International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 1s, 2025
https://www.theaspd.com/ijes.php

Immunophysiological comparison for induced hepatocellular carcinoma treated by alcoholic extract and nano-extract of *opuntia ficus indica*

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Abstract
Objective: This study aimed to assess the preventive effects of alcoholic and nano extracts of cactus pear fruit (Opuntia ficus-indica) pulp and peel against thioacetamide (TAA)-induced hepatic toxicity, oxidative stress, and inflammation in male albino rats.
Methods: The rats used in this investigation were randomly distributed into six groups with 6 animals per group, taking into account weights as follows: Group 1: given only (NaCl % 0.9) for 14 weeks. The group2: is injected with thioacetamide (TAA) at a dosage of 200 mg/kg dissolved in distilled water and given for 14 weeks to induce Hepatic cancer. The group3: is given the alcoholic extract of prickly pear (pulp) at a dose of 100 mg/kg, after which TAA is given until the end of the experiment. The group4: given the alcoholic extract of prickly pear (peels) at a dose of 100g/kg, after which TAA is given until the end of the experiment. The group5: given extract in the nano form (pulp) (54 mg/kg), after which TAA is given until the end of the experiment. The group6: given extract in the nano form (peels) (50 mg/kg), after which TAA is given until the end of the.
Results: TAA exposure markedly raised liver enzymes (ALP, AST, ALT) and total bilirubin, reduced antioxidant markers (GSH, SOD, COX), and increased malondialdehyde (MDA) and pro-inflammatory cytokines (TNF-α, IL-6, IL-1β, MCP-1) in comparison with control group. Treatment with alcoholic and nano extracts markedly increased liver function parameters, reinstated antioxidant status, and decreased oxidative damage and inflammatory cytokine levels.
Conclusion: These findings suggest that prickly pear fruit extracts demonstrate significant hepatoprotective, antioxidant, and anti-inflammatory properties, potentially hindering the advancement of TAA-induced hepatocellular carcinoma.
Keywords: Prickly pear; Thioacetamide; hepatocellular carcinoma; nanotechnology; Inflammation.

INTRODUCTION

Cancer is still the most lethal disease in humans [1], with higher death rates seen in developing countries [2], even with major breakthroughs in therapy. A serious health concern, liver cancer (LC) is the third most deadly malignancy worldwide and the sixth most diagnosed [3,4,5]. During 2020, liver cancer accounted for 7.69% of all cancer-related deaths [6]. Among males, it ranks as the fourth most prevalent cancer type and the second leading cause of cancer-related death. The incidence of liver cancer is higher in men than in women [7]. Liver cancer has recently seen a sharp uptick in cases in Iraq, which calls for urgent action to curb the disease's spread [8]. Chemotherapeutics for cancer used in clinical settings are expensive and high and have major adverse effects such as nephrotoxicity, and myelosuppression. Anemia, nephrotoxicity, and myelosuppression. As a result, it becomes imperative to develop new, safe, and efficient alternatives [9]. This led to the utilization of medicinal plants, which are a significant source of therapeutic chemicals. They have been used since ancient times and can be said to be the origin of modern medicine [10]. Among the medicinal plants of great importance, the prickly pear Opuntia ficus-indica stands out with its unique properties. The prickly pear plant contains many chemical compounds, as scientific research has confirmed that the fruits of the prickly pear contain large amounts of ascorbic acid, vitamin E, carotenoids, fiber, amino acids, and antioxidant compounds (phenols, flavonoids, betaxanthin, and betacyanin), organic acids, betalains, biothiols, taurine, saponins, fatty acids, and phytosterols[11], which are used as medicine

to treat many diseases, such as lowering blood sugar and lipids and antioxidant properties. According to [12], the fruits of the prickly pear plant are valuable sources of nutrients and have anti-ulcer, antioxidant, anti-cancer, neuroprotective, and hepatoprotective properties. Because betalains are found in only a few plant species—beets and prickly pears are the primary producers of this kind of pigment—prickly pear fruits have a high level of antioxidant activity. The primary pigments that give the fruits their color are betalains, which have stronger antioxidant properties than ascorbic acid and reduce blood pressure, cholesterol, and cancer risk in addition to adding color. They also have properties that enhance insulin sensitivity in the human body and act as antioxidants to protect cells from damage [13]. The pulp and peel of prickly pear fruit contain phenolic chemicals that are beneficial and effective at protecting DNA from damage caused by free radicals, improve blood flow, reduce the risk of heart disease, lessen the risk of neurological disorders, reduce the risk of oxidative stress and free radical-induced cell death, and improve cognitive function [14]. The present research sought to assess the preventive effects of alcoholic and nano-extracts of cactus pear fruit in mitigating hepatic damage and illnesses induced by thioacetamide exposure in white male rats.

MATERIALS AND METHODS

2.1 Animals

Albino male rats, averaging 200-250 g and aged 8-10 weeks, were utilised, sourced from the animal facility of the College of Pharmacy, University of Karbala, and maintained under normal circumstances of temperature (22±2°C) and dampness (50-60%), the photoperiod makes up 12 hours of darkness and 12 hours of light. Food and water were supplied gratuitously to the rats during the trial.

2.2 Preparation of Plant Extracts

Fresh prickly pear fruit was acquired from a shop in Karbala, thoroughly cleaned and washed. The fruit was peeled, the pulp was extracted from the skin, and thereafter allowed to desiccate at ambient temperature for six weeks. Subsequent to drying out, the pulp and skin were pulverised to get a finely ground substance.

Alcoholic extract: The alcoholic extract was prepared by combining 50 g of dry powder with 250 ml of ethanol and extracting it using a Soxhlet apparatus for 24 hours, as per the method outlined in [15]. Subsequently, the extract was concentrated with a rotary evaporator to yield a dry extract, which was administered orally to the animal at a dose of 100 mg/kg after mixed in the distilled water.

