

Effect of *Prosopis farcta* L. Fruits on Methotrexate Induced Nephrotoxicity in Rats

Amer Hakeem Chyad¹, Ayat abdulmahdi Altmimi², Saif A. Muhson³

1. Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Baghdad, Iraq.
2. Department of Pathology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.
3. Department of Anatomy and histology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

Corresponding Email: ayat.abd1107a@covm.uobaghdad.edu.iq

ORCID: <https://orcid.org/0009-0001-3722-1160>

Important dates: Received: 01-September-2025; Accepted: 20-January-2026; Published: 15-February-2026

Abstract:

Background: Methotrexate (MTX) has been an excellent chemotherapeutic agent, but its nephrotoxic potential developed by oxidative stress and tubular impairment limit its using. Medicinal plants like *Prosopis farcta* (the fruit) can be proposed as an antioxidant to protect the kidney cells.

Objectives: The purpose of this study was to assess the nephroprotective effect of *Prosopis farcta* fruit extract (PFE) against MTX-induced nephrotoxicity in a rat model. Adult rats were divided into five groups: control, MTX (20 mg/kg, i.p.), and MTX + PFE (300, 400 or 500 mg/kg per orally) for 30 days. The levels of renal function markers (BUN, creatinine, cystatin C and albumin), oxidative stress indicators (MDA, SOD) and urinary NAG were detected. Histopathological analysis was also performed.

Results: MTX resulted in significantly ($P < 0.05$) renal dysfunction as witnessed by increased levels of BUN, creatinine, cystatin C, MDA and NAG with decreased albumin and SOD. PFE therapy ameliorated these parameters in a dose-dependent fashion. The 500 mg/kg dose confirmed the maximum protection, as indicated by biochemical markers and renal histology closer to normalcy.

Conclusions: Fruit extract of *Prosopis farcta* has a potent renal-protective impact, which may be due to its antioxidant and tubular protective activities. Moreover, it can be a cheap natural adjunct for the alleviation of chemotherapy-induced renal toxicity.

Keyword: Methotrexate, Nephrotoxicity, *Prosopis farcta*, Antioxidant, Oxidative stress, Histopathology



This is an open access article licensed under a [Creative Commons Attribution- NonCommercial 4.0 International License](https://creativecommons.org/licenses/by-nc/4.0/).

Introduction:

Folate antagonist methotrexate (MTX) is one of the most frequently used drugs for anti-cancer or autoimmune treatments. However, its application is restricted in clinics due to dose-dependent nephrotoxicity that may be predominantly associated with oxidative stress, lipid peroxidation, inflammation and apoptosis observed in renal tissues. This pathophysiological cascade emphasizes the significance of determining safe and effective methods to preserve kidney function (Abiola, 2018). In recent years, more and more medicinal plants have been studied to explore their potential use in the repair of drug-related organ damage. *Prosopis farcta* L. (Syrian mesquite) is a deciduous leguminous shrub, native to desert and semi-desert regions of the Middle East. It has long been used in traditional medicine to treat diabetes, digestive problems, kidney stones and skin diseases. (Agirman *et al.*, 2022). The *Prosopis* genus is rich in phenolics, flavonoids, tannins, and alkaloids, including vitexin, isovitexin, apigenin, luteolin, and quercetin, which are associated with antioxidant, anti-inflammatory, antidiabetic, antimicrobial, and wound-healing activities. (Ahmed, *et al.*, 2020). To be more precise; *P. farcta* fruit extracts have shown to fight free radicals particularly well. Ethanolic fruit extracts are rich in total phenols (61.55 mg GAE/g) and flavonoids (17 mg QE/g); and they can free the system of DPPH, anion from ABTS⁺, and FRAP assays (Aladaileh *et al.*, 2019). Furthermore, *P. farcta* root extracts possess good antibacterial and free radical scavenging activities (IC₅₀ values are as the same as synthetic antioxidants in β-carotene-linoleic acid systems) (Aziznia *et al.*, 2019). In living diabetic rats, extracts of *P. farcta* fruit and seeds reduced oxidative stress and improved blood sugar and kidney function markers. But, some doses of seed extract have proven hepatotoxic (Jahromi *et al.*, 2018). Until now, the nephroprotective effects of *P. farcta* fruit extract on MTX-induced renal injury have not been systematically investigated in any study. According to the importance of oxidative stress in MTX induced nephrotoxicity and the considerable antioxidant activity of *P. farcta* components, fruit extract of this plant is proposed to ameliorate MTX related nephrotoxicity probably by its antioxidants and anti-inflammatory activities. The objective of this study is to investigate whether *P. farcta* fruit extract can prevent MTX-induced renal injury in adult rats by examining the functional, oxidative stress, and histopathological parameters.

