Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 02 May 2026
Revised 14 June 2026
Accepted 27 June 2026

Natural Resources for Human Health 2026, Vol. 6 (5): 756–763

KEYWORDS:
Mangifera indica; Cyclophosphamide; Hepatotoxicity; Mango seed extract; Liver enzymes; Histopathology; Antioxidants.

Biochemical and Histopathological Evaluation of Mango Seed Extract Against Cyclophosphamide-Induced Hepatotoxicity in Mice

[1]Nibal Kh. Mousa, [2]Amena G. Abd, [3]Milad A. Hussein, [4]Sarab S. Mustafa, [5]Mina R.Abdullah, [6]Auday A. Yaqub, [7]Shahad S. Sabbar, [8]Maha A. Hussein

[1,2,3,4,5,6,7,8]Scientific Research Commission, Baghdad 10070, Iraq
*nibal.mousa@mail.co.uk
[1*]0000-0002-6542-4192
[2]0009-0005-6531-4830
[3]0000-0001-7415-7316
[4]0009-0005-9207-8351
[5]0009-0006-1456-9304
[6]0009-0003-1379-6976
[7]0009-0006-2412-1722
[8]0009-0000-1730-229x

ABSTRACT:

Background: The present study aimed to evaluate the hepatoprotective effect of hot alcoholic extract of Mangifera indica seeds against cyclophosphamide-induced hepatotoxicity in mice.

Methods: Fifty adult male Swiss albino mice were randomly allocated into four groups (n = 10/group): control, cyclophosphamide, mango seed extract administered before cyclophosphamide, and mango seed extract administered after cyclophosphamide. Cyclophosphamide was administered as a single dose of 100 mg/kg body weight by the intraperitoneal route, while mango seed extract was administered at 100 mg/kg body weight by oral gavage for 6 consecutive days before and 6 consecutive days after cyclophosphamide administration, according to the treatment schedule. Serum ALT, AST, and ALP activities were measured as biochemical indicators of hepatic injury, and liver tissues were examined histopathologically. Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post-hoc test, with statistical significance set at $P < 0.05$.

Results: Cyclophosphamide significantly increased serum ALT, AST, and ALP activities compared with the control group ($P < 0.001$), indicating marked hepatic injury. Mango seed extract attenuated these alterations, with the cyclophosphamide followed by mango seed extract group showing the greatest improvement in biochemical parameters and hepatic architecture. Histopathological examination revealed hepatocellular degeneration, inflammatory cell infiltration, sinusoidal congestion, fatty degeneration, and focal necrosis following cyclophosphamide administration, whereas treatment with mango seed extract reduced these pathological alterations.

Natural Resources for Human Health
eISSN: 2583-1194

Conclusion: The hot alcoholic extract of Mangifera indica seeds at a dose of 100 mg/kg body weight demonstrated a hepatoprotective effect against cyclophosphamide-induced liver injury in mice, with the post-cyclophosphamide treatment schedule showing the most pronounced improvement. These findings support the potential hepatoprotective activity of mango seed extract against drug-induced hepatic injury.

INTRODUCTION

Due to its wide spectrum of potent cytotoxicity and immunosuppressive effect, Cyclophosphamide (CP), a nitrogen mustard alkylating agent, is commonly used in the treatment of several malignancies, autoimmune disorders and hematological diseases. Cyclophosphamide is converted after hepatic metabolism carried out by cytochrome P450 enzymes to active metabolites, mainly phosphoramide mustard and acrolein. Phosphoramide mustard, the active metabolite involved in the antineoplastic activity of the drug, and acrolein, a highly reactive species that promotes extreme oxidative stress, lipid peroxidation (Forman & Zhang, 2021), mitochondrial dysfunction (Sies & Jones, 2020), and inflammatory responses (Forman & Zhang, 2021) ultimately causing hepatocellular injury (Villanueva-Paz et al., 2021). Oxidative stress and inflammatory mechanisms in drug-induced liver injury: Recent advances and therapeutic perspectives (Villanueva-Paz et al., 2021). Cyclophosphamide is an important chemotherapeutic agent but its high-dose-dependent hepatotoxicity continues to pose a significant clinical challenge and a potential obstacle to achieving maximum therapeutic effect with safety in patients.

