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Protective biological effects of pan bread fortified with opuntia ficus-indica cladodes against hepatic toxicity in rats

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Abstract

This study evaluated the nutritional, sensory, and biological properties of pan bread fortified with cactus cladode powder (*Opuntia ficus-indica*) and its protective effects against methotrexate (MTX)-induced toxicity in rats. GC-MS analysis identified 15 bioactive compounds in the cactus extract, including oleic acid, n-hexadecanoic acid, and various trimethylsilyl esters. Chemical composition analysis revealed that increasing the levels of cactus powder improved fiber and ash content but reduced protein and carbohydrate levels. Sensory evaluation showed that pan bread containing 14% cactus powder (PBCC 14%) had the highest acceptability, indicating this level as optimal for consumer preference. In vivo, PBCC significantly mitigated MTX-induced hepatotoxicity, nephrotoxicity, and oxidative stress, as demonstrated by improvements in liver and kidney function markers, antioxidant enzyme activities, and histopathological observations. Combined treatment with silymarin and cactus powder further enhanced these protective effects. These findings suggest that cactus-fortified pan bread, particularly at 14% inclusion, offers nutritional benefits and potential protective properties against chemotherapy-induced organ toxicity.

Keywords *Opuntia ficus-indica*, Pan bread, Sensory evaluation, GC-MS, MTX

1 Introduction

Functional foods and nutraceuticals are increasingly recognized for their role in preventing chronic conditions, such as cardiovascular disease and dyslipidemia [1]. Epidemiological evidence links plant-based diets to lower risks of diseases driven by oxidative stress. Enhancing commonly consumed products, such as wheat-based bread—which typically lacks antioxidants—with natural compounds may significantly increase their health-promoting potential [2].

Nopal (*Opuntia* spp.), a cactus native to the arid regions of Mexico, has emerged as a promising candidate for such enhancements. In Egypt, the cultivation of *Opuntia ficus-indica*, also known as prickly pear or nopal, has been steadily growing, particularly in arid regions. This cactus thrives in desert conditions, requiring minimal water and maintenance, making it well-suited to the Egyptian climate [3]. The economic and



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agricultural value of *Opuntia* cultivation is significant, as it can improve soil quality, generate new income opportunities for farmers, and provide a sustainable source of food and raw materials for various industries [3]. The decision to expand its cultivation in Egypt is driven by its drought resilience, low water requirements, and high nutritional and economic benefits [4].

The cactus cladodes, which function as photosynthetic stems, are nutritionally dense, environmentally sustainable, and economically viable. Rich in bioactive compounds, these cladodes have shown potential in managing metabolic disorders, including diabetes [5], hypercholesterolemia [6], obesity [7], and hypertension [8]. With growing global interest in naturally health-promoting diets, there is a shift toward incorporating functional ingredients directly into everyday food products. This approach offers a practical and non-invasive alternative to pharmaceutical supplementation, aligning with traditional dietary habits and consumer preferences [9]. Recent studies, such as those by Shah et al. [10], further highlight the increasing demand for integrating plant-based compounds into food products to enhance their functional value, which aligns with the goals of our study.

Methotrexate (MTX) is a folic acid analog that disrupts nucleotide synthesis by inhibiting enzymes such as dihydrofolate reductase and thymidylate synthase, thereby impairing DNA replication and cell division. Due to its antiproliferative and immunosuppressive properties, MTX is widely used in the treatment of autoimmune diseases and various cancers [11]. However, MTX administration is frequently associated with multiple adverse effects, including hepatotoxicity, nephrotoxicity, gastrointestinal disturbances, hematological suppression, and mucocutaneous damage. Chronic use or high doses may lead to persistent liver enzyme elevation, fibrosis, or cirrhosis [12]. Moreover, genetic variability among patients may increase their susceptibility to MTX toxicity, often resulting in therapy discontinuation [13].

This study aims to evaluate the protective role of pan bread fortified with *Opuntia ficus-indica* cladodes against MTX-induced hepatic toxicity in rats. The inclusion of this cactus, rich in antioxidants and anti-inflammatory compounds, is hypothesized to mitigate liver damage and improve hepatic function under MTX exposure.

2 Results and discussion

2.1 GC-MS analysis

Figure 1 illustrates that GC-MS analysis of the cactus cladode extract identified multiple bioactive compounds with varying retention times (RTs). Table 1 provides comprehensive details regarding the chemical formulas, molecular weights, RTs, and percentage

