltr., ameliorates rheumatoid arthritis by targeting TNF-α. *J Inflamm Res*. 2025;18:2879–98. https://doi.org/10.2147/JIR.S497166.

How to cite this paper:

Alghamdi RA, Al-Attar AM, Alomar MY, Alghamdi YG, Almuntashiri SA, Alzahrani DM, Abu Zeid IM. Hepatoprotective potential of *Malus domestica* seed oil against thioacetamide-induced toxicity in rats: biochemical, histopathological, and in silico study. *J Biol Insights*. 2025;1(1):60–76.

Arabic translation of title page

القدرة الوقائية لزيت بذور Malus domestica ضد السمية الكبدية الناتجة عن الثيوأسيتاميد في الجرذان: دراسة بيوكيميائية، ونسيجية مرضية، ومحاكاة حاسوبية

ريان أحمد الغامدي[١]، عاطف محمد العطار[١، ٣]، محمد يحيى أل عمر[١]، يوسف جمعان الغامدي[٤]، صالح أحمد المنتشري[١]، ضيف الله محمد الزهراني[١]، عصام محمد الأمين أبو زيد[١، ٢، ٣*]

[١] قسم علوم الأحياء، كلية العلوم، جامعة الملك عبد العزيز، جدة، المملكة العربية السعودية.
[٢] مركز التميز في علم النانو الحيوي، جامعة الملك عبد العزيز، جدة، المملكة العربية السعودية.
[٣] مركز الأميرة الدكتورة نجلاء بنت سعود آل سعود للتميز البحثي في التقنية الحيوية، جامعة الملك عبد العزيز، جدة، المملكة العربية السعودية.
[٤] قسم الكيمياء، كلية العلوم، جامعة الملك عبد العزيز، جدة، المملكة العربية السعودية.

المؤلف المراسل: **ialmuan@kau.edu.sa**

الملخص

الهدف: تقييم التأثيرات الوقائية لزيت بذور التفاح ضد الاصابة الكبدية المستحدثّة بواسطة الثيوأسيتاميد في ذكور الجرذان، وتقييم آلية ارتباط TNF-α باستخدام تطبيقات حاسوبية. **الطُرق:** وُزّعت جرذان ويستر الذكور (n = 40) عشوائيًا إلى أربع مجموعات متساوية (المجموعة الضابطة، الثيوأسيتاميد، زيت بذور التفاح + الثيوأسيتاميد، زيت بذور التفاح فقط). أُعطي الثيوأسيتاميد بجرعة ٣٠٠ ملجم/كجم داخل الصفاق مرتين أسبوعيًا، وأُعطي زيت بذور التفاح فمويًا بجرعة ٨٠٠ ملجم/كجم/يوم على مدى ٦ أسابيع وفق البروتوكول. تم قياس انزيمات الكبد والبيليروبين الكلي والألبومين والبروتين الكلي؛ وتم تقييم النسيج الكبدي. وتم اجراء التقنية الحاسوبية للمركبات النشطة ضد TNF-α لتقييم قدرتها على الارتباط بالموقع الفعّال وتثبيطه. **النتائج:** سبّب اعطاء الثيوأسيتاميد للجرذان ارتفاعًا معنويًا في انزيمات الكبد والبيليروبين الكلّي وانخفاضًا في الألبومين والبروتين الكلّي مقارنةً بالمجموعة الضابطة. وأظهر العلاج بزيت بذور التفاح تحسنًا في العوامل الكيموحيوية في موضع الدراسة، مع اقتراب القيم من المجموعة الضابطة؛ وكانت مجموعة زيت بذور التفاح وحدها مماثلةً للمجموعة الضابطة. وأكّد التحليل النسيجي الحماية من أضرار الثيوأسيتاميد مثل سلامة الجيوب الدموية في مجموعات زيت بذور التفاح مقارنةً بمجموعة الثيوأسيتاميد فقط. وأظهرت نتائج التطبيق الحاسوبي أن عدة مركبات نشطة من زيت بذور التفاح أظهرت طاقة ارتباط عالية لبروتين TNF-α. **الاستنتاج:** يُظهر زيت بذور التفاح حماية كبدية قوية في الجرذان المعرضة للثايواسيتامايد، ويُرجَّح أن يكون ذلك بسبب احتواء الزيت على مواد مضادة للاكسدة والالتهابات.

الكلمات المفتاحية: التفاح | الثيوأسيتاميد | الكبد | المعايير الكيموحيوية | التقنية الحاسوبية.