Materials and Methods:

Animals and Experimental Design

The present study was conducted using adult male rats (n = 60) obtained from the Animal House of the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq. Animals were housed under standard laboratory conditions (22 ± 2 °C, 12 h light/dark cycle, 50–60% relative humidity) with free access to food and water. After a one-week acclimatization period, the rats were randomly allocated into five experimental groups.

Group I: Rats received sterile water,

Group II: (MTX group): the rat received a single dose of MTX (20 mg/kg) on day 1 (Aladaileh *et al.*, 2019).

Group III: Rats received a single dose of MTX (20 mg/kg) intraperitoneally on day 1 then rats will receive extract *prosopis farcta* (300mg/kg) for 30 days.

Group IV: Rats received a single dose of MTX (20 mg/kg) intraperitoneally on day 1 then rats will receive extract *prosopis farcta* (400mg/kg) for 30 days

Group V: Rats received a single dose of MTX (20 mg/kg) intraperitoneally on day 1 then rats will receive extract *prosopis farcta* (500mg/kg) for 30 days.

Drug and Plant Extract Administration

Methotrexate was freshly prepared in sterile saline and administered intraperitoneally. *Prosopis farcta* fruit extract was dissolved in distilled water and administered orally by gavage at the recommended doses.

Biochemical Analysis

Blood samples were collected under anesthesia by cardiac puncture at the end of the experiment. Serum was separated and analyzed for renal function parameters (creatinine, BUN, cystatin C, and albumin) using commercial diagnostic kits.

Oxidative Stress Biomarkers

The kidney tissue homogenates were made in normal saline phosphate (PBS, pH 7.4). Malondialdehyde (MDA), an index of lipid peroxidation, was measured by the Thio barbituric acid reactive substances (TBARS) assay. The activity of superoxide dismutase (SOD) was assayed spectrophotometrically according to the method of pyrogallol autoxidation.

N-acetyl-β-D-glucosaminidase (NAG) Activity Assay

Urinary N-acetyl-β-D-glucosaminidase (NAG) activity was measured by a spectrophotometric procedure using the hydrolysis of p-nitrophenyl-N-acetyl-β-D-glucosaminide (pNP-GlcNAc) as substrate. This approach was inspired by the Horak *et al.* (1981) with some changes. Briefly, urine samples were centrifuged at 3,000 rpm for 10 minutes to remove detritus. Aliquots of the supernatant were mixed with substrate solution in citrate-phosphate (pH 4.5) buffer at 37 °C for 15–30 min. It was then stopped by adding sodium carbonate (0.2 M) which converted the released pNp into yellow ionized form. The absorbance of the reaction mixture was measured at 405 nm with a spectrophotometer. Prepared a calibration curve by serially diluting p-nitrophenol standard solutions and then calculated NAG activity from the curve. There were expressed enzyme activity as units per L/g of urinary creatinine (U/L/g creatinine) in order to adjust for urine concentration. One unit (U) of enzyme activity was defined as the amount of enzyme required to hydrolyze 1 μmol substrate per minute under test conditions.

Histopathological-Examination

Tissue sections from the kidney were fixed in 10% neutral-buffered formalin, dehydrated, paraffin-embedded and 5 μ m thickness sliced; these were stained with hematoxylin-eosin (H&E). We then observed the sections under the microscope to determine whether tubular necrosis, glomerular injury, or structural changes had occurred. (Luna and Lee, 1968)

Statistical-Analysis

All data were displayed as mean \pm SD. For the first step of the statistical analysis. Analysis of Variance (ANOVA) was performed followed by an LSD post hoc test in the second stage. Values of $p < 0.05$ were considered to be statistically significant.