The liver, being the organ with the higher capacity for biotransformation and detoxification of xenobiotics also tends to suffer oxidative damage due to toxic metabolized products. Increased membrane permeability is frequently related to hepatocellular injury with either intracellular enzyme release into at least the circulation of the seabed. For this reason, hepatic dysfunction or drug induced liver injury have a good established relationship with serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (Thakur et al., 2024). In addition to the aforementioned biochemical alterations, ROS-induced oxidative stress further increases lipid peroxidation and DNA damage as well as mitochondrial dysfunction and activation of inflammatory signaling pathways, thereby leading to necrosis and degeneration of tissue architecture (LeFort et al., 2024; Lai et al., 2024). Histopathological examination thus remains an essential auxiliary evidence combined with biochemical findings to determine the degree of hepatic injury (Clouston et al., 2024).

Due to the increase in attractiveness of bioactive compounds of plant origin, researchers have begun evaluating natural products that can progressively attenuate oxidative stress as well as organ damage induced by chemo-genic toxicity. Of these medicinal plants, Mangifera indica L. (mango) has gained significant scientific attention due to its large amount of biologically active phytochemicals obtained mainly from mango seeds that are frequently discarded as agricultural waste, even though they demonstrate high therapeutic value. Mango seeds are rich in some antioxidant compounds, including mangiferin, gallic acid, ellagic acid, flavonoids, tannins, and phenolic acids (Saleem et al., 2023), all of which exhibit strong free-radical scavenging activities and anti-inflammatory, antimicrobial and cytoprotective activity . Indeed, these phytochemicals have shown attenuation of oxidative stress by neutralizing reactive oxygen species, augmenting the innate antioxidant defense system and maintaining membrane integrity which can be protective to tissue damage in pathophysiological settings (Saleem et al., 2023).

Several recent experimental studies have explored how polyphenol-rich plant extracts prevent and ameliorate liver injury induced by different classes of xenobiotics via several mechanisms: intrinsic antioxidant activity, inhibition of pro-inflammatory mediators, and modulation of apoptotic pathways as well as enhancement in mitochondrial function (Lai et al., 2024). Evidence accumulating over the last few years has shown that mangiferin and structural analogues have considerable hepatoprotective effects, including reducing lipid peroxidation, pro-inflammatory cytokines, and levels of oxidative stress via improved antioxidant enzymatic activities such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) (Saleem et al., 2023; Li et al., 2023). Collectively, these results endorse the considerable potential of exploring various bioactive substances from mango (Mangifera indica L.) seed as low-cost natural antioxidant agents for the treatment of liver injury caused by oxidative or free radical stress (Kabbashi et al., 2024).

There is, however, very limited information on the hepatoprotective effect of hot alcoholic extract of mango seeds in cyclophosphamide-induced liver injury using biochemical and histopathological assessment, although more number of studies describe the antioxidant and pharmacological properties of Mangifera indica (Naresh et al., 2016). Moreover, few studies have investigated the protective benefits of this extraction process in animal models against experimental liver damage.

Understanding this gap in knowledge could help with the discovery of inexpensive natural products able to attenuate adverse hepatic events associated with chemotherapy and improve supportive care practices.

Thus, aiming to assess the protective ability of hot alcoholic extract of Mangifera indica seeds against cyclophosphamide-induced hepatotoxicity in mice through determining serum liver enzymes (ALT, AST and ALP) activities, besides the histopathological changes for hepatic tissues.

MATERIALS AND METHODS

Experimental Animals

Forty healthy adult male Swiss albino mice (Mus musculus), weighing 20–25 g and aged 6–8 weeks, were used in this study. The animals were obtained and housed at the Iraqi Center for Genetic and Cancer Research (Baghdad, Iraq) under laboratory conditions (22 ± 2°C), relative humidity (50–60%), and a 12 h light/12 h dark photoperiod (National Research Council, 2011).

