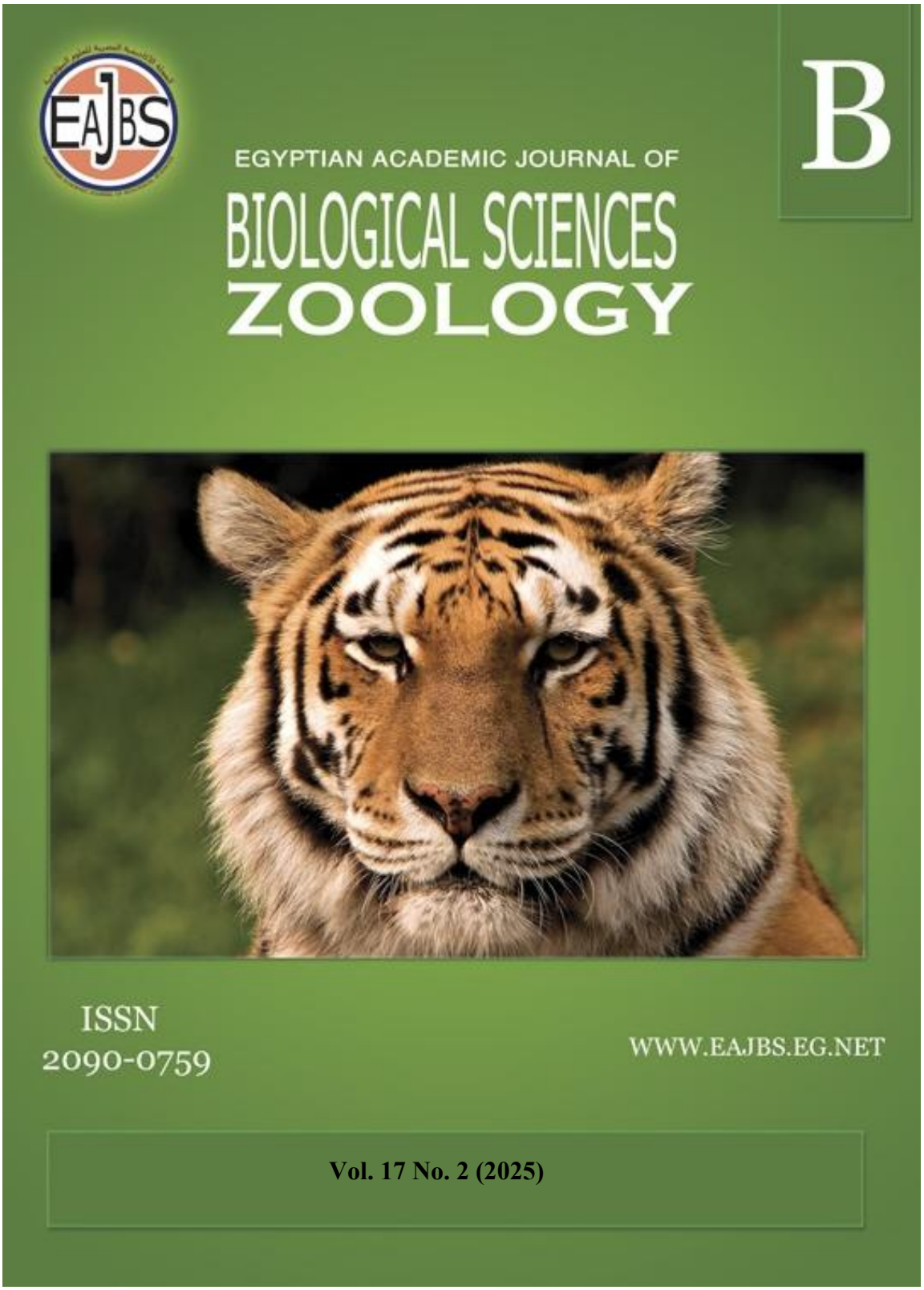


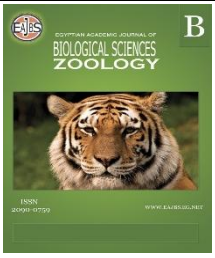
.

www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci. (B. Zoology) Vol. 17(2) pp:115-122 (2025)*
DOI: 10.21608/EAJBSZ.2025.460587

Egypt. Acad. J. Biolog. Sci., 17(2):115-122 (2025)

Egyptian Academic Journal of Biological Sciences
B. Zoology
ISSN: 2090 – 0759
Res. Ethics No.: 03-2025-0020
http://eajbsz.journals.ek b.eg/



Hepatoprotective and Antioxidant Effects of Avocado Oil (*Persea americana*) on Thioacetamide-Induced Toxicity in Male Rats

Saleh A. Al Muntashiri[1], Abdulkader M. Shaikh Omar [1,2], Atef M. Al-Attar[1,2], Rayan A. Alghamdi[1], Mohammed Y. Alomar [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 Distinguished Research Centre for Biotechnology, King Abdulaziz University, Jeddah, Saudi Arabia.