Results:

Biochemical Parameters

Administration of methotrexate (20 mg/kg) deteriorated kidney function significantly in comparison to the control group. Serum blood urea nitrogen (BUN), creatinine, and cystatin C levels were significantly elevated in rats of the MTX group ($p < 0.05$) with a significant reduction observed in serum albumin levels. These changes indicate that MTX was tough on the kidneys. *Prosopis farcta* fruit extract significantly attenuated renal function in a dose-dependent manner. Both the groups of rats receiving 300 mg/kg of extract performed better than MTX group. Their BUN, creatinine and cystatin C levels decreased significantly, while their albumin level increased slightly. At the dose of 400 mg/kg, a clearer protective effect was observed in which kidney biomarkers were improved and levels of albumin approached normal. The biggest change was observed in the 500 mg/kg group, where levels of kidney markers reached control values and albumin levels almost recovered to normal ($p < 0.05$). as shown in table (1).

Oxidative Stress Biomarkers

The rats that received methotrexate had higher renal malondialdehyde (MDA) levels and significantly lower SOD activity than control rats ($p < 0.05$). This showed that the endogenous antioxidant defense systems of body were challenged and lipid peroxidation was increased. These changes were far less pronounced when *P. farcta* fruit extract was applied. Both 300 mg/kg and 400 mg/kg increased SOD activity, reduced MDA level but all of these remained lower/higher than those in control group. At 500 mg/kg, MDA levels and SOD activity were almost restored to normal ($p < 0.05$), this is unbelievable. See table (2).

N-acetyl- β -D-glycosaminidase (NAG) Activity

Methotrexate-treated (20 mg/kg) group is just opposite to the control group with significantly ($P < 0.05$) less urinary NAG activity indicating that there are many tubules were severely damaged. The group treated with *Prosopis farcta* fruit extract had a significant decrease in the amount of NAG, and the more we gave them, the better it was. The 300 mg/kg and 400 mg/kg exerted a weak effect, yet the enzyme activity was still significantly higher than that of the control group, while it was much

less than that of the MTX group. The maximum dose (500 mg/kg) of the extracts normalized NAG activity and no significant difference in the values was observed compared to the control group. Such results can suggest that prevention of tubular injury and stabilization of renal function during MTX-induced stress in the kidney via *P. farcta* fruit extract anticipated its potential nephroprotective activity. As shown in table (3)

Histopathological Findings

Histopathological examination of kidney tissues supported the biochemical and oxidative stress data by showing changes in tissue architecture. The control group had normal renal morphology with intact glomeruli and preserved tubules (Fig. 1). In contrast, the MTX-treated group revealed significant pathological changes (glomerular congestion, tubular epithelial degeneration and vacuolation and interstitial inflammatory lesions) that indicate severe injury of the kidneys (fig. 2). *Prosopis farcta* fruit extract treatment ameliorated these histopathological alterations in a dose-dependent manner. There was a partial improvement in the 300 mg/kg group that there was less tubular and lower amounts of glomerulus noticeable other than MTX (fig. 3). 4) in renal histoarchitecture due to less tubular degeneration with improved glomerulus in 400 mg/kg groups. The 500 mg/kg group presented almost normal renal glomeruli and tubules which appeared very similar to those in the control group (fig. 5). Overall, the histopathological examination revealed that *Prosopis farcta* fruit extract significantly ameliorated MTX-induced nephrotoxicity and the higher dose (500 mg/kg) contributed to better protection.

Table (1): Effect of *Prosopis farcta* fruit extract on serum renal function biomarkers in methotrexate-treated rats (mean ± SD)

Groups	Blood urea nitrogen	Creatinine	Cystatin c	Albumin
(CONTROL)	14.84±1.33 c	0.54±0.26 d	0.37±0.70 d	4.07±0.43 c
(20MG OF MTX)	45.62±3.72 a	2.92±0.87 a	1.92±0.15 a	2.35±1.06 a
PFE (300MG/KG)	33.85±2.66 b	1.54±0.62 b	1.31±0.55 b	3.12±0.94 b
PFE (400MG/KG)	25.64±3.64 b	0.96±0.50 c	0.79±0.36 c	3.44±1.01 b
PFE (500MG/KG)	20.72±1.32 bc	0.62±0.47 d	0.57±0.78 d	3.84±1.01 c
LSD	4.1	0.34	0.52	0.43

Table (2): Effect of *Prosopis farcta* fruit extract on renal oxidative stress biomarkers (MDA and SOD) in methotrexate-treated rats (mean ± SD).