Ethical Approval

Before the experiment began, every animal experiment was examined and approved by the Scientific Research Commission in Baghdad, Iraq (Ethical Approval No**:** SRC/297 /0014).

Preparation of Hot Alcoholic Extract of Mango Seed

We purchased ripe Mangifera indica fruits and separated the seeds from the pulp. The seeds were cleaned with distilled water, air-dried for seven to ten days at 25 ± 2°C, and the inner kernels were extracted. After cooking the kernels until dry, they were ground into a fine powder using an electric grinder, with the outer seed coat removed. The powder was stored in sealed containers until extraction (Santos-Buelga & González-Paramás, 2026).

The powdered mango seed kernels were extracted using 70% ethanol in a Soxhlet apparatus for 6–8 hours at 60–70 °C, following established extraction methods for Mangifera indica. Insoluble materials were filtered out using Whatman No. 1 filter paper. The filtrate was concentrated under vacuum at 40 °C with a rotary evaporator to yield a yellow/brown semi-solid extract, which was stored in sterile amber glass containers at 4 °C until further use (Lim et al., 2019; Azwanida, 2015) .Upon administration, the crude extract was resuspended in distilled water for oral gavage, following internationally recognized hydroalcoholic extraction protocols with slight adaptations (Organisation for Economic Co-operation and Development [OECD], 2008).

Experimental Design

Fifty healthy male Swiss albino mice were randomly allocated into five experimental groups (n = 10/group): Group I served as the control and received normal saline orally; Group II received a single dose of cyclophosphamide (100 mg/kg, i.p.); Group III received mango seed extract alone (100 mg/kg.); Group IV received mango seed extract (100 mg/kg) for 6 consecutive days before cyclophosphamide administration; and Group V received a single dose of cyclophosphamide (100 mg/kg), followed by mango seed extract (100 mg/kg) for 6 consecutive days. Cyclophosphamide was administered as a single dose.

The mango seed extract dose (100 mg/kg) was selected based on previous evidence of the hepatoprotective activity of ethanolic Mangifera indica seed extract (Kabbashi et al., 2024). The cyclophosphamide dose (100 mg/kg) was selected based on its reported ability to induce hepatic injury in Swiss albino mice (Bhat et al., 2018).Ten animals per group were used based on comparable experimental studies [10,17].

Sample Collection and Biochemical Analysis

Mice were anesthetised at the end of the experimental period, and blood was drawn by cardiac puncture into plain tubes. Serum was obtained by allowing blood samples to clot and then centrifuging at 3000 rpm for 10 min (Parasuraman et al., 2010) and stored at −20°C until analyzed. Standard commercial diagnostic kits (Al-Markaz Scientific Supplies) were used to determine serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities using standard methods in accordance with the manufacturer's protocols. They were chosen as biomarkers to assess hepatocellular damage and liver function (McGill, 2016), next, blood samples were obtained and the liver was rapidly excised for histopathological processing after washing in ice-cold normal saline.

Natural Resources for Human Health
eISSN: 2583-1194

Histopathological Examination

Immediately after blood collecting, liver tissues from each group were removed, washed with cold saline solution, and fixed in 10% neutral buffered formalin for 24 h. The tissues were then dehydrated in ethanol, cleared with xylene and paraffin-embedded. Hematoxylin-eosin (H & E) –stained thin sections were counted in the light microscope for assessment of the following parameters: Hepatocellular degeneration, inflammatory cell influx, sinusoidal congestion and necrotic change (Suvarna et al., 2019).

Statistical Assay

Statistical analyses were performed using IBM SPSS Statistics. Data were expressed as mean ± standard deviation (SD). Differences among experimental groups were assessed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post-hoc test. A P-value < 0.05 was considered statistically significant (Field, 2018; Montgomery, 2020).