***E-mail:** salmuntashiri0010@stu.kau.edu.sa

ARTICLE INFO

Article History
Received:4/8/2025
Accepted:20/10/2025
Available:24/10/2025

Keywords:
P. americana oil, TAA, Biochemical parameters, antioxidant markers, Liver histology.
.

ABSTRACT

Thioacetamide (TAA) is a strong hepatotoxin that causes hepatocellular necrosis, apoptosis, pathological extracellular matrix fibrosis, and elevated levels of oxidative stress. *Persea americana* (*P. americana*) is a dietary palm fruit, it is well-known for its antioxidant properties, high monounsaturated fat content, and various other health benefits. This study aimed to evaluate the hepatic and antioxidant effects of *P. americana* oil in TAA-induced liver damage. Biochemical analysis, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, total bilirubin, albumin, glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT), alongside the histological examination for liver tissue. Biochemical analysis of the blood serum showed that TAA exposure impaired liver function, as evidenced by elevations in the activities of ALT, AST, and ALP, total bilirubin, and antioxidant biomarkers, along with reductions in serum albumin and antioxidant enzyme activities. Treatment with *P. americana* oil effectively normalizes serum hepatic markers, with significant reductions in MDA, and elevations in GSH, CAT and SOD levels. Histopathological examination revealed that *P. americana* oil treatment was hepatoprotective with improved architecture, decreased hepatocyte degeneration, and maintained sinusoidal integrity in treated groups. These findings underscore that *P. americana* oil may serve as a therapeutic agent for oxidative stress-related hepatic disorders.

INTRODUCTION

Thioacetamide triggers liver injury by bioactivation of metabolites, which increases oxidative stress, disrupts redox balance, and promotes inflammation and lipid peroxidation (Ezhilarasan, 2023). It also triggers hepatotoxicity in animal models, characterized by an increase in liver enzymes, including ALT, AST, ALP, and total bilirubin, along with histological deteriorations, such as fibrosis, cirrhosis, and potential hepatocellular carcinoma (Saad *et al*., 2020; Faisul & Al-Saidya, 2023). These changes reduce cellular integrity, weaken

antioxidant defenses, and cause severe hepatocellular damage, fibrosis, and organ dysfunction (Zhang & Xu, 2024). The oxidative stress and abnormal lipid profile biomarkers observed in TAA toxicity promote the need for therapeutic interventions targeting oxidative stress and pathological pathways.

Persea americana, commonly known as avocado, is native to Central America with various medicinal applications (Zakaria *et al*., 2022). The natural bioactive substances present in its oil are considered therapeutic agents for managing the context of toxin-induced injury (Abozaid *et al*., 2021). It is also a rich source of monounsaturated fatty acids, particularly oleic acid (Nascimento *et al*., 2025). Additionally, *P. americana* oil contains phytosterols, tocopherols, carotenoids, and polyphenols, which collectively exert potent antioxidant and hepatoprotective effects (Okorie *et al*., 2024). Oxidative stress is mitigated by bioactive molecules through the enhancement of antioxidant enzyme activity and restoring lipid homeostasis in different toxicity models (Ehikioya *et al*., 2023).

The protective role of *P. americana* oil in reducing oxidative stress, lipid profile abnormalities, and liver toxicity caused by TAA is still being studied, despite mounting evidence of its therapeutic benefits. The current study sought to determine how *P. americana* oil affected antioxidant markers, lipid profiles, and liver function in male rats exposed to TAA. It also sought to determine whether the oil could reverse histopathological alterations in hepatic tissue.

MATERIALS AND METHODS

Chemicals:

Thioacetamide was obtained from Sigma Aldrich Co. (St. Louis, MO, USA), and *P. americana* oil was purchased from the local market.

Experimental Design:

The rats were obtained from the Experimental Animal Unit of King Fahd Medical Research Center, King Abdulaziz University, weighing 140-160 g. The rats were divided into four groups (n=10), The following treatments with *P. americana* were administered daily for six consecutive weeks as follows:

- **Group I:** The control group received orally NaCl (0.9 %)
- **Group II:** TAA-treated group, received orally TAA (300 mg/kg)
- **Group III:** TAA-treated group, received orally TAA (300 mg/kg, per orally) + *P.* americana (800 g/kg)
- **Group IV:** PSO *P.* americana -treated group, received *P. amer**ica**na* (800 g/kg)

Liver Function Parameters:

Serum ALT and AST were measured using the Reitman and Frankel (1957) method. ALP was determined using the Bowers Jr and McComb (1966) method. Albumin was measured using the Busher (1990) method. Total bilirubin was determined according to the Doumas *et al*. (1985) method. Total protein was assessed by the method of Peters Jr (1968).