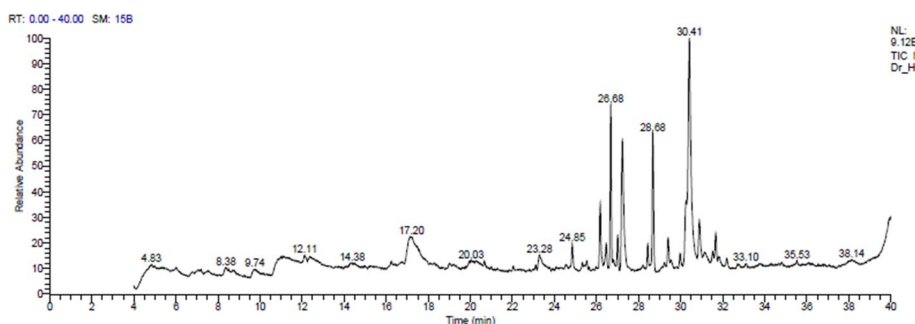


Fig. 1 Compounds Identified by GC-MS Analysis of Cactus cladodes

Table 1 Compounds identified by GC-MS analysis of cactus cladodes

No.	RT	Compound Name	Molecular formula	Mo- lecular weight	Area %
1	17.13	Melezitose	C ₂ H ₇ NO ₃ S ₂	157	3.61
2	17.18	3-O-Hexopyranosylhex-2-ulofuranosyl hexopyranoside #	C ₁₈ H ₃₂ O ₁₆	504	4.69
3	24.85	Myristic acid, TMS derivative	C ₁₇ H ₃₆ O ₂ Si	300	2.41
4	26.17	α-D-Galactopyranoside, methyl 2,3-bis-O-(trimethylsilyl)-, cyclic butylboronate	C ₁₇ H ₃₇ BO ₆ Si ₂	404	5.08
5	26.46	Galactopyranose, 5TMS derivative	C ₂₁ H ₅₂ O ₆ Si ₅	540	2.35
6	26.68	d-Glucose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxyme, (1Z)-	C ₂₂ H ₅₅ NO ₆ Si ₅	569	9.05
7	27.01	d-Mannose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, o-methyloxyme, (1Z)-	C ₂₂ H ₅₅ NO ₆ Si ₅	569	2.12
8	27.23	n-Hexadecanoic acid	C ₁₆ H ₃₂ O ₂	256	14.00
9	28.44	α-L-(-)-Fucopyranose, (R, S,R, R,S)-4TMS derivative	C ₁₈ H ₄₄ O ₅ Si ₄	452	1.86
10	28.68	Hexadecanoic acid, Trimethylsilyl ester	C ₁₉ H ₄₀ O ₂ Si	328	8.87
11	29.40	9-Octadecenoic acid (z)-,Methyl ester	C ₁₉ H ₃₆ O ₂	296	2.36
12	30.26	9-Octadecynoic acid	C ₁₈ H ₃₂ O ₂	280	5.35
13	30.41	Oleic Acid	C ₁₈ H ₃₄ O ₂	282	31.52
14	30.89	9-Octadecenoic acid	C ₁₈ H ₃₄ O ₂	282	4.47
15	31.67	Oleic Acid, (Z)-, TMS derivative	C ₂₁ H ₄₂ O ₂ Si	354	2.26

Table 2 Chemical composition of Pan bread fortified with varying percentages of cactus cladodes powder

Constituents (%)	CC	WF	PB	PBCC 7%	PBCC 14%	PBCC 21%
Moisture	10.32 ± 1.25 ^d	15.13 ± 0.51 ^c	43.03 ± 0.023 ^b	44.79 ± 0.253 ^a	44.60 ± 0.22 ^a	45.05 ± 0.08 ^a
Ash	27.66 ± 0.16 ^a	0.65 ± 0.10 ^f	1.59 ± 0.012 ^e	1.75 ± 0.028 ^d	2.34 ± 0.010 ^c	3.29 ± 0.002 ^b
Fiber	18.58 ± 0.31 ^a	0.09 ± 0.01 ^e	0.29 ± 0.01 ^d	0.81 ± 0.004 ^c	1.01 ± 0.002 ^c	1.42 ± 0.048 ^b
Protein	9.68 ± 0.22 ^d	14.10 ± 0.04 ^a	11.87 ± 0.025 ^b	10.53 ± 0.004 ^c	9.74 ± 0.030 ^d	9.35 ± 0.089 ^e
Fat	2.07 ± 0.04 ^c	1.67 ± 0.22 ^d	3.16 ± 0.00 ^a	2.45 ± 0.005 ^b	2.31 ± 0.027 ^b	2.34 ± 0.015 ^b
NFE	31.68 ± 1.55 ^d	68.36 ± 0.33 ^a	40.06 ± 0.025 ^b	39.68 ± 0.22 ^c	39.99 ± 0.18 ^b	38.54 ± 0.025 ^c

peak areas of the identified compounds. Fifteen components were detected in the cactus extract, with the predominant ones being oleic acid (31.52%), n-hexadecanoic acid (14%), D-glucose, 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, O-methyloxyme, (1Z)- (9.05%), hexadecanoic acid, trimethylsilyl ester (8.87%), 9-octadecynoic acid (5.35%), α-D-galactopyranoside, methyl 2,3-bis-O-(trimethylsilyl)-, cyclic butylboronate (5.08%), 3-O-hexopyranosylhex-2-ulofuranosyl hexopyranoside (4.69%), and 9-octadecenoic acid (4.47%). These compounds highlight the diverse chemical composition of the cactus extract, which may contribute to its various biological activities and potential health benefits.

