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# Current Insights into the biochemical and antimicrobial properties of *Prosopis farcta* plant extract in rats with induced diarrhea

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**Abstract**

The research concentrated on developing a water-based extract from Prosopis farcta fruits and analyzing its constituents through Gas Chromatography Mass Spectrometry (GC MS). The study revealed that the plant contains 25 compounds with characteristics based on their molecular structure. The most abundant compound was n Hexadecanoic acid, making up 15,48%, followed by cis 13 Octadecenoic acid at 10,94% and Hexadecanoic acid methyl ester at 10,55%. The impact of the extract properties was measured at a dosage of 200 mg/kg, on animals experiencing diarrhea induced by castor oil for a span of 10 and 20 days of treatment sessions. The results showed that the therapy led to levels of vitamin C and increased activity in the enzyme glutathione peroxidase (GPX) well as enhancements, in insulin and manganese levels. Furthermored, reductions in glucose levels and insulin resistance (HOMA-IR) were observed, alongside a significant decrease in the frequency of daily diarrhea episodes. Additionally, the actived compounds in the extract contributed to repairing liver tissue, by treating necrotic degeneration (cellular swelling) in hepatocytes, reduction in sinusoidal and portal vein congestion, and restoration of normal hepatocyte functions. The extract also reduced inflammatory cell infiltration and tissue congestion in affected areas.

**Keywords:** *Prosopis farcta* plant extract, diarrhea, parameters in blood, tissues liver, GC-MASS.

# رؤى حالية حول الخواص الكيميائية الحيوية والمضادة للميكروبات لمستخلص نبات ( *Prosopis Farcta*) في الجرذان المصابة بالاسهال المستحدث

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**الخلاصة**

ركزت الدراسة إلى تحضير مستخلص مائي من ثمار نبات *Prosopis farcta* وتحليل مكوناته الكيميائية الفعالة باستخدام تقنية كروماتوكراف الغاز–مطياف الكتلة .(GC–MS) أشارت النتائج أن النبات يحتوي على 25 مركباً فعالاً .

وُجد أن المركب الأكثر وفرة هو حامض n–Hexadecanoic بنسبة 15.48%، يليه حمض–13–cis Octadecenoicبنسبة 10.94%، بالإضافة إلى مركب حمض Hexadecanoic methyl ester بنسبة 10.55.% تم تقييم التأثير العلاجي للمستخلص بتركيز 200 ملغم/كغم على حيوانات مصابة بالإسهال المستحث بزيت الخروع، وذلك بعد فترة علاج استمرت 10 و20 يوماً. أشارت النتائج إلى أن العلاج أدى إلى زيادة تركيز فيتامينC ، وارتفاع نشاط إنزيم الجلوتاثيون بيروكسيداز (GPX) ، بالإضافة إلى تحسين مستويات الأنسولين والمنغنيز. كما لوحظ انخفاض في مستويات الجلوكوز ومؤشر مقاومة الأنسولين(HOMA–IR) ، مع تقليل تكرار نوبات الإسهال اليومية بشكل ملحوظ. علاوة على ذلك، بيّنت الدراسة أن المركبات النشطة في المستخلص ساهمت في تحسين أنسجة الكبد، حيث ساعدت على علاج التنكس النخري (التورم الخلوي) في الخلايا الكبدية، وتقليل احتقان الجيوب الكبدية والوريد البابي. كما أسهمت في استعادة الوظائف الطبيعية للخلايا الكبدية، مع تقليل التسلل الالتهابي والاحتقان في الأنسجة المتضررة.

## 1. Introduction

Diarrhea is one of the leading causes of mortality worldwide, particularly among children It is characterized by pathological symptoms such as intestinal disturbances and changes in stool volume. The primary causes of diarrhea are categorized into two main types: the first involves an imbalance in the intestinal microbiota, leading to gastrointestinal damage, especially in the intestines, accompanied by stomach muscle spasms, dehydration, poor nutrient absorption, and disruptions in water and nutrient balance within the body. The second type is caused by gastrointestinal infections due to bacteria or parasites, which are often accompanied by increases in certain biochemical markers, exacerbating diarrhea and causing significant fluid loss [1-4].