Antioxidant parameters

Following the collection of blood samples, the oxidative stress markers selected for this experiment were analyzed using the following procedures: GST, based on the protocol described by Moron *et al*. (1979); MDA was determined according to Draper and Hadley (1990). SOD and catalase were measured based on the method of Misra and Fridovich (1972); CAT were assessed according to Aebi (1974) method.

Histological Examination of Liver Tissues:

Liver tissues were fixed in a 10% formalin solution, dehydrated in varying alcohol concentrations, cleared with xylol, and embedded in paraffin wax. For histological examination, 5 µM sections were stained with Harris hematoxylin and eosin.

Statistical Analysis:

The results were presented as mean ± standard deviation (SD) for comparison. The data were statistically analyzed using Prism® software for Windows, version 9.50 (GraphPad, USA), using one-way ANOVA, with $P<0.05$.

RESULTS

Liver Function Measurements:

Fig.1 shows the liver function biomarkers, including ALT, AST, ALP, and total bilirubin, significantly demonstrated an elevation following TAA administration while markedly reducing total protein and albumin levels compared to the control group ($P < 0.05$), indicating hepatocyte membrane rupture. However, treatment with *P. americana* oil significantly mitigated these changes, as demonstrated by the reduction of ALT, AST, ALP, and total bilirubin, alongside the restoration of total protein and albumin levels compared to the TAA group ($P < 0.05$) was observed. Additionally, treatment with *P. americana* oil showed no significant change, indicating its hepatoprotective potential.