2.2 Chemical composition

Table 2 presents the average values of the main chemical constituents—moisture, ash, fiber, protein, fat, and carbohydrates—in cactus cladode powder (CC), wheat flour (WF), and pan bread fortified with varying levels of cactus cladode powder. Increasing the cactus powder content led to higher moisture levels; PBCC 21% had a moisture content of 45.05%, compared with 43.03% in the control pan bread (PB) ($p < 0.05$; Table 2). Ash (27.66%) and fiber (18.58%) were highly concentrated in cactus powder, and accordingly,

PBCC 21% showed higher levels of these components (ash 3.29%, fiber 1.42%) compared with PB (ash 2.03%, fiber 1.88%).

Conversely, protein and carbohydrate contents declined with increasing cactus powder. PB exhibited the highest protein content (11.87%), while PBCC 21% had the lowest (9.35%) ($p < 0.05$; Table 2). These changes are attributed to the lower protein and carbohydrate content of cactus cladodes compared to wheat flour, causing a dilution effect when incorporated. Additionally, the high fiber content of cactus cladodes can interfere with gluten network formation and starch gelatinization during baking, limiting the full integration of these macronutrients into the dough. These results are consistent with previous studies reporting similar nutrient composition changes in bakery products enriched with high-fiber plant-based flours, including cactus and legume flours [14–16].

2.3 Sensory evaluation

The PBCC 14% formulation scored significantly higher in appearance, aroma, texture, and softness ($p < 0.05$), indicating acceptable sensory performance for an enriched product (Table 3). However, when the fortification level was increased to 21%, appearance, flavor, softness, and overall preference decreased significantly ($p < 0.05$), suggesting that excessive replacement can negatively affect sensory quality (Table 3).

The improved sensory performance at the 14% level is likely due to the moderate addition of cactus cladode powder, which enhances moisture retention and imparts a pleasant greenish color and mild herbal aroma without disrupting the gluten structure. At higher inclusion levels (PBCC 21%), the increased fiber and ash content probably interfered with gluten network formation, reducing the dough's ability to trap gas and resulting in a denser crumb and reduced softness effects commonly observed in fiber-enriched bakery products.

In this study, PBCC 7% achieved the highest overall acceptability, but PBCC 14% remained within the acceptable sensory range while offering greater functional enrichment. Therefore, 14% was selected as the optimal level for the nutrition study, representing a balance between sensory acceptability and nutritional benefits. These findings align with previous studies by Sciacca et al. [15] and Rodrigues et al. [16], which reported that moderate cactus ingredient replacement improves texture and aroma, whereas excessive inclusion leads to sensory unacceptability. Overall, a fortification level of 14% appears most reasonable, providing enhanced nutritional value while maintaining favorable sensory characteristics (Table 3).

Table 3 Sensory evaluation scores of Pan bread fortified with different levels of cactus cladodes powder

Sensory attributes	PB	PBCC 7%	PBCC 14%	PBCC 21%
Appearance	8.86 ± 0.38 ^{ab}	9.00 ± 0.00 ^a	8.29 ± 0.36 ^b	7.43 ± 0.53 ^c
Crust color	8.86 ± 0.38 ^a	9.00 ± 0.00 ^a	8.57 ± 0.53 ^a	7.29 ± 0.49 ^b
Crumb color	8.71 ± 0.75 ^a	8.57 ± 0.53 ^a	8.43 ± 0.78 ^a	7.29 ± 0.76 ^b
Aroma	8.86 ± 0.38 ^a	8.71 ± 0.49 ^a	8.57 ± 0.53 ^a	7.71 ± 1.11 ^b
Flavor	8.57 ± 0.79 ^a	8.43 ± 0.53 ^a	8.00 ± 0.58 ^{ab}	7.29 ± 0.76 ^b
Texture	8.43 ± 0.53 ^{ab}	9.00 ± 0.00 ^a	8.14 ± 0.38 ^b	8.00 ± 1.00 ^b
Sponginess when opening	8.29 ± 0.75 ^{ab}	9.00 ± 0.00 ^a	8.43 ± 0.53 ^{ab}	8.00 ± 1.00 ^b
Sponginess in the mouth	8.29 ± 0.76 ^a	8.57 ± 0.53 ^a	8.29 ± 0.49 ^a	7.43 ± 0.75 ^b
Overall acceptability	8.29 ± 0.76 ^a	8.86 ± 0.38 ^a	8.29 ± 0.49 ^{ab}	7.57 ± 0.98 ^b

Table 4 Effect of PBCC on final weight, body weight gain, daily body weight gain, daily feed intake, and feed efficiency against MTX-induced toxicity in rats

Groups	Final weight	Body weight gain	Daily Body weight gain	Daily feed intake	Feed efficiency ratio
Control	203.88 ± 8.31	63.55 ± 0.62	3.03 ± 0.03	16.91 ± 0.12	17.92
PB	214.77 ± 1.77	75.10 ± 5.25	3.58 ± 0.25	16.82 ± 0.12	21.28
PBCC	212.52 ± 4.33	71.85 ± 0.49	3.42 ± 0.02	16.93 ± 0.11	20.20
MTX	179.03 ± 4.43 ^a	27.86 ± 1.81 ^a	1.33 ± 0.08 ^a	13.29 ± 0.49 ^a	9.98 ^a
MTX + PBCC	183.81 ± 7.61 ^b	44.15 ± 4.04 ^b	2.10 ± 0.19 ^b	15.91 ± 0.71 ^b	13.04 ^b
MTX + SILY	186.30 ± 3.81 ^b	45.63 ± 1.13 ^b	2.17 ± 0.05 ^b	16.12 ± 0.56 ^b	13.46 ^b
MTX + PBCC + SILY	194.95 ± 6.04 ^{ab}	55.62 ± 2.74 ^{ab}	2.65 ± 0.13 ^{ab}	16.11 ± 0.55 ^{ab}	16.45 ^{ab}