Nanoparticle extraction: The nano-extract was prepared according to the method of [16] By incorporating 1 g of zinc oxide into 50 ml of distilled deionised water, followed by the addition of 1 g of an alcoholic extract of prickly pear. The mixture was agitated using a magnetic stirrer for 24 hours, subsequently transferred to a shaking incubator at sixty degrees Celsius for 18 hours. The pH is modified to approximately 12 by incrementally adding NaOH solution while maintaining agitation until a nano zinc oxide precipitate is generated. The precipitate was further isolated by centrifuged at 10,000 rpm for 20 minutes, thereafter washed with distilled water and ethanol to eliminate contaminants. The precipitate is dried in an oven at 60–100°C and subsequently calcined at 300 to 600°C in a muffle furnace to enhance particle crystallinity. The ZnO nanoparticles were pulverised post-drying to yield a fine powder, which was subsequently stored in the refrigerator until required. Three methods were used to confirm the biosynthesis of nano zinc oxide using prickly pear fruit: nanomolecular atomic force microscopy (AFM), infrared spectroscopy (FTIR), and scanning electron microscopy (SEM).

2.3 Induction of Liver Cancer

Induction of Hepatic Malignancy using Thioacetamide: Liver cancer was induced in rats utilising thioacetamide (TAA) at a concentration of 200 mg/kg, which was injected by subcutaneous injection twice weekly for 14 weeks.

2.4 Experimental design and treatment protocol

The rats used in this investigation were randomly distributed into six groups with 6 animals per group, taking into account weights as follows: Group 1: The negative control group was given only normal saline (NaCl % 0.9) for 14 weeks. The second group, designated as the positive control group (the infected group), is injected with thioacetamide (TAA) at a dosage of 200 mg/kg dissolved in distilled water and given for 14 weeks to induce cancer. The third group, the prevention group, is given the alcoholic extract of prickly pear (pulp) at a dose of 100 mg/kg for a period, after which TAA is given until the end of the experiment, which is 14 weeks. The fourth group, the prevention group is given the alcoholic extract of prickly pear (peels) at a dose of 100g/kg for a period, after which TAA is given until the end of the experiment, which is 14 weeks. The fifth group is the prevention group for the extract in the nano form (pulp) (54 mg/kg), after which TAA is given until the end of the experiment, which is 14 weeks. The sixth group is the prevention group for the extract in the nano form (peels) (50 mg/kg), after which TAA is given until the end of the experiment, which is 14 weeks.

2.5 Sample Collection and Analysis

Blood samples were collected by cardiac puncture and examined to evaluate liver functions such as liver enzymes Levels of ALT, AST, ALP, and total bilirubin. To assess the immunological response, immune markers including tumor necrosis factor (TNF-α), interleukin 6 (IL-6), interleukin 1-beta (IL-1β), and MCP-1 were measured, and oxidative stress was evaluated by determining GSH, MDA, and SOD levels. Cytochrome oxidase (COX) activity was assessed.

Statistical Analysis

Data was analyzed utilising SPSS and one-way analysis of variance (ANOVA). succeeded by a least significant difference (LSD) test to compare differences between groups at a significance level ($P<0.05$).

RESULTS

3.1 The impact of thioacetamide, alcoholic extract, and nanoparticles derived from prickly pear fruit on hepatic enzymes and total bilirubin concentrations.

The results of Table (4-1) revealed a statistically significant elevation ($P<0.05$) in the levels of hepatic enzymes (ALP, AST, ALT) and total bilirubin (T-BIL) in the infected control group (G2) treated with thioacetamide in comparison to the untreated negative control group (G1). However, there was a significant decrease ($P<0.05$) in enzyme levels and total bilirubin in all groups treated with the alcoholic and nanoparticle extracts compared to the positive-controlled group. The findings indicated no statistically significant variations ($P>0.05$) between groups G4 and G3, as well as between groups G6 and G5. However, there were substantial variations ($P<0.05$) between groups (G4, G3) and (G6, G5).

Table (4-1) The impact of alcoholic and nanoparticle extracts of cactus pear fruit on the concentrations of hepatic enzymes AST, ALT, ALP (U/L) and (T-BIL) in the blood of male albino rats administered Thioacetamide.

group	S.E ± Means			
	ALP	AST	ALT	T-BIL
G1 Negative control group	3.94± 232.17 B	1.64± 130.17 B	2.44± 46.33 B	0.01± 0.166 B
G2 Positive control (200 mg/kg TAA)	16.34± 510.83 A	16.04± 396.33 A	3.22± 98.66 A	0.05± 0.673 A
G3 Alcoholic extract group (pulp) (100 mg/kg) + (200 mg/kg TAA)	5.49± 290.17 C	0.83± 251.17 C	2.65± 66.83 C	0.01± 0.25 C
G4 Alcoholic extract group (peel) (100 mg/kg) + (200 mg/kg TAA)	5.64± 288.50 C	6.34± 245.50 C	2.45± 60.33 C	0.01± 0.203 C

G5 Nano-extract group (pulp) (54 mg/kg) + (200 mg/kg TAA)	2.82± 260.83 D	3.63± 200.83 D	2.66± 48.83 B	0.009± 0.171 B
G6 Nano-extract group (peel) (50 mg/kg) + (200 mg/kg TAA)	3.32± 256.17 D	18.18± 159.00 B	1.14± 47.66 B	0.01± 0.168 B
L.S. D	**22.496**	**29.953**	**7.2398**	**0.0715**

The Impact of Thioacetamide, Alcoholics Extract, and Nano-Extracts from Prickly Pear Fruit on Oxidative Stress

The results of Table (4-2) indicate a statistically significant reduction ($P<0.05$) in the concentrations of reduced glutathione (GSH), superoxide dismutase (SOD), and cytochrome oxidase (COX) injected with thioacetamide comparable to the untreated control group (G1), which was not exposed to any treatment. Simultaneously, there was a statistically elevation ($P<0.05$) in the levels of reduced glutathione (GSH), superoxide dismutase (SOD), and cytochrome oxidase (COX) in all groups administered to alcoholics and nano-extracts relative to the infected group. The results indicated no statistically significant variance ($P>0.05$) between groups G4 and G3, as well as between groups G6 and G5. However, there were significant variations. ($P<0.05$) between (G4, G3) and (G6, G5).