The study and understanding of plants are crucial because humans historically depended on them for both sustenance and medicinal purposes. Traditionally, various parts of plants have been used as remedies, with their applications evolving over time. In some cultures, toxic plants were used in wars or for hunting prey, which helped humans discover the relationship between wild plants and the diseases they can address. The use of medicinal plants has become one of the most common methods for treating diarrhea due to their positive therapeutic effects and lack of side effects.

Plants, in this situation, are valuable for their properties. Are commonly utilized in areas like the Middle East. Notably, in Iraq, Morocco, and Iran.

Prosopis farcta is highly regarded as a commonly utilized plant, for managing diarrhea issues in herbal remedies and traditional medicine practices. They also include components such, as tannins and gallic acid along with antioxidants and vitamins, like Vitamin C and Vitamin E [5,-7].

The goal of this research is to assess the effects of the extract from the fruits of *Prosopis farcta*, on specific biochemical factors across different treatment durations and its influence on liver tissues, in animals afflicted with watery diarrhea.

2. **Experimental Design**

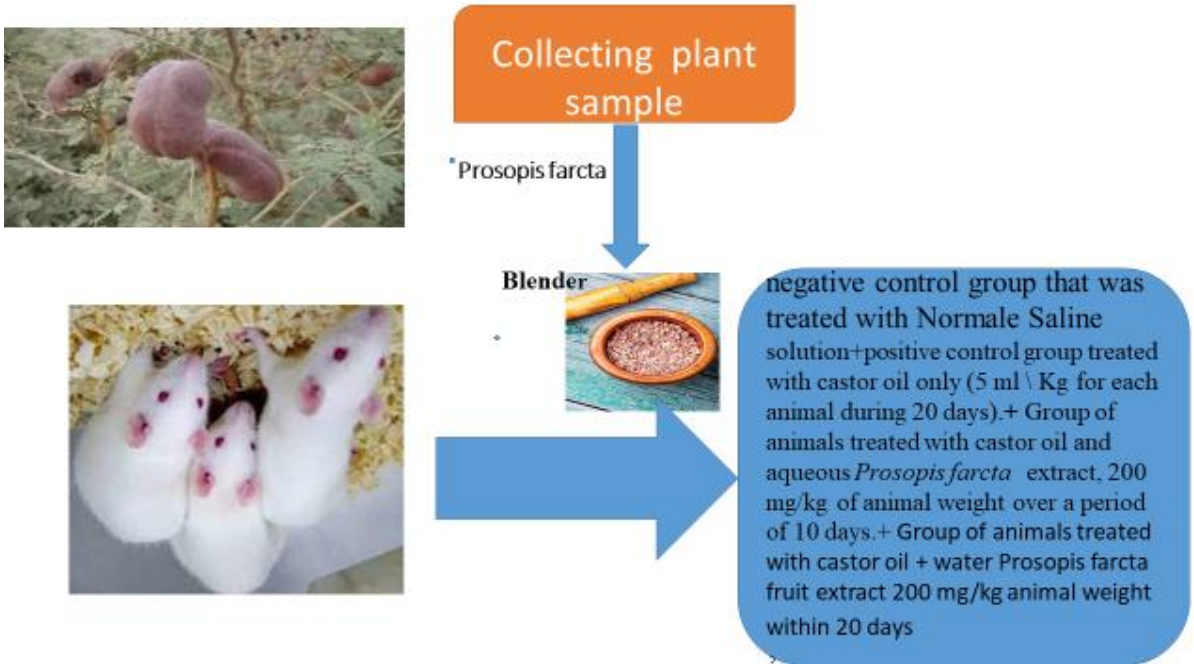


**Figure 1:** Illustration of the practical part

**1. Ethical Approval**

Ethical clearance was granted by the Animal Care Committee, at the College of Veterinary Medicine in Mosul University under reference number UM.VET.2023.041. The committee internationally acknowledged its support for the research and ethical use of laboratory animals.

**2.2 Preparation of the Aqueous Extract *of Prosopis farcta* Fruits**

Fruits of the *Prosopis farcta* plant were collected from markets in Mosul city, Iraq. A 1000-gram sample of the fruits were was mechanically pulverized for 15 minutes using a blender. The crushed material was mixed with ionized water in a 1 to 5 ratio (volume to volume) then frozen and left to thaw at room temperature repeating this procedure several times for a thorough blend. The mixture underwent blending for two hours with the help of a blender and a cooling system utilizing a water bath when needed. After the blend was filtered through layers of gauze and the liquid was. Freeze dried to produce the water based extract [8].