Table 5 Effect of PBCC on liver functions against MTX-induced toxicity in rats

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Control	28.48 ± 2.2	34.44 ± 2.4	125.00 ± 9.3	22.33 ± 2.2	1.31 ± 0.1	7.50 ± 1.5
PB	26.70 ± 2.8	37.94 ± 2.7	130.90 ± 8.4	25.21 ± 2.9	1.26 ± 0.2	8.50 ± 1.4
PBCC	27.81 ± 2.6	36.19 ± 2.6	136.35 ± 10.3	22.40 ± 2.2	1.28 ± 0.3	7.00 ± 1.4
MTX	52.56 ± 4.7 ^a	97.78 ± 6.4 ^a	354.51 ± 12.2 ^a	51.11 ± 3.8 ^a	2.97 ± 0.4 ^a	16.50 ± 1.5 ^a
MTX + PBCC	40.84 ± 3.5 ^b	69.36 ± 5.9 ^b	316.33 ± 13.4 ^b	35.21 ± 2.3 ^b	2.40 ± 0.4 ^b	12.00 ± 1.4 ^b
MTX + SILY	35.18 ± 3.2 ^b	57.62 ± 5.2 ^b	299.97 ± 15.2 ^b	34.14 ± 2.5 ^{ab}	2.36 ± 0.5 ^{ab}	13.50 ± 1.9 ^{ab}
MTX + PBCC + SILY	33.40 ± 2.1 ^{ab}	48.89 ± 3.9 ^{ab}	283.61 ± 14.4 ^b	30.11 ± 2.6 ^{ab}	2.16 ± 0.5 ^{ab}	10.50 ± 1.5 ^{ab}

2.4 Biological activities

2.4.1 Body weight and feed efficiency

Table 4 illustrates the effects of PBCC on body weight and feed efficiency in rats subjected to MTX-induced toxicity. No significant differences were observed in weight gain or feed efficiency between the control and PB groups (Table 4). Although the PBCC group showed slight improvements in both parameters, these changes were not statistically significant.

In contrast, the MTX group exhibited a significant reduction in body weight gain and feed efficiency compared to the control group ($p < 0.05$; Table 4), consistent with previous reports of MTX-induced metabolic deterioration [17]. Notably, rats in the MTX + PBCC group demonstrated marked improvements in both parameters relative to the MTX-only group ($p < 0.05$; Table 4), suggesting a protective effect of cactus supplementation. Similar trends were observed in the MTX + SILY and MTX + PBCC + SILY groups, with the latter showing the most pronounced recovery, indicating a potential synergistic interaction between cactus cladodes and silymarin.

These findings are consistent with earlier studies highlighting the growth-promoting and protective effects of cactus cladodes in disease-induced models [18].

2.4.2 Liver and kidney functions

The results indicate that PBCC exerted a protective effect on liver and kidney functions in rats administered MTX, as shown in Table 5. Liver enzymes, including ALT, AST, and ALP, were significantly elevated in the MTX group, reflecting hepatocellular damage consistent with previous reports of MTX toxicity [19]. In contrast, the MTX + PBCC group exhibited significantly lower enzyme levels compared to the MTX group

($p < 0.05$), suggesting that cactus powder alleviates liver injury, likely due to its antioxidant properties that reduce oxidative stress [20]. The hepatoprotective effect was further enhanced when PBCC was combined with silymarin, resulting in an additional reduction in liver enzyme levels.

Regarding renal function, MTX treatment caused significant increases in serum urea, creatinine, and uric acid ($p < 0.05$), indicating nephrotoxicity [21]. These elevations were mitigated by co-treatment with PBCC, demonstrating its potential to protect against MTX-induced kidney damage. The combination of PBCC and silymarin (MTX + PBCC + SILY) produced the strongest reduction in renal biomarkers, indicating a synergistic protective effect [22, 23]. These findings suggest that PBCC may serve as a beneficial dietary intervention to attenuate liver and kidney toxicity in MTX-treated rats.

2.4.3 Metabolic disturbances

The effects of treatments on glucose and lipid profiles in rats are presented in Table 6. No significant changes were observed in the control, PB, and PBCC groups, indicating that the diet did not produce any adverse effects. Administration of MTX resulted in significant increases in glucose, total cholesterol, and triglycerides, along with a marked reduction in HDL cholesterol ($p < 0.05$), consistent with its role as a metabolic toxicant [24].

These metabolic disturbances were significantly ameliorated in rats co-treated with MTX and either PBCC or silymarin (SILY). Notably, the combination treatment (MTX + PBCC + SILY) provided the strongest protective effect, suggesting a synergistic interaction between cactus and silymarin [25].