The results of Table (4-2) demonstrated a significant elevation ($P<0.05$) in malondialdehyde (MDA) levels in the positive group (G2) given thioacetamide in contrast to the negative control collection (G1) that was not exposed to any treatment. However, there was a notable reduction ($P<0.05$) in the concentrations of MDA in across all groups administered alcoholic-extracts and nano extracts comparing to the positive control group. The results indicated no statistically significant variations ($P>0.05$) between groups G4 and G3, as well as between groups G6 and G5. However, there were notable variations ($P<0.05$) between (G3) and (G6, G5, G4).

Table (4-2) Effect of alcoholic and nano-extracts of prickly pear fruit on the level of antioxidants (SOD, GSH, MDA, COX) in the blood of male albino rats treated with Thioacetamide.

group	S.E ± Means			
	GSH	MDA	SOD	COX
G1 Negative control group	± 17.03 0.20 A	0.01± 0.335 C	3.66± 64.16 A	0.80± 220.81 A
G2 Positive control (200 mg/kg TAA)	0.73± 8.61 C	0.05± 0.871 A	3.07± 31.33 C	6.44± 165.68 C
G3 Alcoholic extract group (pulp) (100 mg/kg) + (200 mg/kg TAA)	14.86 0.21± B	0.05± 0.550 B	2.18± 54.16 B	1.05± 206.85 B
G4 Alcoholic extract group (peel) (100 mg/kg) + (200 mg/kg TAA)	15.35 0.35± D	0.02± 0.401 BC	2.08± 55.83 B	1.68± 210.65 A
G5 Nano-extract group (pulp) (54 mg/kg) + (200 mg/kg TAA)	16.51 0.21± A	0.02± 0.386 C	3.01± 63.50 A	4.04± 214.98 A
G6 Nano-extract group (peel) (50 mg/kg) + (200 mg/kg TAA)	16.81 0.34± A	0.01± 0.378 C	2.26± 64.33 A	0.46± 218.51 A
L.S. D	**1.122**	**0.1037**	**8.0164**	**9.3391**

The Influence of Thioacetamide, Alcoholics Extract, and Nano-Extracts of Prickly Pear Fruit on Inflammatory Mediators

The results of Table (4-3) suggest a significant rise (P<0.05) in the concentrations of tumor necrosis factor alpha (TNF-α), interleukin six (IL-6), interleukin one beta (IL-1β), and monocyte chemoattractant protein one (MCP-1) in the positive control group (G2) treated with thioacetamide in relation to the untreated negative control group (G1), which was not exposed to any treatment. Nonetheless, there was a notable reduction (P<0.05) in the levels of inflammatory mediators across all groups administered the alcoholic extracts and nano-extracts in comparison to the infected control collection (G2). The findings indicated no statistically variations (P>0.05) among groups G4, G3, and G6, G5; nevertheless, significant variations (P<0.05) were observed between these groups.

Table (4-3) illustrates the effect of alcoholic and nano-extracts of cactus pear fruit on the concentrations of inflammatory mediators (MCP-1, IL-1β, IL-6, TNF-α) in the bloodstream of male albino rats administered Thioacetamide.

group	S.E ± Means			
	TNF α (ng/ml)	IL-6 (pg/ml)	IL-1β (ng/L)	MCP-1 (ng/L)
G1 Negative control group	0.16± 3.06 B	0.60± 35.15 B	1.64± 87.61 C	1.37± 45.95 A
G2 Positive control (200 mg/kg TAA)	0.31± 9.93 A	5.45± 58.11 A	± 144.81 2.29 A	4.79± 93.55 B
G3 Alcoholic extract group (pulp) (100 mg/kg) + (200 mg/kg TAA)	0.08± 4.53 C	0.37± 42.81 C	2.00± 95.23 B	1.23± 60.65 C
G4 Alcoholic extract group (peel) (100 mg/kg) + (200 mg/kg TAA)	0.18± 4.13 C	0.52± 40.13 C	1.32± 94.90 B	1.08± 52.30 D
G5 Nano-extract group (pulp) (54 mg/kg) + (200 mg/kg TAA)	0.16± 3.31 B	1.18± 37.73 B	1.37± 90.00 C	1.19± 47.86 AD
G3 Nano-extract group (peel) (50 mg/kg) + (200 mg/kg TAA)	0.08± 3.23 B	0.43± 36.66 B	0.73± 88.01 C	2.51± 46.21 A
L.S. D	0.5312	6.683	4.7418	7.1