RESULTS

Biochemical analysis revealed marked alterations in serum liver enzymes following cyclophosphamide administration (Table 1; Figure 2). Compared with the control group, cyclophosphamide markedly increased ALT, AST, and ALP activities. Mango seed extract attenuated these alterations, with the Cyclo + Mango group showing the greatest improvement and enzyme activities approaching control values.

Table 1. Serum liver enzyme activities in the experimental groups.

Group	ALT (U/L)	AST (U/L)	ALP (U/L)
Control	32 ± 4^{a}	45 ± 5^{a}	110 ± 8^{a}
Cyclophosphamide	85 ± 6^{d}	120 ± 10^{d}	210 ± 12^{d}
Mango seed extract	35 ± 4^{a}	48 ± 5^{a}	118 ± 8^{a}
Mango + Cyclo	48 ± 5^{b}	65 ± 6^{b}	145 ± 9^{b}
Cyclo + Mango	40 ± 5^{c}	55 ± 6^{c}	130 ± 9^{c}

Values are expressed as mean ± SD (n = 10). Different lowercase superscript letters within the same column indicate statistically significant differences among groups (Tukey HSD, $P < 0.05$).

Histopathology analysis

Histopathological examination of liver sections revealed marked differences among the experimental groups (Figure 1). The control and mango-only groups showed preserved hepatic architecture, with well-organized hepatic cords, intact hepatocytes, and normal hepatic sinusoids. In contrast, cyclophosphamide administration resulted in marked hepatic alterations, including hepatocellular degeneration, inflammatory cell infiltration, sinusoidal congestion, fatty degeneration, and focal necrosis. Treatment with mango seed extract attenuated these pathological alterations. Notably, the Cyclo + Mango group showed the greatest histological improvement, with hepatic architecture approaching that of the control group. The Mango + Cyclo group also showed improvement compared with the cyclophosphamide group, although the histological recovery was less pronounced than that observed in the Cyclo + Mango group.

Natural Resources for Human Health
eISSN: 2583-1194

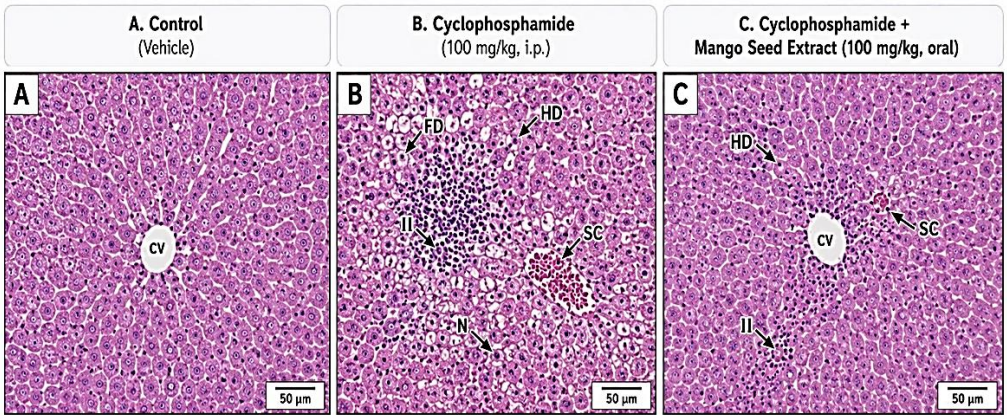

Fig. 1. Representative histopathological changes in liver tissues of the experimental groups stained with hematoxylin and eosin (H&E). (A) Control group showing normal hepatic architecture with intact hepatocytes and a normal central vein. (B) Cyclophosphamide-treated group showing hepatocellular degeneration (HD), inflammatory cell infiltration (II), sinusoidal congestion (SC), and focal necrosis (N). (C) Cyclophosphamide followed by mango seed extract treatment showing marked improvement in hepatic architecture with reduced inflammatory infiltration and mild hepatocellular degeneration. Abbreviations: HD, hepatocellular degeneration; II, inflammatory cell infiltration; SC, sinusoidal congestion; N, necrosis; CV, central vein. Magnification: ×200; scale bar = 50 µm.