MTX-induced dyslipidemia is characterized by elevated total cholesterol, triglycerides, and LDL cholesterol, along with reduced HDL cholesterol, which increases the risk of cardiovascular disease due to hepatic dysfunction and oxidative stress [26, 27]. The phenolic compounds and flavonoids in cactus exhibit antihyperglycemic properties by enhancing insulin sensitivity and reducing free radical activity [15]. Additionally, cactus contains unsaturated fatty acids that inhibit α -glucosidase activity and protect pancreatic β -cells [28]. Its high fiber content further reduces fat absorption, helping to prevent dyslipidemia and providing protection against atherosclerosis and diabetes-related complications [29].

2.4.4 Oxidative stress

Table 7 shows the effects of cactus cladode-fortified pan bread (PBCC) on oxidative stress indicators and antioxidant enzyme activities in rats subjected to methotrexate (MTX). No significant differences were observed in the levels of superoxide dismutase

Table 6 Effect of PBCC on lipid profile and glucose against MTX-induced toxicity in rats

Groups	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Control	84.49 ± 2.50	100.00 ± 4.1	80.00 ± 4.9	40.50 ± 1.2	43.5 ± 4.38
PB	76.11 ± 2.56	117.14 ± 5.1	82.86 ± 5.4	35.00 ± 1.9	65.57 ± 5.55
PBCC	80.14 ± 3.43	108.57 ± 5.4	85.43 ± 2.9	38.25 ± 2.1	53.23 ± 5.81
MTX	285.00 ± 5.00 ^a	185.71 ± 5.7 ^a	158.57 ± 4.8 ^a	19.00 ± 1.3 ^{ab}	135.0 ± 5.92 ^a
MTX + PBCC	225.21 ± 8.30 ^{ab}	156.43 ± 4.8 ^{ab}	122.86 ± 5.7 ^{ab}	23.00 ± 1.08 ^{ab}	108.86 ± 5.05 ^{ab}
MTX + SILY	215.16 ± 2.50 ^{ab}	144.29 ± 5.0 ^{ab}	104.26 ± 5.0 ^{ab}	27.50 ± 1.4 ^{ab}	95.94 ± 5.29 ^{ab}
MTX + PBCC + SILY	190.11 ± 4.86 ^{ab}	135.00 ± 5.5 ^{ab}	97.71 ± 5.3 ^{ab}	29.25 ± 1.1 ^{ab}	86.21 ± 5.71 ^{ab}

Table 7 Effect of cactus cladode-fortified Pan bread against methotrexate-induced oxidative stress

Groups	SOD (U/g)	CAT (U/g)	MDA (nmol/g)
Control	3.08 ± 0.9	3.44 ± 0.8	0.95 ± 0.1
PB	3.21 ± 0.7	3.63 ± 0.5	0.91 ± 0.2
PBCC	3.14 ± 0.6	3.26 ± 0.9	0.87 ± 0.2
MTX	0.96 ± 0.4 ^a	1.15 ± 0.6 ^a	3.47 ± 0.5 ^a
MTX + PBCC	2.31 ± 0.7 ^b	2.16 ± 0.6 ^b	2.12 ± 0.9 ^b
MTX + SILY	2.53 ± 0.8 ^b	2.40 ± 0.8 ^b	1.94 ± 0.3 ^b
MTX + PBCC + SILY	2.98 ± 0.7 ^{ab}	3.52 ± 0.9 ^{ab}	1.02 ± 0.5 ^{ab}

(SOD), catalase (CAT), or malondialdehyde (MDA) among the control, PB, and PBCC groups, indicating that the diet had no adverse effects ($p < 0.05$). In contrast, the MTX group exhibited decreased SOD and CAT activities and elevated MDA levels, reflecting heightened oxidative stress ($p < 0.05$). These findings are consistent with previous reports, including Khafaga and El-Sayed [30], which attributed MTX-induced liver toxicity to oxidative damage.

MTX-induced impairment of SOD and CAT suggests disruption of cellular redox homeostasis and increased production of reactive oxygen species (ROS) [31], leading to lipid peroxidation and elevated MDA levels [32]. In contrast, feeding PBCC restored antioxidant enzyme activities and significantly reduced MDA levels compared to the MTX group ($p < 0.05$; Table 7), indicating a protective effect. Similarly, the MTX + SILY group exhibited improved antioxidant defenses and reduced MDA levels, supporting the known antioxidant properties of silymarin ($p < 0.05$).

Notably, the combined treatment (MTX + PBCC + SILY) resulted in the greatest recovery of SOD and CAT activities and the lowest MDA levels, suggesting a synergistic effect of cactus and silymarin in alleviating MTX-induced oxidative stress ($p < 0.05$). These results align with the findings of Chekkal et al. [33], who reported decreased lipid peroxidation and enhanced antioxidant defenses following cactus supplementation. Additional evidence from studies on cactus cladodes and fruit, rich in polyphenols and antioxidants, further supports their effectiveness in reducing oxidative damage [34].