DISCUSSION

The study results demonstrated that the induction of hepatocellular carcinoma using thioacetamide (TAA) led to a significant increase (P<0.05) in hepatic enzyme levels (ALP, ALT, AST), consistent with findings reported in studies [17,18,19]. This escalation is ascribed to several toxicological pathways, particularly the heightened generation of reactive oxygen forms by thioacetamide, resulting in peroxidation of lipids of cellular membranes, resulting in membrane injury, augmented permeability, and subsequent leakage of enzymes into the circulation [20]. In addition, the deterioration of antioxidant defense systems, such as glutathione (GSH), contributes to the exacerbation of oxidative damage. Furthermore, an elevation in bilirubin levels was seen, serving as a critical indicator of impaired liver function. Exposure to TAA is associated with hepatocellular injury and the development of fibrotic scarring, which obstructs normal blood and bile flow, thereby intensifying bilirubin accumulation [21,22] In contrast, the results showed that treatment with alcoholic and nano-extracts of the pulp and peel of prickly pear (Opuntia ficus-indica) resulted in a significant decrease in ALT, AST, and ALP levels, consistent with the results of [23,24]. This improvement is attributed to the plant containing bioactive compounds with antioxidant and anti-

inflammatory properties, which enhance the stability of hepatic cell membranes and improve the efficiency of detoxification [25,26]. The study also recorded a significant decrease in total bilirubin levels in treated animals, confirming the improvement of liver function and the enhancement of bile acid secretion, which is in line with what was reported by studies [27]. [24] indicated that prickly pear extract enhances the processes of bilirubin conjugation and secretion, which supports the rebalancing of liver function.

The study's findings indicated that Thioacetamide injection resulted in a substantial significant reduction ($P<0.05$) in the concentrations of glutathione and the superoxide dismutase enzyme, while simultaneously causing a substantial rise ($P<0.05$) in malondialdehyde concentrations, corroborating the results of [28,29]. The diminished levels of superoxide dismutase and glutathione enzymes are ascribed to the harmful impact of thioacetamide. Thioacetamide induces oxidative stress and inflammation in tissues, resulting in a reduction of antioxidant enzymes such as SOD and GSH. [29, 30] TAA promotes lipid peroxidation, as seen by increased levels of thiobarbituric acid reactive compounds (TBARS). This process utilizes GSH to neutralize peroxides, resulting in the depletion of GSH reserves. [30] Lipid oxidation generates various reactive chemical species, including malondialdehyde, isoprostanes, and 4-hydroxy-2-nonenal (HNE). MDA is the principal aldehyde product of lipid oxidation and is frequently utilized as a marker for this phenomenon. [32] The results indicated that the injection of thioacetamide resulted in a considerable ($P<0.05$) reduction in cytochrome oxidase concentration. Reduced levels of cytochrome oxidase can be ascribed to the toxic effects of thioacetamide, which induces hepatic damage that may impair mitochondrial function. The liver serves as a principal location for metabolic activities, and injury to liver cells may result in diminished COX levels [33]. The findings of the present study that the oral administration of alcoholic and nano-extracts of prickly pear pulp and peel led to a substantial ($P<0.05$) elevation in glutathione and superoxide dismutase levels, corroborating the results of [34, 23], and a significant ($P<0.05$) reduction in malondialdehyde levels, aligning with [35, 36]. The elevation in reduced glutathione and superoxide dismutase levels is ascribed to the bioactive components in the prickly pear (Opuntia ficus-indica) fruit extract, which activate the cellular mechanisms responsible for synthesizing endogenous antioxidants. The Nrf2 pathway is particularly significant, as nuclear factor erythroid 2-related factor 2 (Nrf2) interacts with antioxidant response elements (AREs) in DNA, consequently stimulating the expression of genes that encode enzymes like superoxide dismutase and those involved in glutathione synthesis, including glutamate-cysteine ligases [37]. The reduction in MDA levels following administration of alcoholic and nano-extracts of prickly pear fruit is ascribed to the active chemicals in prickly pear, which donate electrons to free radicals, so obstructing the lipid peroxidation process [38]. The findings of the present investigation indicated that treatment with alcoholic and nano extracts of prickly pear pulp and peel led to a notable elevation ($P<0.05$) in the levels of Cytochrome oxidase. The fruit of the prickly pear is rich in bioactive chemicals that have potent antioxidant effects [39]. The association between bioactive substances and the COX enzyme is primarily comprehended through their antioxidant properties and their capacity to safeguard mitochondria.[40 ,41]

The research demonstrated that intraperitoneal injection of thioacetamide elicits an initial hepatic inflammatory response, marked by substantial elevations in TNF-α, IL-6, and IL-1β levels consistent with studies [42, 43, 19]. This inflammation arises from the conversion of TAA by CYP450 enzymes into hazardous compounds that provoke cellular necrosis, particularly in the central zone of the hepatic lobule, resulting in the release of damage-associated molecular patterns such as HMGB1 and the production of reactive oxygen forms. These signals stimulate Kupffer cells and macrophages to adopt an M1 inflammatory phenotype [49], activating NF-κB pathways through TLR4 and RAGE [46] or directly via reactive oxygen species [50, 42], in addition to MAPK pathways [51, 52]. Reactive oxygen species also induce the stimulation of NLRP3 and the release of mature IL-1β [53, 54]. MCP-1 is raised through NF-κB and MAPK passageway, facilitating macrophage infiltration as well as exacerbating liver damage. Increased concentrations of TNF-α, IL-6, and IL-1β are frequently linked to hepatic impairment and the progression of fibrosis. The oral administration of alcoholic and nano-extracts from the pulp and peel of prickly pear (Opuntia ficus-indica) resulted in a substantial reduction in TNF-α, IL-1β, IL-6, and MCP-1 levels, indicating anti-inflammatory effects. The preventive properties are ascribed to the abundance of bioactive substances in prickly pear, particularly betalains, polyphenols, and Isorhamnetin derivatives [60, 61]. These compounds synergistically neutralize ROS [62], reducing oxidative stress and indirectly preventing the stimulation of the NF-κB pathway (by preventing IκB degradation and translocation of p65 to the nucleus) [63, 64], reducing the activation of NLRP3 [65] and MAPK pathways [66], as well as inhibiting COX-2 [67], thus limiting TAA-induced inflammation and liver damage [68].