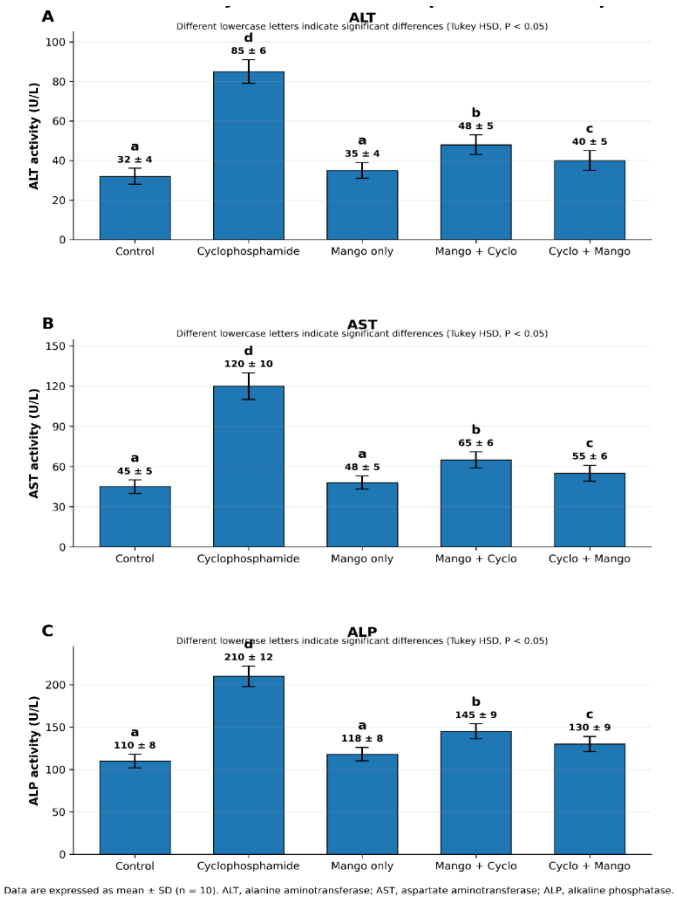

Fig. 2. Serum ALT, AST, and ALP activities in the five experimental groups following cyclophosphamide administration and mango seed extract treatment. Data are expressed as mean ± SD (n = 10). Different lowercase letters indicate statistically significant differences among groups (Tukey HSD, $P < 0.05$).

DISCUSSION

The data from the current study showed that cyclophosphamide caused significant hepatotoxicity, as indicated by increased serum ALT, AST, and ALP activities, together with evident pathological changes in the liver tissues. These findings are consistent with previous studies reporting hepatotoxic effects associated with cyclophosphamide administration and supporting the use of biochemical and histopathological parameters for the evaluation of drug-induced liver injury (Prajapati et al., 2025; Maleki et al., 2024).

Cyclophosphamide is a cytotoxic prodrug that undergoes hepatic bioactivation to 4-hydroxycyclophosphamide, which subsequently gives rise to reactive metabolites, including acrolein and phosphoramide mustard. These metabolites may contribute to oxidative stress and tissue injury and may therefore play an important role in cyclophosphamide-induced hepatic damage (Hasanah et al., 2021). The histopathological alterations observed following cyclophosphamide administration in the present study are consistent with previous findings describing biochemical, oxidative, inflammatory, and structural changes associated with cyclophosphamide-induced hepatic injury (ZaaZaa & Abd El-Motlep, 2020).

Treatment with mango seed extract largely ameliorated the biochemical alterations induced by cyclophosphamide, as indicated by the reduction in serum ALT, AST, and ALP activities. The hepatoprotective effect of the extract may be associated with its antioxidant and free-radical-scavenging properties. Mango (Mangifera indica) contains several bioactive polyphenolic compounds, including mangiferin, gallic acid, quercetin, and catechins, which have been reported to contribute to antioxidant activity and protection against oxidative cellular damage (Prajapati et al., 2025; Masibo & He, 2008). These properties may help maintain hepatocyte integrity and attenuate the biochemical consequences of cyclophosphamide-induced hepatic injury.