2.4.5 Histopathological assessment

Microscopically, liver tissue from rats in the control group displayed normal hepatic histoarchitecture (Fig. 2). Similarly, liver tissues from the Pan bread (PB) and Pan bread fortified with cactus cladode powder (PBCC) groups showed no histopathological lesions (Fig. 2), indicating the safety of cactus-fortified pan bread.

In contrast, the MTX group exhibited significant histopathological alterations, including hepatocellular vacuolar degeneration (black arrow), Kupffer cell proliferation, fibroblast strands in the portal triad, and newly formed bile ductules (blue arrow) (Fig. 2), consistent with MTX-induced hepatic injury [35]. These changes are largely attributed to oxidative damage from MTX metabolism, which promotes reactive oxygen species (ROS) generation and disrupts folate pathways, resulting in hepatocellular injury [18, 36].

Liver tissues from rats treated with MTX + PBCC or MTX + SILY showed only mild hepatocellular vacuolation and limited Kupffer cell proliferation (black arrow), indicating partial protection (Fig. 2). Remarkably, the MTX + PBCC + SILY group displayed near-normal histology with minimal alterations (Fig. 2), reflecting a synergistic protective effect. These findings are consistent with previous reports attributing the

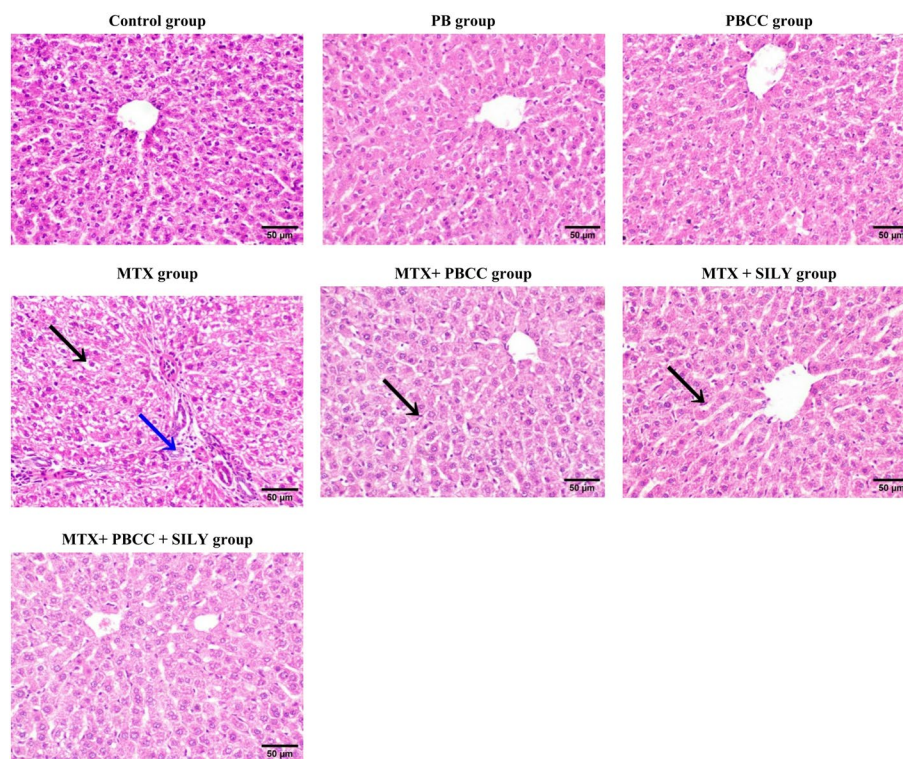


Fig. 2 Histopathological examination of liver: (H & E X 200, scale bar 50 μ m). **Control group:** Photomicrograph of liver of rat from group 1 showing the normal histoarchitecture of hepatic tissue. **PB group:** Photomicrograph of liver of rat from group 2 showing no histopathological lesions. **PBCC group:** Photomicrograph of liver of rat from group 3 showing no histopathological lesions. **MTX group:** Photomicrograph of liver of rat from group 4 showing hepatocellular vacuolar degeneration (black arrow) and newly formed bile ductules in the portal triad (blue arrow). **MTX + PBCC group:** Photomicrograph of liver of rat from group 7 showing slight Kupffer cells proliferation (black arrow). **MTX + SILY group:** Photomicrograph of liver of rat from group 6 showing slight Kupffer cells proliferation (black arrow). **MTX + PBCC + SILY group:** Photomicrograph of liver of rat from group 8 showing normal histology. *PB* Pan Bread, *PBCC* Pan bread fortified with Cactus Cladode, *SILY* Silymarin, *MTX* Methotrexate

hepatoprotective effects of cactus cladode extract to its antioxidant and metal-chelating properties [37].

3 Limitations of the study

This study demonstrated the hepatoprotective effects of cactus cladode powder and silymarin against methotrexate-induced toxicity through biochemical and histological evaluations. However, some limitations should be considered. The work mainly depended on measuring enzyme activities and oxidative stress parameters, which do not fully explain the molecular events responsible for the observed protection. The absence of gene or protein expression studies related to antioxidant and inflammatory pathways limits the understanding of the precise biological mechanisms. In addition, the small number of animals and the short duration of treatment may not reflect long-term hepatic adaptation. Future studies are recommended to include molecular and cellular analyses, such as PCR and Western blot, to verify these findings and clarify the underlying protective pathways.