CONCLUSION

Alcoholic and nano-extracts of prickly pear pulp and peel demonstrated clear protective effects against thioacetamide-induced liver damage, by improving biofunctional markers, enhancing antioxidant defenses, and reducing inflammation. These results support the potential of prickly pear extracts as a natural preventive option, warranting further clinical studies.

REFERENCES

1. Nassif, A. B., Talib, M. A., Nasir, Q., Afadar, Y., & Elgendy, O. (2022). Breast cancer detection using artificial intelligence techniques: A systematic literature review. Artificial intelligence in medicine, 127, 102276.
2. El-Aassar MR, Saad EA, Habib SA, Waly HM. Loading of some quinoxaline derivatives in poly (l-lactic) acid/Pluronic® F-127 nanofibers enhances their anticancer efficiency and induces a p53 and p21 apoptotic-signaling pathway. Colloids Surf B Biointerfaces.
2019;183:110444. https://doi.org/10.1016/j.colsurfb.2019.110444.
3. Yang, C., Jia, J., Yu, Y., Lu, H., & Zhang, L. (2024). Temporal trends in prevalence of liver cancer and etiology-specific liver cancer from 1990 to 2019. *Clinics and Research in Hepatology and Gastroenterology*, *48*(8), 102451.
4. Attallah AM, El-Far M, Abdel Malak CA, Farid K, Omran MM, Yahya RS, et al. A simple diagnostic index comprising epithelial membrane antigen and fbronectin for hepatocellular carcinoma. Ann Hepatol. 2015;14:869–80. https://doi.org/10.5604/16652681.1171774.
5. World Health Organization-Cancer. https://www.who.int/news-room/ factsheets/detail/cancer, 3 March 2021.
6. Hassona SM, Saad EA, Kiwan HA, Hassanien MM. Palladium(II) Schiff base complex arrests cell cycle at early stages, induces apoptosis, and reduces Ehrlich solid tumor burden: a new candidate for tumor therapy. Invest New Drugs. 2022;40(4):681–9.
7. Kulik L, El-Serag HB. Epidemiology and Management of Hepatocellular Carcinoma. Gastroenterology. 2019;156(2):477-491.e1.
8. Hussain, A. M., & Lafta, R. K. (2021). Cancer trends in Iraq 2000–2016. *Oman medical journal*, *36*(1), e219.
9. Saad EA, Elsayed SA, Hassanien MM, AL-Adl MS. The new iron(III) 3-oxo-N-(pyridin-2-yl)butanamide complex promotes Ehrlich solid tumor regression in mice via induction of apoptosis. Appl Organomet Chem. 2020;34(1):e5282.
10. Salmerón-Manzano, E., Garrido-Cardenas, J. A., & Manzano-Agugliaro, F. (2020). Worldwide research trends on medicinal plants. International Journal of Environmental Research and Public Health, 17(10), 3376.
11. Slimen, I. B., Najar, T., & Abderrabba, M. (2021). Correction to: Bioactive Compounds of Prickly Pear [Opuntia ficus-indica (L.) Mill.]. In Reference series in phytochemistry (p. C1). https://doi.org/10.1007/978-3-030-57415-4_39
12. El-Beltagi, H. S., Mohamed, H. I., Elmelegy, A. A., Eldesoky, S. E., & Safwat, G. (2019). Phytochemical screening, antimicrobial, antiaxidant, anticancer activities and nutritional values of cactus (Opuntia ficus indicia) pulp and peel.
13. Shoukat, R., Cappai, M., Pia, G., & Pilia, L. (2023). An Updated Review: Opuntia ficus indica (OFI) Chemistry and Its Diverse Applications. Applied Sciences, 13(13), 7724. https://doi.org/10.3390/app13137724
14. Martins, M., Ribeiro, M. H., & Almeida, C. (2023). Physicochemical, Nutritional, and Medicinal Properties of Opuntia ficus-indica (L.) Mill. and Its Main Agro-Industrial Use: A Review. *Plants*, *12*(7), 1512. https://doi.org/10.3390/plants12071512
15. Kulkarni, S. J., Maske, K. N., Budre, M. P., & Mahajan, R. P. (2012). Extraction and purification of curcuminoids from Turmeric (Curcuma longa L.). International Journal of Pharmacology and Pharmaceutical Technology, 1(2), 81–84.