GROUPS	MDA (NMOL/100G)	SOD (U/G TISSUE)
(CONTROL)	19.63± 1.88 c	42.53 ± 3.02c
(20MG OF MTX)	62.86± 4.62 a	14.58 ± 2.51a
PFE (300MG/KG)	43.11± 2.72 b	33.29 ± 2.81 b
PFE (400MG/KG)	35.16± 3.56 b	35.46 ± 2.87 b
PFE (500MG/KG)	24.23± 2.92 c	39.77 ± 3.62 c
LSD	6.35	2.95

Table (3): Effect of *Prosopis farcta* fruit extract on urinary N-acetyl-β-D-glycosaminidase (NAG) activity in methotrexate-treated rats (mean ± SD).

GROUPS	NEGATIVE CONTROL	(20 MG OF MTX)	PFE (300MG/KG)	PFE (400MG/KG)	PFE (500MG/KG)
NAG (U/G CREATININ E)	1.27 ± 0.23 a	8.64 ± 1.21 c	6.13 ± 0.78 b	4.92 ± 0.56 b	2.43± 0.74 a
LSD	2.23				

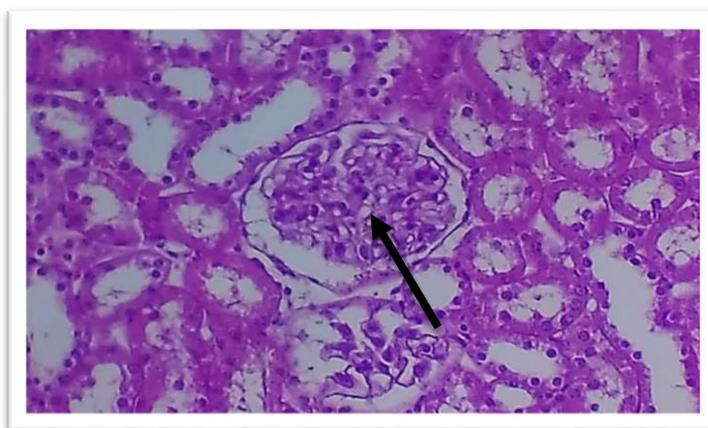


Figure 1. Kidney section from the control group showing normal renal histology, with intact glomeruli, well-organized renal tubules, and no evidence of necrosis, degeneration, or interstitial inflammation. (H&E staining, ×400)

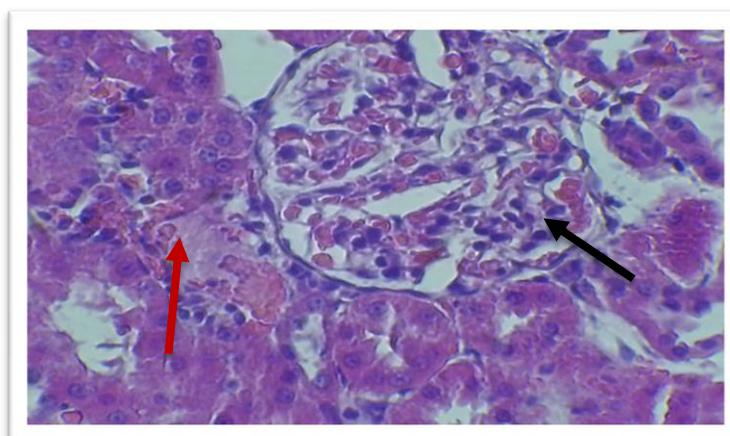


Figure 2. Kidney section from the MTX-treated group demonstrating severe pathological alterations, including glomerular congestion with red blood cell accumulation (black arrow), tubular epithelial degeneration (red arrow), and interstitial changes (H&E stain, 400×).