4 Conclusion

This study highlights the potential of cactus cladode powder (CP) as a valuable ingredient for fortifying pan bread, enhancing its nutritional content without compromising sensory quality. Incorporation of CP significantly increased fiber and mineral levels, particularly in the 21% fortified bread, although protein and carbohydrate contents were slightly reduced. Sensory evaluation indicated that a 14% inclusion provided the optimal balance between nutritional enhancement and desirable taste and texture. Moreover, cactus-fortified pan bread demonstrated protective effects against methotrexate (MTX)-induced toxicity in rats, improving body weight, liver and kidney function, and antioxidant enzyme activities. Notably, the combination of CP with silymarin produced a synergistic effect, further enhancing these protective properties. These findings support the potential of cactus-fortified pan bread as a functional dietary strategy to promote health and mitigate toxicity-induced damage.

5 Materials and methods

5.1 Preparation of cactus cladodes powder

Fresh cladodes of *Opuntia ficus-indica* were collected from a local prickly pear cactus farm located on the Giza-Aswan Western Desert Road, Beni Suef, Egypt. Undamaged, mature cladodes aged 12–15 months were manually harvested in July 2023. The cladodes were thoroughly washed with water and carefully cleaned to remove impurities, and any remaining spines were gently removed using a soft brush to avoid damaging the plant tissue [38]. The cleaned cladodes were blotted with absorbent paper and sliced uniformly to a thickness of 7–8 mm using a slicing machine. Drying was performed under direct sunlight at approximately 36–40 °C with 80–82% relative humidity for 18–20 h. The dried cladodes were then ground in an electric laboratory mill and sieved through a 60-mesh screen to obtain fine powders. The resulting powders were stored in airtight containers at 4 °C until further analysis and use. The collection of herbs complies with relevant institutional, national, and international guidelines.

5.2 GC–MS analysis

Five grams of cactus cladode powder were extracted with 50 mL of ethanol under magnetic stirring for 3 h. The mixture was filtered using Whatman paper, and the filtrate was concentrated under vacuum to obtain the extract used for GC–MS analysis. The analysis was performed using a Trace GC1300-TSQ MS (Thermo Scientific, USA) with a TG–5MS capillary column (30 m × 0.25 mm, 0.25 µm film thickness). The temperature program began at 60 °C (2 min), increased at 8 °C/min to 300 °C, then to 310 °C at 20 °C/min (held for 2 min). The injector and transfer line were set at 250 °C and 260 °C, respectively. Helium served as the carrier gas at 1 mL/min. Samples (1 µL) were injected in split mode with a 3-min solvent delay. Spectra were recorded in full scan mode (50–650 m/z) at 70 eV, and compounds were identified using WILEY 09 and NIST 14 libraries.

5.3 Preparation of pan bread

The pan bread preparation followed the procedure described by Amjad et al. [39] with modifications. Wheat flour with a 72% extraction rate was used as the base ingredient, and part of it was substituted with cactus cladodes powder at levels of 0, 7, 14, and 21%, respectively.

5.4 Chemical analysis

Moisture, crude protein, crude fat, ash and crude fiber contents were determined according to the methods of the AOAC [40]. Nitrogen free extract (NFE) was calculated by difference $100 - (\text{Moisture} + \text{protein} + \text{fat} + \text{ash} + \text{fibers})$.

5.5 Sensory evaluation

Sensory evaluations for the appearance, crust color, crumb color, aroma, flavor, texture, sponginess when opening, sponginess in the mouth, and overall acceptability of pan bread were performed to evaluate consumers' preferences. A numerical hedonic scale ranging from one to nine (9-point hedonic scale) was used for sensory evaluation (9 = like extremely, 8 = like very much, 7 = like moderately, 6 = like slightly, 5 = neither like nor dislike, 4 = dislike slightly, 3 = dislike moderately, 2 = dislike very much, 1 = dislike extremely). The sensory evaluation was performed following the ethical standards approved by Minia University (Approval No. MU/FA011/01/25). The panelists (23 men and 23 women, 19–58 years old) comprised of academic staff (Department of Food Science), postgraduates who previously experienced sensory evaluation as well as regular pan bread consumers. They were seated under white light in a separate well-ventilated room during lunch break sessions. The samples were coded with random three-digit codes and presented to the panelists in a randomized order to evaluate their preferences [41].

5.6 Experimental design

A total of forty-two adult male Sprague-Dawley albino rats, each weighing approximately 140 ± 5 g, were used in this study. The animals were obtained from the Animal House at the Faculty of Pharmacy, Nahda University, Egypt. The animals were housed under standard laboratory conditions (21 ± 2 °C) in plastic cages and provided with a commercial balanced diet and tap water ad libitum for two weeks prior to the experimental phase.

After the acclimatization period, the rats were randomly divided into seven groups (six rats per group) as follows:

Control group Received a standard basal diet.

PB group Fed a basal diet supplemented with 20% control pan bread (PB) [42] throughout the 21-day study.