16. Bashi, A. M., Hussein, M. Z., Zainal, Z., & Tichit, D. (2013). Synthesis and controlled release properties of 2, 4-dichlorophenoxy acetate–zinc layered hydroxide nanohybrid. Journal of Solid State Chemistry, 203, 19–24.
17. Ali, O., Amer, F., & Elrahany, M. (2024). Hepatoprotective potential of vitamin D on liver fibrosis induced in rats by Thioacetamide. Deleted Journal, 0(0), 56–67.
18. Ali, S. A., & Datusalia, A. K. (2024). Protective effects of Tinospora cordifolia miers extract against hepatic and neurobehavioral deficits in thioacetamide-induced hepatic encephalopathy in rats via modulating hyperammonemia and glial cell activation. Journal of Ethnopharmacology, 323, 117700. https://doi.org/10.1016/j.jep.2023.117700
19. Othman, O. A., Amer, F., Elrehany, M., & Ali, E. (2024). Therapeutic impact of telmisartan against the thioacetamide induced hepatic fibrosis in rats. Delta Journal of Science, 49(1), 68–78. https://doi.org/10.21608/djs.2024.321762.1189
20. Ezhilarasan, D. (2023). Molecular mechanisms in thioacetamide-induced acute and chronic liver injury models. Environmental toxicology and pharmacology, 99, 104093.
21. Guerra Ruiz, A. R., Crespo, J., López Martínez, R. M., Iruzubieta, P., Casals Mercadal, G., Lalana Garcés, M., ... & Morales Ruiz, M. (2021). Measurement and clinical usefulness of bilirubin in liver disease. Advances in Laboratory Medicine/Avances en Medicina de Laboratorio, 2(3), 352-361.
22. Megahed, A., Gadalla, H., Filimban, W. A., Albukhari, T. A., Sembawa, H., Bagadood, R. M., Sindi, G., Abdelhamid, F. M., El-Boshy, M. E., & Risha, E. F. (2024). Hesperidin ameliorates thioacetamide-induced liver fibrosis via antioxidative and anti-inflammatory mechanisms targeting TGF-β/α-SMA pathways in rats. International Journal of Immunopathology and Pharmacology, 38. https://doi.org/10.1177/03946320241309004
23. Ghanem, R., El-beltagy, A., Kamel, K., Brakat, E., and Elsayyad, H. (2023). Adverse effects of MSG and aspartame on the liver of female albino rats and their offspring and the possible ameliorative role Opuntia ficus-indica fruit. Delta Univ. Sci. J. 6 (2), 1–32. doi:10.21608/dusj.2023.318628
24. Hafeez, H., Israr, B., Butt, M. S., & Faisal, M. N. (2024). Therapeutic Intervention of Opuntia Ficus Indica (L.) Fruit and Seed Powder against CCl 4-Induced Acute Liver Injury in Wistar Rats. Pakistan Veterinary Journal, 44(2).
25. Besné-Eseverri, I., Trepiana, J., Gómez-Zorita, S., Antunes-Ricardo, M., Cano, M. P., & Portillo, M. P. (2023). Beneficial effects of Opuntia spp. on liver health. Antioxidants, 12(6), 1174. https://doi.org/10.3390/antiox12061174
26. Wang, J., Rani, N., Jakhar, S., Redhu, R., Kumar, S., Devi, B., Simal-Gandara, J., & Singla, R. K. (2023). Opuntia ficus-indica (L.) Mill.: Anticancer properties and phytochemicals: Current trends and future perspectives. Frontiers in Plant Science, 14, 1236123. https://doi.org/10.3389/fpls.2023.1236123
27. Gutierrez, O. G. H., Ríos, G. C. S., Gutierrez, Z. J. H., & Miranda, V. H. T. (2024). Efecto del fruto Opuntia ficus indica (tuna morada) frente a la toxicidad hepática por paracetamol en ratas. Nutrición Clínica Y Dietética Hospitalaria/Nutrición Clínica, Dietética Hospitalaria, 44(4). https://doi.org/10.12873/444huaman
28. Kundu, A., Gali, S., Sharma, S., Kacew, S., Yoon, S., Jeong, H. G., Kwak, J. H., & Kim, H. S. (2023). Dendropanoxide alleviates thioacetamide-induced hepatic fibrosis via inhibition of ROS production and inflammation in BALB/C mice. *International Journal of Biological Sciences*, *19*(9), 2630–2647. https://doi.org/10.7150/ijbs.80743
29. Abdelrahman, R. S., & Abdelmageed, M. E. (2024). Hepatoprotective effects of the xanthine oxidase inhibitor Febuxostat against thioacetamide-induced liver injury in rats: The role of the Nrf2/HO-1 and TLR4/NF-κB pathways. *Food and Chemical Toxicology*, *194*, 115087.
30. Türkmen, N. B., Yüce, H., Taşlidere, A., ŞahïN, Y., & ÇïFtçï, O. (2022). The ameliorate effects of nerolidol on thioasteamide-induced oxidative damage in heart and kidney tissue. *Turkish Journal of Pharmaceutical Sciences*, *19*(1), 1–8. https://doi.org/10.4274/tjps.galenos.2021.30806
31. Zhang, X., Hou, L., Guo, Z., Wang, G., Xu, J., Zheng, Z., ... & Guo, F. (2023). Lipid peroxidation in osteoarthritis: focusing on 4-hydroxynonenal, malondialdehyde, and ferroptosis. *Cell death discovery*, *9*(1), 320.
32. Rizzo, M. (2024). Measurement of malondialdehyde as a biomarker of lipid oxidation in fish. *American Journal of Analytical Chemistry*, *15*(9), 303-332.