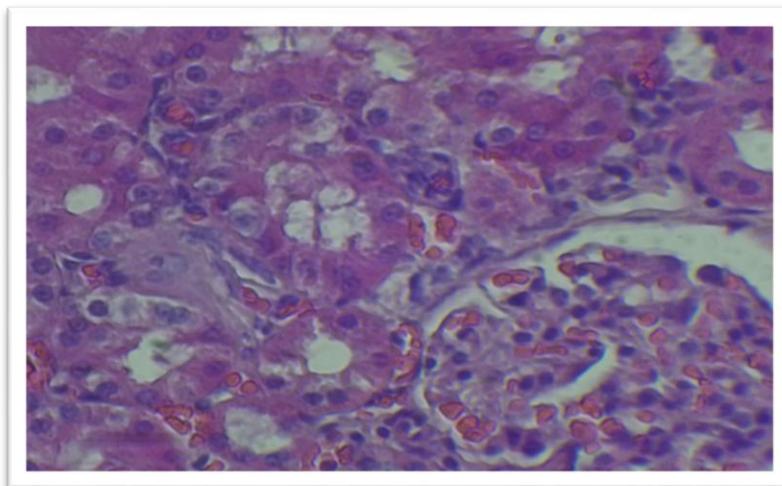


Figure 3. Kidney section from the MTX + *Prosopis farcta* (300 mg/kg) group showing partial improvement in tubular architecture, with reduced epithelial degeneration and fewer necrotic cells compared to the MTX group. Some proteinaceous casts and mild interstitial infiltration are still visible, indicating moderate protection against MTX-induced nephrotoxicity. (H&E stain, 400×).

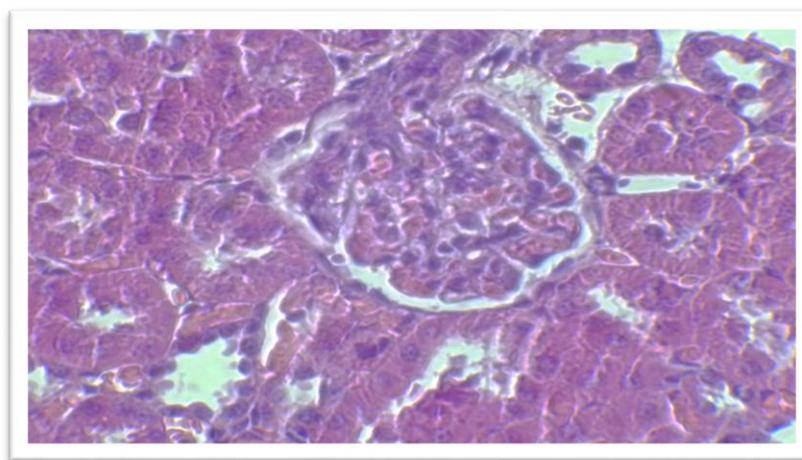


Figure 4. Kidney section from the MTX + *Prosopis farcta* (400 mg/kg) group showing marked tubular epithelial degeneration and necrosis, pyknotic nuclei, proteinaceous casts within the tubular lumen, and interstitial inflammatory infiltration, consistent with acute tubular injury induced by MTX (H&E stain, 400×).

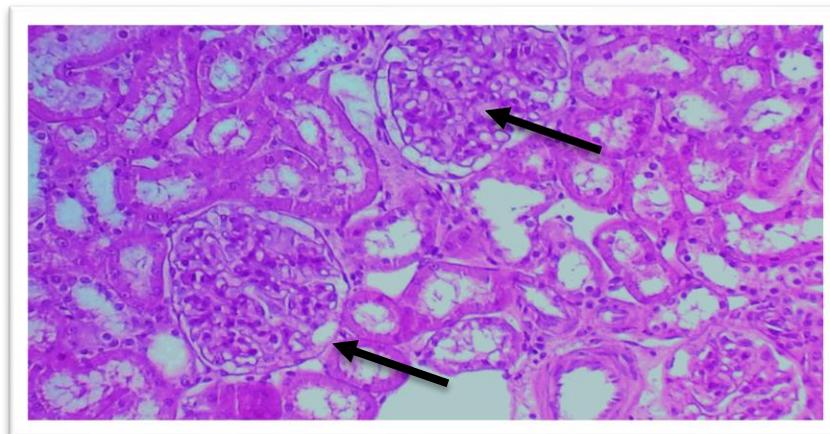


Figure 5. Kidney section from the PFE (500 mg/kg) + MTX group demonstrating near-normal renal architecture, with well-preserved glomeruli and tubular epithelial cells, minimal vacuolar degeneration, and absence of significant proteinaceous casts or inflammatory infiltration. These findings indicate substantial protection against MTX-induced nephrotoxicity at the higher PFE dose. (H&E staining, $\times 400$).