PBCC group Received a diet containing 20% pan bread fortified with 14% cactus cladodes powder (PBCC) throughout the 21-day study.

MTX group Received a diet containing 20% pan bread, and on day 15, a single intraperitoneal dose of methotrexate (MTX) at 20 mg/kg was administered to induce toxicity [30].

MTX + PBCC group Received a diet containing 20% pan bread fortified with 14% cactus cladodes powder (PBCC) and injected with a single dose of MTX (20 mg/kg, *i.p.*) on day 15.

MTX + SILY group Received a diet containing 20% pan bread and administered Silymarin orally at a dose of 50 mg/kg body weight once daily for 21 days [43]. On day 15, the rats also received a single MTX injection (20 mg/kg, *i.p.*).

MTX + PBCC + SILY group Received a diet containing 20% pan bread fortified with 14% cactus cladodes powder (PBCC) and treated orally with silymarin (50 mg/kg, orally

once daily for 21 days). On day 15, the rats received a single MTX injection (20 mg/kg, *i.p.*).

5.6.1 Body weight and food intake

The rats' body weights were recorded weekly throughout the three-week experimental period. Daily food intake was monitored by calculating the difference between the feed provided and the residual amount remaining in the bin, as described by Ennouri et al. [6].

5.6.2 Blood collection and biochemical analysis

At the end of the experimental phase, rats were anesthetized with intraperitoneal injections of ketamine (75 mg/kg) and xylazine (16 mg/kg). Blood samples were drawn from the retro-orbital venous plexus, allowed to clot at room temperature, and centrifuged at $1200 \times g$ for 15 min. The separated serum was preserved at -20°C for subsequent biochemical assessments.

Serum uric acid was analyzed using the method by Caraway et al. [44], while creatinine levels were measured according to Bartels and Böhmer [45]. Urea concentration was evaluated following the procedure by Marsch et al. [46]. The activities of hepatic enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined as outlined by Bergmeyer and Harder [47]. Serum total cholesterol (TC) was measured following the method of Henry et al. [48], while triglycerides (TG) were assessed according to Fossati and Prencipe [49]. High-density lipoprotein cholesterol (HDL-C) was determined using the procedure described by Burstein [50]. The concentration of low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation:

$$\text{LDL-C (mg/dL)} = \text{TC} - \text{HDL-C} - (\text{TG}/5).$$

5.6.3 Organ collection and preparation

Following blood collection, rats were euthanized via cervical dislocation. The abdominal cavity was carefully opened, and the liver was excised, rinsed in saline, blotted dry with filter paper, and weighed. Liver samples were stored at -20°C for further biochemical evaluation [51].

5.6.4 Oxidative stress biomarkers in liver tissue

Liver samples (0.5 g) were homogenized in ice-cold 50 mM sodium phosphate buffer (5 ml, pH 7.4) at a 1:10 (w/v) ratio using a tissue homogenizer to ensure efficient cell disruption and minimize enzymatic degradation. The homogenates were centrifuged at 10,000 rpm for 20 min at 4°C to remove cell debris, following the method of Zaid et al. [52]. The resulting supernatants were collected and stored at -20°C for the analysis of oxidative stress parameters. Superoxide dismutase (SOD) and catalase (CAT) activities were determined according to Beutler et al. [53] and Cohen et al. [54], respectively, while lipid peroxidation was assessed by measuring malondialdehyde (MDA) levels using the procedure described by Ohkawa et al. [55].

5.6.5 Histopathological examination

Samples from the liver were collected and fixed in 10% formal saline for 24 h. The tissues were washed with tap water, dehydrated through graded alcohols (methyl, ethyl, and absolute), and cleared in xylene. They were then embedded in paraffin at 56 °C for 24 h. Paraffin blocks were sectioned at 4 µm using a rotary microtome. The sections were mounted on glass slides, deparaffinized, stained with hematoxylin and eosin, and examined under a light microscope [56].

5.7 Statistical analysis

The obtained data were statistically analyzed using SPSS software. Differences among treatment groups were evaluated through one-way analysis of variance (ANOVA), followed by Duncan's multiple range test to determine significant differences. A *p*-value of less than 0.05 was considered statistically significant.

Author contributions

Marwa W. Abohleka, Sanaa M. Abdel-Hameed, Hanaa S. S. Gazwi, Osama I. A. Soltan designed and performed the experiments. Marwa W. Abohleka analyzed the data. Marwa W. Abohleka, Sanaa M. Abdel-Hameed, Hanaa S. S. Gazwi, Osama I. A. Soltan wrote the manuscript. Marwa W. Abohleka, Sanaa M. Abdel-Hameed, Hanaa S. S. Gazwi, Osama I. A. Soltan contributed to the manuscript by engaging in critical analysis, revising, and ultimately granting final clearance. The manuscript was prepared with the contributions of all authors.

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Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All animal procedures adhered to the ethical guidelines approved by the Institutional Animal Ethics Committee of Minia University, Egypt (Approval No. MU/FA010/01/25). The ARRIVE guidelines have been followed in this animal study.

Competing interests

The authors declare no competing interests.

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