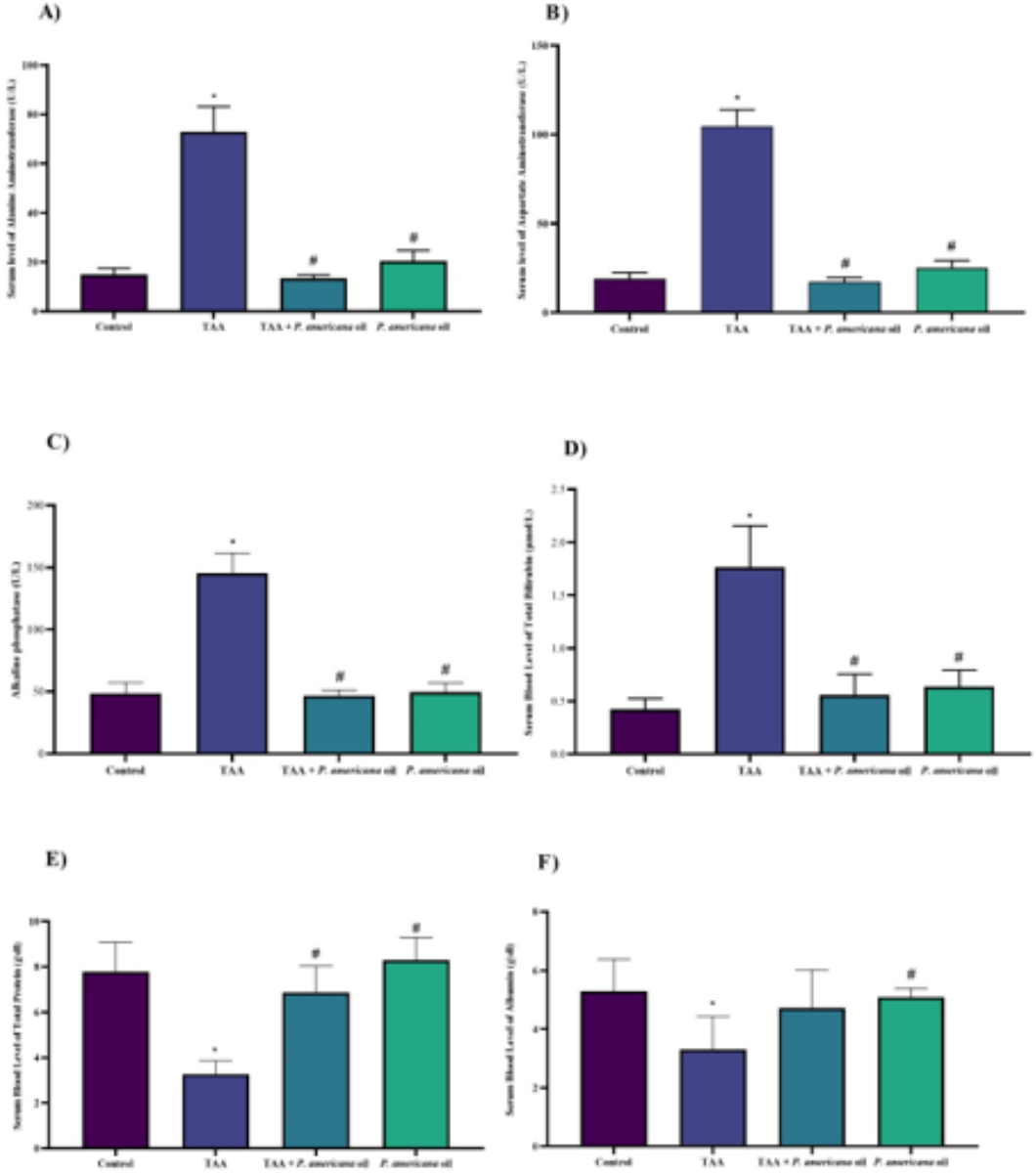


Fig. 1. Effect of *P. americana* oil on liver function biomarkers in the different studied groups. (A) ALT, (B) AST, (C) ALP, (D) total bilirubin, (E) total protein, and (F) albumin levels. Results are presented as mean ± SD. *: Significant change compared to the control group. #:

Significant change compared to the TAA group.

Antioxidant Measurements:

Fig.2 demonstrates the TAA-treated group showed a significant decrease in the serum levels of antioxidant biomarkers, including GSH, SOD, and CAT while markedly increasing MDA levels compared to the control group ($P < 0.05$), indicating oxidative stress. Conversely, treatment with *P. americana* oil restored the levels of GSH, SOD and CAT, while reducing MDA levels compared to TAA group ($P < 0.05$). Moreover, treatment with *P. americana* oil demonstrated no significant change comparable to the control group across all measured parameters, confirming its potent antioxidants and protective properties against TAA-induced oxidative damage.