Discussion

The present study revealed that rats receiving MTX developed severe nephrotoxicity; as reflected by a significant increase in serum BUN and creatinine, cystatin C and a decrease in albumin levels to the extent comparable to those who receive only CYC. Concomitantly with these biochemical alterations, a significant increase in malondialdehyde (MDA), a decrease in superoxide dismutase (SOD) activity, and a significant increase of urinary N-acetyl- β -D-glycosaminidase (NAG) activity were observed. All those alterations indicate oxidative stress on the tubules. Histopathologically, glomerular congestion, tubular degeneration and interstitial injury were confirmed. These results are consistent with the prior literatures, which reported that MTX can influence renal function by stimulating oxidative stress, inflammation and cellular apoptosis in renal tissues (Sharifi-Rad *et al.*, 2019 and Aladaileh *et al.*, 2019). Oral administration of *Prosopis farcta* fruit extract notably mitigated MTX induced renal injury in dose-dependent manner. The administration of the extract significantly improved the rats' renal function markers, decreased MDA levels and increased SOD activity while decreasing urine NAG with highest effect being recorded at the highest dose used (500 mg/kg). Histological studies supported the biochemical findings, revealing partial to near complete restoration of the renal architecture at the escalating doses of the extract. The nephroprotective effect of *Prosopis farcta* is attributed to the high content of phytochemicals. In previous studies on phytochemicals of the fruits, phenolic acids, flavonoids including quercetin, luteolin and vitexin, and alkaloids were reported. Each of these is appreciated for its medicinal properties, as they counteract inflammation and free radicals (Tapas *et al.*, 2008 and Khatibi *et al.*, 2020). Flavonoids are important for the protection of renal tissue from oxidative stress. They work by scavenging ROS, up-regulating the activity of endogenous antioxidant defense systems e.g. superoxide dismutase (SOD) and suppressing lipid peroxidation. All these activities act together to minimize oxidative stress and

preserve the kidneys' original morphology (Sharifi-Rad *et al.*, 2019 and Vardi *et al.*,2010). The decrease in N-acetyl- β -D-glycosaminidase (NAG) activity of the extract-treated groups also indicates that *Prosopis farcta* has a nephroprotective effect on renal tubules, maintaining normal cellular structure and decreasing lysosomal enzyme release. These results are in accordance with earlier reports showing that *posopis* species have antioxidant and organ protectant traits. Fruits of *P. farcta* have been reported to possess significant radical scavenging activity in vitro using ethanolic extracts, and in vivo studies have confirmed their antioxidant effects against oxidative stress biomarkers in STZ-induced diabetic rats (Azizniz *et al.*, 2019 and Vardi *et al.*, 2010). Current results augment our understanding by showing the orientation of a *P. farcta* fruit extract protection for the first time against MTX nephrotoxicity. From a mechanistic perspective the observed benefits could reflect several interacting actions: (Ahmed *et al.*, 2020) possible direct anti-oxidant effect in removing ROS, (Agirman *et al.*, 2022) augmenting the endogenous antioxidant defenses—such as SOD, (Ahmed *et al.*, 2020) inhibition of lipid oxidative stress and (Aladaileh *et al.*, 2019) reduction in tubular injury as evidenced by reduced NAG levels. As demonstrated histologically, these effects resulted in the conservation of the configuration of the renal tissue.

Conclusions:

In conclusion, PF fruit extract significantly ameliorated MTX-induced renal damage in rats via its antioxidant potential, decreasing tubular damage and preserving renal histoarchitecture. These findings indicate that *P. farcta* is a potential natural source for production of an inexpensive harmless method for kidney preservation.