The biochemical improvement was accompanied by favorable histopathological changes in the liver. Compared with the cyclophosphamide-treated group, which showed hepatocellular degeneration, inflammatory cell infiltration, sinusoidal congestion, fatty degeneration, and focal necrosis, mice treated with mango seed extract exhibited better-preserved hepatic architecture, with reduced inflammatory infiltration and less tissue degeneration. Similar histopathological changes, including hepatocellular degeneration, necrotic alterations, fatty changes, and sinusoidal congestion, have been reported in experimental models of chemically induced hepatotoxicity, while treatment with plant-derived extracts was associated with attenuation of these structural lesions (Kaushal et al., 2019). These findings support the potential of plant-derived antioxidant compounds to protect hepatic tissue against chemically induced injury (Hari Prasath et al., 2023).

Importantly, the histopathological findings in the present study were consistent with the biochemical changes observed in serum ALT, AST, and ALP activities, providing complementary evidence for the hepatoprotective effect of mango seed extract against cyclophosphamide-induced hepatic injury. Previous experimental research has similarly demonstrated that attenuation of cyclophosphamide-induced biochemical abnormalities can be accompanied by improvement in hepatic tissue architecture following treatment with protective agents (Banerjee & Sen, 2020). Furthermore, the reported hepatoprotective properties of mango-derived polyphenols provide a plausible basis for the protective effects observed in the present study (Prajapati et al., 2025; Masibo & He, 2008).

Overall, the present findings indicate that mango seed extract may attenuate cyclophosphamide-induced hepatic injury at both the biochemical and histopathological levels. The protective effect may be related, at least in part, to the antioxidant and free-radical-scavenging properties of bioactive constituents present in mango seeds. Nevertheless, further studies evaluating oxidative stress markers, inflammatory mediators, apoptotic pathways, and the individual bioactive constituents of the extract are warranted to clarify the precise mechanisms underlying its hepatoprotective activity.

CONCLUSION

In the present study, cyclophosphamide administration at a dose of 100 mg/kg induced marked biochemical and histopathological alterations in the liver of mice, as evidenced by increased serum ALT, AST, and ALP activities and disruption of hepatic architecture. Administration of hot alcoholic extract of *Mangifera indica* seeds at a dose of 100 mg/kg attenuated these biochemical and histopathological alterations. The mango seed extract-only group showed biochemical values comparable to the control group, suggesting no evident hepatic injury under the experimental conditions. Notably, administration of mango seed extract following cyclophosphamide treatment produced the most pronounced improvement in biochemical and

histopathological parameters. These findings support the hepatoprotective potential of mango seed extract against cyclophosphamide-induced hepatic injury.

Funding source: No funding received this paper.

Acknowledgments: The Authors want to thank and appreciate the help, advice, and support of the Iraqi Center for Genetic and Cancer Research (Baghdad, Iraq) throughout the experiment and supply equipment's and solvents.

REFERENCES

[1] Azwanida, N. N. (2015). A review on the extraction methods used in medicinal plants: Principles, strengths and limitations. *Medicinal & Aromatic Plants, 4*(3), 196. https://doi.org/10.4172/2167-0412.1000196

[2] Banerjee, S., & Sen, K. K. (2020). Preparation and evaluation of surface modified nanoparticles of calcium phosphate as extract carrier. *International Journal of Applied Pharmaceutics, 12*(4), 248–257. https://doi.org/10.22159/ijap.2020v12i4.38126

[3] Bhat, N., Kalthur, S. G., Padmashali, S., & Monappa, V. (2018). Toxic effects of different doses of cyclophosphamide on liver and kidney tissue in Swiss albino mice: A histopathological study. *Ethiopian Journal of Health Sciences, 28*(6), 711–716. https://doi.org/10.4314/ejhs.v28i6.5

[4] Clouston, A. D., Gouw, A. S. H., Tiniakos, D., Bedossa, P., Brunt, E. M., Callea, F., Dienes, H.-P., Goodman, Z. D., Hubscher, S. G., Kakar, S., Kleiner, D. E., Lackner, C., Park, Y. N., Roberts, E. A., Schirmacher, P., Terracciano, L., Torbenson, M., Wanless, I. R., Zen, Y., & Burt, A. D. (2024). Severe acute liver disease in adults: Contemporary role of histopathology. *Histopathology, 85*(4), 549–561. https://doi.org/10.1111/his.15212

[5] Field, A. (2018). *Discovering statistics using IBM SPSS Statistics* (5th ed.). SAGE Publications.