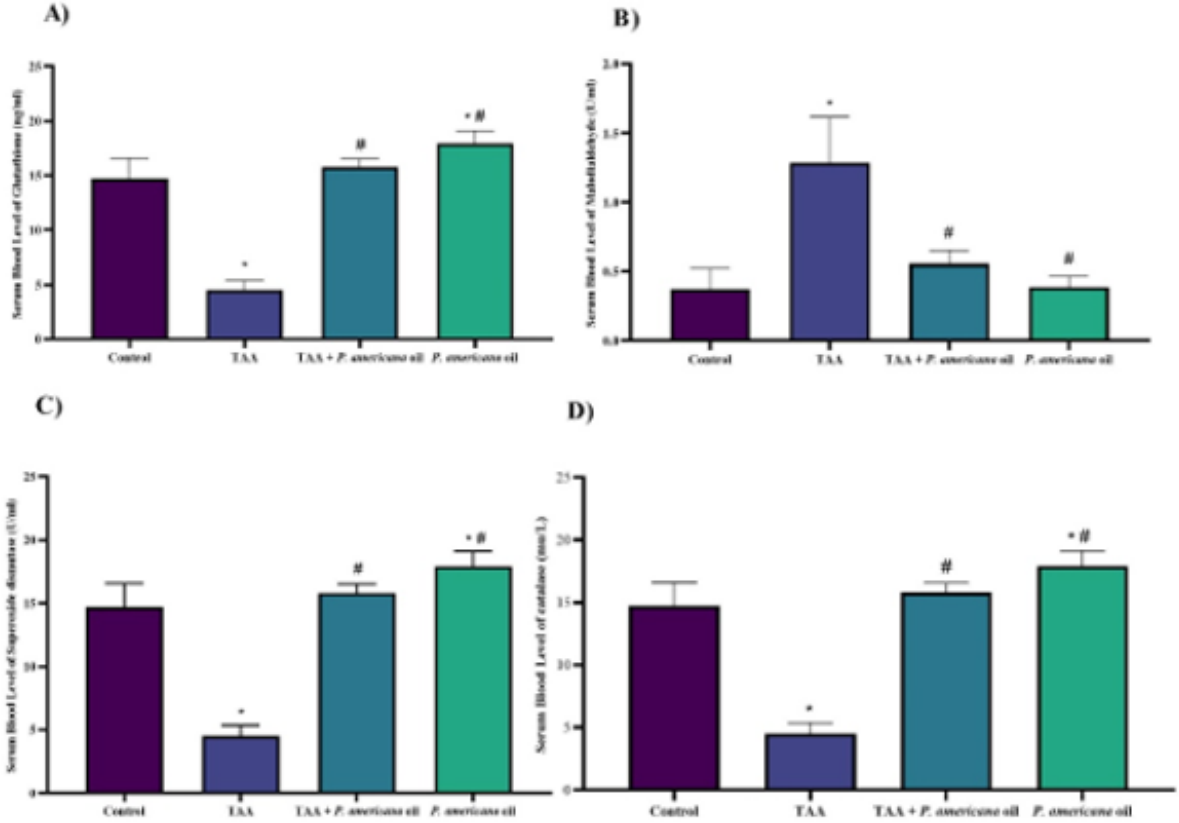


Fig. 2. Effect of *P. americana* oil on antioxidant parameters, including (A) GSH, (B) MDA, (C) SOD, and (D) CAT) in different studied groups. Results are presented as mean ± SD. *: Significant change compared to the control group. $^{\#}$: Significant change compared to TAA group.

Histological Examination of Liver Tissue:

Fig.3 shows the histological analysis revealed that the control group demonstrated normal liver architecture with intact hepatocytes, central veins, and sinusoids. The TAA-treated group showed significant hepatocellular damage, including disrupted hepatocytes, dilated central veins, and sinusoidal congestion, indicative of severe liver injury. Treatment with *P. americana* oil exhibited considerable restoration of liver structure, with reduced hepatocellular damage and improved sinusoidal organization, suggesting a protective effect of *P. americana* oil against TAA-induced damage. The *P. americana* oil group displayed liver architecture like the control group, confirming its hepatoprotective properties in maintaining normal liver histology.