Acknowledgment:

The technical staff of the lab is acknowledged for their assistance during experiments and histopathological analyses.

Conflict of Interest:

There is no conflict of interest

Funding Sources:

The authors received no specific funding for this work.

Authors Contributions:

Each authors contributed equally to conception, data collection, analysis, design, and writing of the manuscript.

References:

- Abiola, T. (2018). Assessment of the Antidiabetic Potential of the Ethanolic Extract of Date Palm (Phoenix Dactylifera) Seed in Alloxan-Induced Diabetic Rats. <https://doi.org/10.4172/2155-6156.1000784>
- Agirman, E., Celik, I., & Dogan, A. (2022). Consumption of the Syrian mesquite plant (Prosopis farcta) fruit and seed lyophilized extracts may have both protective and toxic effects in STZ-induced diabetic rats. *Archives of Physiology and Biochemistry*, 128(4), 887-896. <https://doi.org/10.1080/13813455.2020.1734844>.
- Ahmed, H. M., Taha, S. H., & Al-Mayah, A. A. (2020). Phytochemical constituents and biological activities of Prosopis farcta: A review. *Journal of Medicinal Plants Research*, 14(4), 200–210.
- Aladaileh, S. H., Al-Swailmi, F. K., Abukhalil, M. H., & Al-Sweedan, S. A. (2019). *Protective role of natural compounds against methotrexate-induced toxicity: Biochemical and histological evidence*. *Biomedicine & Pharmacotherapy*, 111, 599–610.
- Aziznia, H., Keramat, J., & Soleimanian-Zad, S. (2019). Antioxidant properties and antimicrobial activity of *Prosopis farcta* root extract on foodborne bacteria. *Food Hygiene*, 9(2 [34]), 47–59. Islamic Azad University, Tabriz Branch.
- Jahromi, Mohammad & Etemadfar, Hamed & Zebarjad, Zahra. (2018). Antimicrobial and Antioxidant Characteristics of Volatile Components and Ethanolic Fruit Extract of Prosopis farcta (Bank & Soland.). *Trends in Pharmaceutical Sciences*.
- Khatibi, S. R., Heidari, R., & Samzadeh-Kermani, A. (2020). Protective effect of Prosopis farcta extract against oxidative stress in streptozotocin-induced diabetic rats. *Iranian Journal of Basic Medical Sciences*, 23(4), 531–538.
- Luna, L.G. (1968) *Manual of histologic staining methods of the Armed Forces Institute of Pathology*. 3rd Edition, McGraw-Hill, New York.
- Middleton, E., Jr, Kandaswami, C., & Theoharides, T. C. (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacological reviews*, 52(4), 673–751.
- Rafique, Z., Aabid, M., Nadeem, H., Rehman, A., Khan, J. Z., Waqas, M., & Irshad, N. (2024). Nephroprotective Potential of 1,3,4-Oxadiazole Derivative Against Methotrexate-Induced Nephrotoxicity in Rats by Upregulating Nrf2 and Downregulating NF-κB and TNF-α Signaling Pathways. *Journal of biochemical and molecular toxicology*, 38(12), e70084. <https://doi.org/10.1002/jbt.70084>
- Sharifi-Rad, J., Kobarfard, F., Ata, A., Ayatollahi, S. A., Khosravi-Dehaghi, N., Jugran, A. K., ... & Martins, N. (2019). Prosopis plant chemical composition and pharmacological attributes: Targeting clinical studies from preclinical evidence. *Biomolecules*, 9(12), 777. <https://doi.org/10.3390/biom9120777>
- Tapas, A., Sakarkar, D., & Kakde, R. (2008). Flavonoids as Nutraceuticals: A Review. *Tropical Journal of Pharmaceutical Research*, 7(3), 1089–1099. <https://doi.org/10.4314/tjpr.v7i3.14693>

Vardi, N., Parlakpinar, H., Cetin, A., Erdogan, A., & Cetin Ozturk, I. (2010). Protective effect of β -carotene on methotrexate-induced oxidative liver damage. Toxicologic pathology, 38(4), 592-597. <https://doi.org/10.1177/0192623310367806>