[6] Forman, H. J., & Zhang, H. (2021). Targeting oxidative stress in disease: Promise and limitations of antioxidant therapy. *Nature Reviews Drug Discovery, 20*(9), 689–709. https://doi.org/10.1038/s41573-021-00233-1

[7] Hari Prasath, C., Divyapriya, S., & Dhana Rangesh Kumar, V. (2023). Physicochemical, formulation, and evaluation of antifungal herbal soap using *Curcuma amada* Roxburgh and *Prunus dulcis*. *Asian Journal of Pharmaceutical and Clinical Research, 16*(4), 28–34. https://doi.org/10.22159/ajpcr.2023.v16i4.46834

[8] Hasanah, Y. I. F., Harahap, Y., & Suryadi, H. (2021). Development and validation method of cyclophosphamide and 4-hydroxycyclophosphamide with 4-hydroxycyclophosphamide-D4 as internal standard in dried blood spots using UPLC-MS/MS. *International Journal of Applied Pharmaceutics, 13*(2), 148–152. https://doi.org/10.22159/ijap.2021v13i2.39590

[9] Kabbashi, A. S., Eltawaty, S. A., Ismail, A. M., Elshikh, A. A., Alrasheid, A. A., Elmahi, R. A., Koko, W. S., & Osman, E. E. (2024). Ethanolic extract of *Mangifera indica* protects against CCl4-induced hepatotoxicity via antioxidant capabilities in albino rats. *Journal of Toxicology, 2024*, 5539386. https://doi.org/10.1155/2024/5539386

[10] Kaushal, K., Singh, H., & Thakur, A. K. (2019). Antioxidant and hepatoprotective effect of *Swertia chirata* on hypoxia-induced oxidative stress in Wistar rats. *Asian Journal of Pharmaceutical and Clinical Research, 12*(1), 76–80. https://doi.org/10.22159/ajpcr.2019.v12i1.28502

[11] Lai, W., Zhang, J., Sun, J., Min, T., Bai, Y., He, J., Cao, H., Che, Q., Guo, J., & Su, Z. (2024). Oxidative stress in alcoholic liver disease, focusing on proteins, nucleic acids, and lipids: A review. *International Journal of Biological Macromolecules, 278*, 134809. https://doi.org/10.1016/j.ijbiomac.2024.134809

[12] LeFort, K. R., Rungratanawanich, W., & Song, B. J. (2024). Contributing roles of mitochondrial dysfunction and hepatocyte apoptosis in liver diseases through oxidative stress, post-translational modifications, inflammation, and intestinal barrier dysfunction. *Cellular and Molecular Life Sciences, 81*, 34. https://doi.org/10.1007/s00018-023-05061-7

[13] Li, L., Dong, Y., Liu, X., & Wang, M. (2023). Mangiferin for the management of liver diseases: A review. *Foods, 12*(13), 2469. https://doi.org/10.3390/foods12132469

[14] Lim, K. J. A., Cabajar, A. A. F., Lobarbio, C. F. Y., Taboada, E. B., & Lacks, D. J. (2019). Extraction of bioactive compounds from mango (*Mangifera indica* L. var. Carabao) seed kernel with ethanol-water binary solvent systems. *Journal of Food Science and Technology, 56*(5), 2536–2544. https://doi.org/10.1007/s13197-019-03732-7