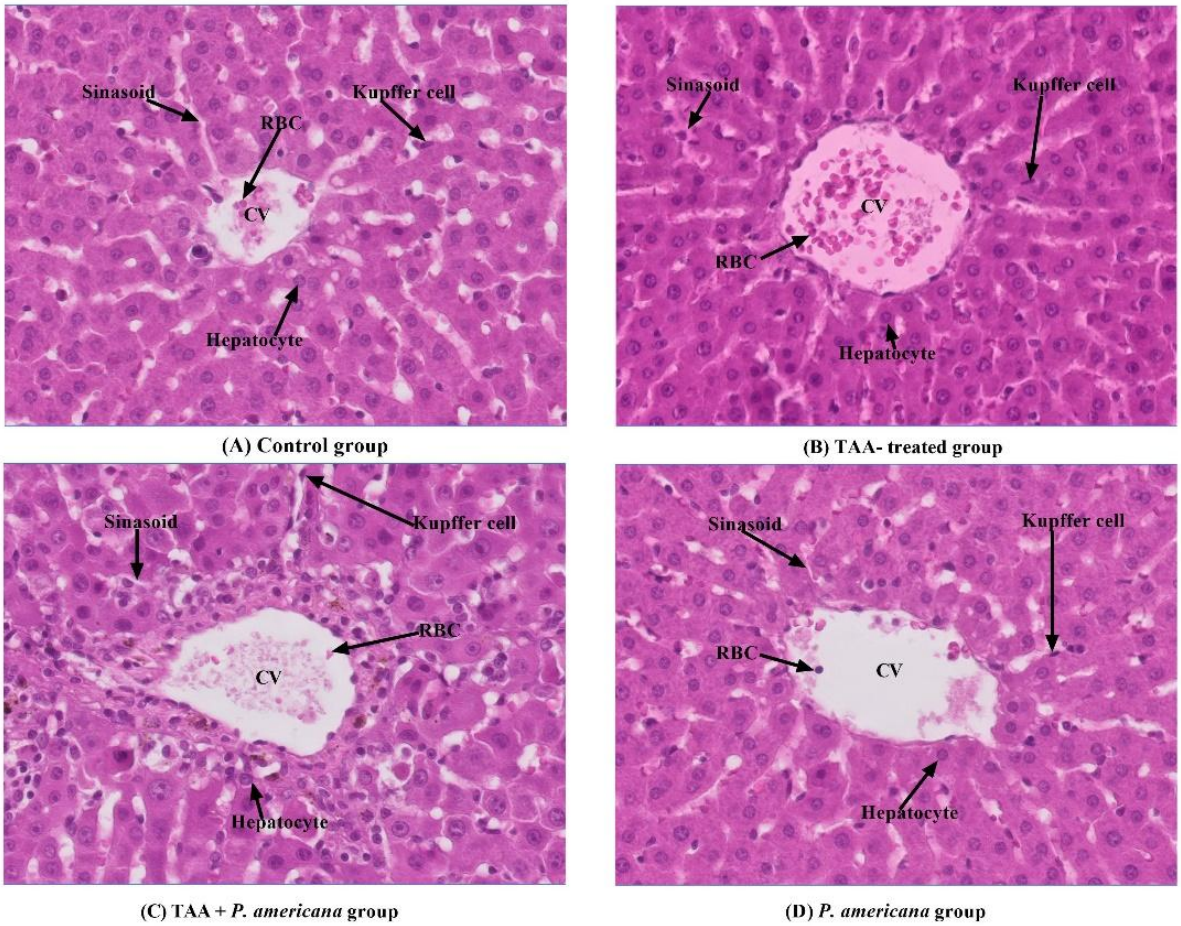


Fig. 3. Histopathological examination of lobe sections of the liver tissues (Hematoxylin & Eosin, 100x magnification) in different studied groups, showing degenerative change. (A) control group: Normal liver architecture with intact hepatocytes, central vein (CV), sinusoids, red blood cells (RBC), and Kupffer cells. (B) TAA-treated group: Marked hepatocellular damage, including disrupted hepatocytes, dilated central vein, and sinusoidal congestion, indicative of liver injury. (C) TAA + *P. americana* oil improved liver architecture with reduced hepatocellular damage, restoration of sinusoidal structure, and relatively normal Kupffer cells. (D) *P. americana* oil group: Liver tissue like the control group with intact hepatocytes, central vein, and sinusoids, demonstrating hepatoprotective effects of *P. americana* oil.

DISCUSSION

Thioacetamide is a hepatotoxin commonly used to induce liver injury due to its ability to trigger oxidative stress, lipid peroxidation, and hepatocellular necrosis (Stefanello *et al*., 2017). The presented findings collectively demonstrate the protective effects of *P. americana* oil against TAA-induced hepatotoxicity and oxidative stress through a multifaceted approach involving biochemical, functional, and histological analysis. TAA-triggered hepatotoxicity was demonstrated by a significant increase in ALT, AST, ALP, and total bilirubin levels. These findings are in line with the previous studies done by Kabiri *et al*. (2013), who showed an increase in liver biomarkers after exposure to TAA. Both Chen *et al*. (2008) and Abd El-Hameed *et al*. (2024) demonstrated that the rise in ALT, AST, and ALP alkaline phosphatase activity in the serum following TAA exposure is due to injury inflicted on the hepatocyte membranes.

For instance, the increase in total bilirubin levels can also demonstrate the obstruction in the bile excretion and the detoxification activity of the liver. This is consistent with the

finding by Joerger *et al*. (2007), who showed that these biomarkers as important indicators of biliary obstruction and liver injury. Additionally, the experimental animals administered with TAA showed a marked decrease in serum albumin and total protein levels, which suggests impairment of hepatic protein synthesis, thus indicating liver function impairment. These findings agree with the findings of Hamad Shareef *et al*. (2022), who reported that TAA administration was associated with increased hepatic enzymes and decreased albumin and total protein levels, indicating profound hepatic injury.

Persa. americana oil treatment greatly reduced elevations of ALT, AST, and ALP, indicating that it may have hepatoprotective potential by restoring hepatocyte membrane integrity and aiding healing processes in the liver. Decreased total bilirubin levels indicate recovering bile duct function and reduced cholestasis. The normalization of albumin and total protein levels confirms the resumption of hepatic protein production.

Changes in antioxidant profile were notable in the TAA-treated group with diminished GSH, SOD, and CAT levels and increased levels of MDA. These changes as a result of severe oxidative stress associated with the TAA exposure, including increased reactive oxygen species (ROS) production and a corresponding cellular redox imbalance (Dawood *et al*., 2022). The increase in MDA levels, as a marker of lipid peroxidation, signals injury to the membrane lipids that affect cell structure and function (Ezhilarasan, 2023). The application of *P. americana* oil mitigated the increase in MDA and restored GSH, SOD, and CAT levels, indicating *P. americana* oil's antioxidative property, which is probably because of its flavonoids, polyphenols, and vitamins that are known to potentiate endogenous antioxidant systems and detoxify ROS (Munthe *et al*., 2023). Antioxidant parameters were altered, showcasing the capacity of *P. americana* oil in oxidative stress mitigation, even in the absence of external factors.

Histological examination confirmed biochemical analysis results, which demonstrated morphologic changes of hepatic injury and tissue regeneration. Liver slices from the control group had their characteristic architecture with normal functioning hepatocytes, central veins, and sinusoids, which means they had preserved hepatic function. However, the hepatic tissues from the TAA exposure group showed marked and pronounced histological changes such as necrosis of liver cells, adenomegaly of central veins, and congestion of sinusoids, which indicate advanced hepatic injury.