33. Mihajlovic, M., & Vinken, M. (2022). Mitochondria as the target of hepatotoxicity and drug-induced liver injury: molecular mechanisms and detection methods. *International journal of molecular sciences*, *23*(6), 3315.
34. El-Hawary, S. S., Sobeh, M., Badr, W. K., Abdelfattah, M. A., Ali, Z. Y., El-Tantawy, M. E., Rabeh, M. A., & Wink, M. (2020). HPLC-PDA-MS/MS profiling of secondary metabolites from Opuntia ficus-indica cladode, peel and fruit pulp extracts and their antioxidant, neuroprotective effect in rats with aluminum chloride induced neurotoxicity. *Saudi Journal of Biological Sciences*, *27*(10), 2829–2838. https://doi.org/10.1016/j.sjbs.2020.07.003
35. Zaman, R., Tan, E. S. S., Bustami, N. A., Amini, F., Seghayat, M. S., Ho, Y. B., & Tan, C. K. (2025). Assessment of Opuntia ficus-indica supplementation on enhancing antioxidant levels. *Scientific Reports*, *15*(1), 3507.
36. Ahmed, S. a. A., El-Rahman, G. I. A., Behairy, A., Beheiry, R. R., Hendam, B. M., Alsubaie, F. M., & Khalil, S. R. (2020). Influence of Feeding Quinoa (Chenopodium quinoa) Seeds and Prickly Pear Fruit (Opuntia ficus indica) Peel on the Immune Response and Resistance to Aeromonas sobria Infection in Nile Tilapia (Oreochromis niloticus). *Animals*, *10*(12), 2266. https://doi.org/10.3390/ani10122266
37. El Kebbaj, R., Bouchab, H., Tahri-Joutey, M., Rabbaa, S., Limami, Y., Nasser, B., ... & Cherkaoui-Malki, M. (2024). The potential role of major argan oil compounds as Nrf2 regulators and their antioxidant effects. *Antioxidants*, *13*(3), 344.
38. Ahmed, F. A., Ibrahim, M. A., El-Azab, M. M., Fahmy, W. G., & Fahmy, D. M. (2024). A REVIEW: OPUNTIA FICUS-INDICA AS A SOURCE OF BIOACTIVE COMPOUND INGREDIENTS FOR FUNCTIONAL FOODS, NUTRITION, HUMAN DISEASE AND HEALTH. Universal Journal of Pharmaceutical Research.
39. Özcan, M. M., Uslu, N., Kara, H. H., & Özcan, M. M. (2023). Variations in Bioactive Properties, Phenolic Compounds and Fatty Acid Compositions of Different Parts of Prickly Pear (Opuntia ficus-indica Spp) Fruits. *Erwerbs-Obstbau*, *65*(4), 1163–1170. https://doi.org/10.1007/s10341-022-00766-8
40. Benramdane, E., Chougui, N., Ramos, P. a. B., Makhloufi, N., Tamendjari, A., Silvestre, A. J. D., & Santos, S. a. O. (2022). Lipophilic Compounds and Antibacterial Activity of Opuntia ficus-indica Root Extracts from Algeria. *International Journal of Molecular Sciences*, *23*(19), 11161. https://doi.org/10.3390/ijms231911161
41. Wang, J., Rani, N., Jakhar, S., Redhu, R., Kumar, S., Kumar, S., Kumar, S., Devi, B., Simal-Gandara, J., Shen, B., & Singla, R. K. (2023). Opuntia ficus-indica (L.) Mill. - anticancer properties and phytochemicals: current trends and future perspectives. *Frontiers in Plant Science*, *14*. https://doi.org/10.3389/fpls.2023.1236123
42. El-Kashef, D. H., Abdel-Rahman, N., & Sharawy, M. H. (2024). Apocynin alleviates thioacetamide-induced acute liver injury: Role of NOX1/NOX4/NF-κB/NLRP3 pathways. *Cytokine*, *183*, 156747. https://doi.org/10.1016/j.cyto.2024.156747
43. Amirshahrokhi, K., & Imani, M. (2025). Edaravone reduces brain injury in hepatic encephalopathy by upregulation of Nrf2/HO-1 and inhibition of NF-κB, iNOS/NO and inflammatory cytokines. *Molecular Biology Reports*, *52*(1). https://doi.org/10.1007/s11033-025-10343-3
44. Dong, X., Liu, J., Xu, Y., & Cao, H. (2019). Role of macrophages in experimental liver injury and repair in mice (Review). *Experimental and Therapeutic Medicine*. https://doi.org/10.3892/etm.2019.7450
45. Abdelmageed, M. E., & Abdelrahman, R. S. (2023). Canagliflozin attenuates thioacetamide-induced liver injury through modulation of HMGB1/RAGE/TLR4 signaling pathways. *Life Sciences*, *322*, 121654. https://doi.org/10.1016/j.lfs.2023.121654
46. Ni, Y., Chen, H., Nie, H., Zheng, B., & Gong, Q. (2021). HMGB1: An overview of its roles in the pathogenesis of liver disease. *Journal of Leukocyte Biology*, *110*(5), 987–998. https://doi.org/10.1002/jlb.3mr0121-277r
47. Alamery, S., Zargar, S., Yaseen, F., Wani, T. A., & Siyal, A. (2022). Evaluation of the effect of wheat germ oil and olmutinib on the Thioacetamide-Induced liver and kidney toxicity in mice. Life, 12(6), 900. https://doi.org/10.3390/life12060900
48. Jorgačević, B., Stanković, S., Filipović, J., Samardžić, J., Vučević, D., & Radosavljević, T. (2022). Betaine modulating MIF-mediated oxidative stress, inflammation and fibrogenesis in thioacetamide-induced nephrotoxicity. Current Medicinal Chemistry, 29(31), 5254-5267.
49. Kuramochi, M., Izawa, T., Kuwamura, M., & Yamate, J. (2021). Involvement of neutrophils in rat livers by low-dose thioacetamide administration. *Journal of Veterinary Medical Science*, *83*(3), 390–396. https://doi.org/10.1292/jvms.20-0581