[15] Maleki, R., Noorbakhsh, M. F., Kazemipour, N., Masudian, M., Namazi, F., & Nazifi, S. (2024). The hepatoprotective effects of sitagliptin against cyclophosphamide-induced hepatotoxicity in rats. *Molecular Biology Research Communications, 13*(4), 193–200. https://doi.org/10.22099/mbrc.2024.49925.1964
[16] Masibo, M., & He, Q. (2008). Major mango polyphenols and their potential significance to human health. *Food Reviews International, 24*(4), 346–370. https://doi.org/10.1080/87559120802089378
[17] McGill, M. R. (2016). The past and present of serum aminotransferases and the future of liver injury biomarkers. *EXCLI Journal, 15*, 817–828. https://doi.org/10.17179/excli2016-800
[18] Montgomery, D. C. (2020). *Design and analysis of experiments* (10th ed.). John Wiley & Sons.
[19] Naresh, K., Varakumar, S., Rajesh, N., Variyar, P. S., & Obulam, V. S. R. (2016). Protective effect of non-irradiated and γ-irradiated mango (*Mangifera indica* L.) juice against ethanol-induced oxidative stress in albino rats. *Asian Journal of Pharmaceutical and Clinical Research, 9*(4), 147–155.
[20] National Research Council. (2011). *Guide for the care and use of laboratory animals* (8th ed.). National Academies Press.
[21] Organisation for Economic Co-operation and Development. (2008). *OECD guidelines for the testing of chemicals: Acute oral toxicity—Up-and-down procedure (Test No. 425).* OECD Publishing. https://doi.org/10.1787/9789264071049-en
[22] Parasuraman, S., Raveendran, R., & Kesavan, R. (2010). Blood sample collection in small laboratory animals. *Journal of Pharmacology and Pharmacotherapeutics, 1*(2), 87–93. https://doi.org/10.4103/0976-500X.72350
[23] Prajapati, J., Bhatt, N., & Rawal, R. (2025). Hepatoprotective effects of phytochemicals and plant extracts against chemotherapy-induced liver damage in animal models: A systematic review. *Archives of Toxicology, 99*(3), 887–914. https://doi.org/10.1007/s00204-024-03928-7
[24] Saleem, K., Hayat, Z., Tariq, Z., Riaz, T., & Azam, M. (2023). Profiling of phenolic compounds, antimicrobial, antioxidant, and hemolytic activity of mango seed kernel using different optimized extraction systems. *Journal of Food Science, 88*(12), 5002–5011. https://doi.org/10.1111/1750-3841.16799
[25] Santos-Buelga, C., & González-Paramás, A. M. (2026). Strategies in the analysis of plant flavonoids: An update. In R. A. Meyers (Ed.), *Encyclopedia of analytical chemistry*. John Wiley & Sons. https://doi.org/10.1002/9780470027318.a9924.pub2
[26] Sies, H., & Jones, D. P. (2020). Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. *Nature Reviews Molecular Cell Biology, 21*(7), 363–383. https://doi.org/10.1038/s41580-020-0230-3
[27] Suvarna, S. K., Layton, C., & Bancroft, J. D. (2019). *Bancroft's theory and practice of histological techniques* (8th ed.). Elsevier.
[28] Thakur, S., Kumar, V., Das, R., Sharma, V., & Mehta, D. K. (2024). Biomarkers of hepatic toxicity: An overview. *Current Therapeutic Research, 100*, 100737. https://doi.org/10.1016/j.curtheres.2024.100737
[29] Villanueva-Paz, M., Morán, L., López-Alcántara, N., Freixo, C., Andrade, R. J., Lucena, M. I., & Cubero, F. J. (2021). Oxidative stress in drug-induced liver injury (DILI): From mechanisms to biomarkers for use in clinical practice. *Antioxidants, 10*(3), 390. https://doi.org/10.3390/antiox10030390
[30] ZaaZaa, A. M., & Abd El-Motlep, B. A. (2020). The modulatory effects of bone marrow-derived mesenchymal stem cells on cyclophosphamide-induced hepatotoxicity in carcinoma mice. *International Journal of Pharmacy and Pharmaceutical Sciences, 12*(4). https://doi.org/10.22159/ijpps.2020.v12i4.36916