This aligns with the observations conducted by Köhn-Gaone *et al*. (2016) and Abd El-Hameed *et al*. (2024), who demonstrated that TAA-exposure oxidative stress, accompanying TAA exposure, is responsible for the hepatocellular injury in rats as seen by the hepatocyte loss, dilated central veins, and sinusoidal changes, which ranged from hypocellularity to congestion within the liver. Unlike the others, *P. americana* oil treatment did improve the histopathological changes attributable to TAA, as the liver moved towards normal architecture. This was marked by restored hepatocytes, normal central veins, and mild sinusoidal congestion, confirming the hepatoprotective efficacy of P. americana oil. In addition, liver sections of rats given *P. americana* oil alone showed improvement of histological changes that were like the control group, proving it fulfilled the role in sustaining normal liver structure and function.

Conclusion

The results of this study indicate a strong hepatoprotective and antioxidative effect of *P. americana* oil against TAA-induced liver injury and oxidative harm, through the reactivation of antioxidant enzyme activity, decline of lipid peroxidation, normalization of liver biochemical tests, and improvement of histological changes in the liver. *P. americana* oil presents its pharmacological significance in preventing and mitigating liver injury. Future studies should aim to understand the molecular pathways that lead to these actions and explore the clinical applicability of *P. americana* oil as a treatment for liver injuries caused by oxidative stress and inflammation.

Declarations:

Ethical Approval: The experiments were processed using the animal ethical rules of King Abdulaziz University's Animal Care and Use Committee (ACUC). Furthermore, all tests adhered to the ARRIVE standards and EU Directive 2010/63/EU regarding animal research.

Competing interests: The authors declare that they have no competing interests.

Authors' Contributions: All the authors are equal in their contributions.

Funding: This research did not receive external funding.

Availability of Data and Materials: The data presented in this study are available upon fair request from the corresponding author.

Acknowledgments: Not applicable.

REFERENCES

Abd El-Hameed, S., Ibrahim, I., Awadin, W., & El-Shaieb, A. (2024). Pathological and Ultrastructural Study on the Chronic Liver Damage Induced by Thioacetamide in Male Rats. *Egyptian Journal of Veterinary Sciences*, 1-9.

Abozaid, O. A. R., Anees, L. M., & Abdel-Hamed, G. R. (2021). Modulatory effect of *Persea americana* oil against diethylnitrosamine-induced hepatotoxicity in rats: a proposed mechanism. *Bulletin of the National Research Centre*, *45*, 1-10.

Aebi, H. (1974). Catalase. In Methods of Enzymatic Analysis (pp. 673-684). Academic Press, 673-7.

Bowers, G. N., Jr, & McComb, R. B. (1966). A continuous spectrophotometric method for measuring the activity of serum alkaline phosphatase. *Clinical Chemistry*, *12*(2), 70–89.

Busher, J. T. (1990). Serum albumin and globulin. *Clinical methods: The History, Physical, and Laboratory Examinations*, *3*, 497-499.

Chen, T. M., Subeq, Y. M., Lee, R. P., Chiou, T. W., & Hsu, B. G. (2008). Single-dose intravenous thioacetamide administration as a model of acute liver damage in rats. *International Journal of Experimental Pathology*, *89*(4), 223–231.

Dawood, A. F., Al Humayed, S., Momenah, M. A., El-Sherbiny, M., Ashour, H., Kamar, S. S., ShamsEldeen, A. M., Haidara, M. A., Al-Ani, B., & Ebrahim, H. A. (2022). MiR-155 Dysregulation is associated with the augmentation of ROS/p53 axis of fibrosis in thioacetamide-induced hepatotoxicity and is protected by resveratrol. *Diagnostics (Basel, Switzerland)*, *12*(7), 1762.

Doumas, B. T., Kwok-Cheung, P. P., Perry, B. W., Jendrzejczak, B., McComb, R. B., Schaffer, R., & Hause, L. L. (1985). Candidate reference method for determination of total bilirubin in serum: development and validation. *Clinical Chemistry*, *31*(11), 1779–1789.

Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol*. 1990;186:421-31.

Ehikioya CO, Osagie AM, Omage SO, Omage K, Azeke MA. Carbohydrate digestive enzyme inhibition, hepatoprotective, antioxidant, and antidiabetic benefits of *Persea americana. Scientific Report*. 2023 Jan 6;*13*(1):284.

Ezhilarasan D. (2023). Molecular mechanisms in thioacetamide-induced acute and chronic liver injury models. *Environmental Ttoxicology and Pharmacology*, *99*, 104093.