50. Ezhilarasan, D. (2023). Molecular mechanisms in thioacetamide-induced acute and chronic liver injury models. *Environmental Toxicology and Pharmacology*, *99*, 104093. https://doi.org/10.1016/j.etap.2023.104093
51. Li, P., & Chang, M. (2021). Roles of PRR-Mediated Signaling Pathways in the regulation of Oxidative Stress and Inflammatory Diseases. International Journal of Molecular Sciences, 22(14), 7688. https://doi.org/10.3390/ijms22147688
52. Lee, Y. H., Son, J. Y., Kim, K. S., Park, Y. J., Kim, H. R., Park, J. H., Kim, K., Lee, K. Y., Kang, K. W., Kim, I. S., Kacew, S., Lee, B. M., & Kim, H. S. (2019). Estrogen deficiency potentiates Thioacetamide-Induced hepatic fibrosis in Sprague-Dawley rats. International Journal of Molecular Sciences, 20(15), 3709. https://doi.org/10.3390/ijms20153709
53. Jiao, F., Wang, Y., Chen, Q., Cao, P., Shi, C., Pei, M., Wang, L., & Gong, Z. (2021). Role of SIRT1 in hepatic encephalopathy: in vivo and in vitro studies focusing on the NLRP3 Inflammasome. Oxidative Medicine and Cellular Longevity, 2021(1). https://doi.org/10.1155/2021/5522708
54. Mai, Z., Huang, Y., Huang, D., Huang, Z., He, Z., Li, P., Zhang, S., Weng, J., & Gu, W. (2020). Reversine and herbal Xiang–Sha–Liu–Jun–Zi decoction ameliorate thioacetamide-induced hepatic injury by regulating the RelA/NF-κB/caspase signaling pathway. Open Life Sciences, 15(1), 696–710. https://doi.org/10.1515/biol-2020-0059
55. Lin, X., Wei, J., Nie, J., Bai, F., Zhu, X., Zhuo, L., Lu, Z., & Huang, Q. (2018). Inhibition of RKIP aggravates thioacetamide-induced acute liver failure in mice. Experimental and Therapeutic Medicine. https://doi.org/10.3892/etm.2018.6542
56. She, S., Ren, L., Chen, P., Wang, M., Chen, D., Wang, Y., & Chen, H. (2022). Functional roles of chemokine receptor CCR2 and its ligands in liver disease. *Frontiers in Immunology*, *13*. https://doi.org/10.3389/fimmu.2022.812431
57. Mori, Y., Izawa, T., Takenaka, S., Kuwamura, M., & Yamate, J. (2009). Participation of functionally different macrophage populations and monocyte chemoattractant protein-1 in early stages of thioacetamide-induced rat hepatic injury. *Toxicologic Pathology*, *37*(4), 463–473. https://doi.org/10.1177/0192623309335634
58. Bagalagel, A., Diri, R., Noor, A., Almasri, D., Bakhsh, H., Kutbi, H. I., & Al-Gayyar, M. M. (2022). Evaluating the anticancer activity of blocking TNF Type 1 receptors in Thioacetamide-Induced hepatocellular carcinoma in a rat model. *Cureus*. https://doi.org/10.7759/cureus.32519
59. Shi, C., Jiao, F., Wang, Y., Chen, Q., Wang, L., & Gong, Z. (2022). SIRT3 inhibitor 3-TYP exacerbates thioacetamide-induced hepatic injury in mice. *Frontiers in Physiology*, *13*. https://doi.org/10.3389/fphys.2022.915193
60. Ahmedah, H. T. (2023). Opuntia ficus-indica and its potential effects on cancer. *Journal of Contemporary Medical Sciences*, *9*(5). https://doi.org/10.22317/jcms.v9i5.1430
61. Madrigal-Santillán, E., Portillo-Reyes, J., Madrigal-Bujaidar, E., Sánchez-Gutiérrez, M., Mercado-Gonzalez, P., Izquierdo-Vega, J., Vargas-Mendoza, N., Álvarez-González, I., Fregoso-Aguilar, T., Delgado-Olivares, L., Morales-González, Á., Anguiano-Robledo, L., & Morales-González, J. (2022). Opuntia genus in Human Health: A Comprehensive Summary on Its Pharmacological, Therapeutic and Preventive Properties. Part 1. *Horticulturae*, *8*(2), 88. https://doi.org/10.3390/horticulturae8020088
62. Filannino, P., Cavoski, I., Thlien, N., Vincentini, O., De Angelis, M., Silano, M., Gobbetti, M., & Di Cagno, R. (2016). Lactic Acid Fermentation of Cactus Cladodes (Opuntia ficus-indica L.) Generates Flavonoid Derivatives with Antioxidant and Anti-Inflammatory Properties. *PLoS ONE*, *11*(3), e0152575. https://doi.org/10.1371/journal.pone.0152575
63. Zeghbib, W., Boudjouan, F., Vasconcelos, V., & Lopes, G. (2022). Phenolic compounds' occurrence in opuntia species and their role in the inflammatory process: a review. *Molecules*, *27*(15), 4763. https://doi.org/10.3390/molecules27154763
64. Liu, T., Zhang, L., Joo, D., & Sun, S. C. (2017). NF-κB signaling in inflammation. *Signal transduction and targeted therapy*, *2*(1), 1-9.
65. Lee, Y. S., Yang, W. K., Park, Y. R., Park, Y. C., Park, I. J., Lee, G. J., ... & Kim, S. H. (2022). Opuntia ficus-indica Alleviates Particulate Matter 10 Plus Diesel Exhaust Particles (PM10D)—Induced Airway Inflammation by Suppressing the Expression of Inflammatory Cytokines and Chemokines. *Plants*, *11*(4), 520.
66. Allegra, M., D'Acquisto, F., Tesoriere, L., Attanzio, A., & Livrea, M. (2014). Pro-oxidant activity of indicaxanthin from Opuntia ficus indica modulates arachidonate metabolism and prostaglandin synthesis through

lipid peroxide production in LPS-stimulated RAW 264.7 macrophages. *Redox Biology*, *2*, 892–900. https://doi.org/10.1016/j.redox.2014.07.004

67. Antunes-Ricardo, M., Gutiérrez-Uribe, J. A., Martínez-Vitela, C., & Serna-Saldívar, S. O. (2015). Topical Anti-Inflammatory Effects of Isorhamnetin Glycosides Isolated fromOpuntia ficus-indica. *BioMed Research International*, *2015*, 1–9. https://doi.org/10.1155/2015/847320

68. Smida, A., Ncibi, S., Taleb, J., Saad, A. B., Ncib, S., & Zourgui, L. (2017). Immunoprotective activity and antioxidant properties of cactus (Opuntia ficus indica) extract against chlorpyrifos toxicity in rats. *Biomedicine & Pharmacotherapy*, *88*, 844-851.