Faisul, A. G., & Al-Saidya, A. (2023). Pathological aspects of rabbits hepatotoxicity and fibrosis experimentally induced by thioacetamide and the role of Silymarin as anti-fibrotic effects. *Egyptian Journal of Veterinary Sciences*, *54*(1), 23-30.

Hamad Shareef, S., Abdel Aziz Ibrahim, I., Alzahrani, A. R., Al-Medhtiy, M. H., & Ameen Abdulla, M. (2022). Hepatoprotective effects of methanolic extract of green tea against

Thioacetamide-Induced liver injury in Sprague Dawley rats. *Saudi Journal of Biological Sciences*, *29*(1), 564–573.

Joerger, M., Huitema, A. D. R., Huizing, M. T., Willemse, P. H. B., De Graeff, A., Rosing, H., ... & Vermorken, J. B. (2007). Safety and pharmacology of paclitaxel in patients with impaired liver function: a population pharmacokinetic–pharmacodynamic study. *British Journal of Clinical Pharmacology*, *64*(5), 622-633.

Kabiri, N., Ahangar-Darabi, M., Setorki, M., & Rafieian-kopaei, M. (2013). The effect of silymarin on liver injury induced by Thioacetamide in rats. *Journal of HerbMed Pharmacology*, *2*(2), 29-33.

Köhn-Gaone, J., Dwyer, B. J., Grzelak, C. A., Miller, G., Shackel, N. A., Ramm, G. A., McCaughan, G. W., Elsegood, C. L., Olynyk, J. K., & Tirnitz-Parker, J. E. E. (2016). Divergent Inflammatory, Fibrogenic, and Liver Progenitor Cell Dynamics in Two Common Mouse Models of Chronic Liver Injury. *The American Journal of Pathology*, *186*(7), 1762–1774.

Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *The Journal of Biological Chemistry*, *247*(10), 3170–3175.

Moron, M. S., Depierre, J. W., & Mannervik, B. (1979). Levels of glutathione, glutathione reductase, and glutathione S-transferase activities in rat lung and liver. *Biochimica et Biophysica Acta*, *582*(1), 67–78.

Munthe, S. W. N., Riskianto, R., Juvi, D., & Novia, J. (2023). Antioxidant, total phenolic, and total flavonoid of 70% ethanol extract of avocado seeds (*Persea americana* Mill.). *Pharmacognosy Journal*, *15*(4), 599-605

Nascimento APS, Duarte MEM, Rocha APT, Barros AN. Bioactive Compounds, Technological Advances, and Sustainable Applications of Avocado (*Persea americana* Mill.): A Critical Review. Foods. 2025 Aug 6;14(15):2746.

Okorie, N. H., Omorevbarhia, O., Umerie, S. C., Aniefuna, E. L., Ujam, N. T., & Ali, I. J. (2024). Proximate, phytochemical, mineral composition, and antioxidant effects of methanol extract of *persea americana* (avocado pear) seed. *Fudma Journal of Sciences*, *8*(3), 166-174.

Peters T., Jr (1968). Proposals for standardization of total protein assays. *Clinical Chemistry*, *14*(12), 1147–1159.

Reitman, S., & Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*, *28*(1), 56–63.

Saad HM, Oda SS, Alexiou A, Papadakis M, Mahmoud MH, Batiha GE, Khalifa E. Hepatoprotective activity of Lactéol® forte and quercetin dihydrate against thioacetamide-induced hepatic cirrhosis in male albino rats. *Journal of Cellular and Molecular Medicine*. 2024 Apr;28(8):e18196.

Stefanello, S. T., Hartmann, D. D., Amaral, G. P., Courtes, A. A., Leite, M. T. B., da Silva, T. C., Gonçalves, D. F., Souza, M. B., da Rosa, P. C., Dornelles, L., & Soares, F. A. A. (2017). Antioxidant protection by β-selenoamines against thioacetamide-induced oxidative stress and hepatotoxicity in mice. *Journal of Biochemical and Molecular Toxicology*, *31*(12), 10.1002/jbt.21974.

Zakaria, R., Vit, P., Wijaya, A., Ahmad, A. H., Othman, Z., & Mezzetti, B. (2022). A bibliometric review of *Persea americana* Mill. (Lauraceae): A green gold in agroindustry. *AIMS Agriculture and Food*, *7*(4), 831-854.

Zhang, H., & Xu, J. (2024). Unveiling thioacetamide-induced toxicity: Multi-organ damage and omitted bone toxicity. *Human & Experimental Toxicology*, *43*, 9603271241241807.