**2.3 Experimental Design**

The study involved 18 rats aged between 12 and 18 weeks, with an average weight of 200 to 250 grams each. These rats were sourced from the Animal House at the College of Veterinary Medicine in Mosul University. Were maintained in lab settings with a temperature, between 21°C to 30°C and a consistent light/dark cycle of 13 hours of light followed by 11 hours of darkness. When trying to find the median lethal dose (LD50), the different high doses did not cause any toxic or lethal effect on the animals, in agreement with another studies that proved this [9,10].

**The animals were split into four groups, in the manner:**

- Negative control group received treatment, with a saline solution.
- Positive control group received castor oil treatment alone at a dosage of 5 mL/kg, per animal, for a period of 20 days.
- The initial test group received a combination of castor oil and an aqueous extract, from Prosopis farcta, administered at a dosage of 200 mg per kilogram of body weight, over a span of 10 days.
- The second experimental group received a combination of castor oil and an aqueous extract of Prosopis farcta at a dosage of 200 mg, per kilogram of body weight, for a duration of 20 days.

### 2.4 Identification of Active Compounds in *Prosopis farcta*

Active compounds, in Prosopis farcta were identified through GC-MS analysis conducted at the Scientific Center for Chemical Analysis Laboratories in Baghdad using an Agilent A7820 GC-MS system. A 1 µL extract sample was injected at 11.933 psi pressure, with temperature control, the injection port maintained at 250°C while helium gas acted as the carrier.

### 2.5 Estimation of Biochemical Parameters in Animal Serum

Serum biochemical parameters were assessed throughout the study period for all four research groups using the methods outlined below:

- **Determination of Vitamin C in Serum**

Serum vitamin C levels were determined through a process that involved oxidizing ascorbic acid with copper to create dehydroascorbic acid and then reacting it with 2，5-Dinitrophenylhydrazone in the presence of thiourea to produce 2，5-Dinitrophenylhydrazone [11].

- **Measurement of Glutathione Peroxidase Enzyme Activity:**

The level of glutathione peroxidase (GPx) an enzyme involved in antioxidant activity was determined using a made assay kit, from BioMerieux, in France that employs an enzymatic approach.

- **Determination of Serum Glucose Levels:**

Serum glucose levels were measured by analyzing the oxidation of glucose molecules aldehyde groups, with the help of glucose oxidase in a kit provided by Biolabo, in France [12].

- **Assessment of Insulin Resistance (HOMA-IR):**

Insulin resistance was calculated using the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR). This was determined using fasting plasma glucose and insulin concentrations, following the formula [13]:

**HOMA-IR=**Fasting Glucose (mg/dL)×Fasting Insulin (µU/mL)\405

**Determination of Manganese in Blood**

Manganese levels in blood were determined using flame-free atomic absorption spectroscopy with Zeeman effect correction. The analysis was conducted after diluting the sample according to the method described in reference [14].

### 2.6 Tissue Sampling

Liver tissue samples were collected following animal euthanasia via decapitation. The samples were preserved in a neutral saline solution (0.9% NaCl). Tissue fixation was performed using 10% neutral buffered formalin following the Luna (1968) protocol [15].

### 2.7 Statistical Analysis

The laboratory test results were analyzed using the SPSS version 25 statistical software. The mean and standard deviation were calculated, and Duncan's multiple range test was employed to compare more than two groups. Results were considered statistically significant at a probability level of $p \leq 0.05$ [16].

## 3. Results and discussion

The GC-MS analysis revealed that the aqueous extract of Prosopis farcta contains 25 bioactive compounds, as detailed in Table 1 and Figure 2.

The major compounds in the extract were distributed as follows: *n*-Hexadecanoic acid: 15.48%, *cis*-13-Octadecenoic acid: 10.94%. and Hexadecanoic acid, methyl ester: 10.55%. These compounds account for more than a quarter of the total plant compounds identified in the extract. They exhibit significant biological properties, including antioxidant activity, free radical scavenging, and anti-inflammatory effects by reducing pro-inflammatory cytokines associated with diarrhea. Additionally, these compounds possess antibacterial properties [17-19].

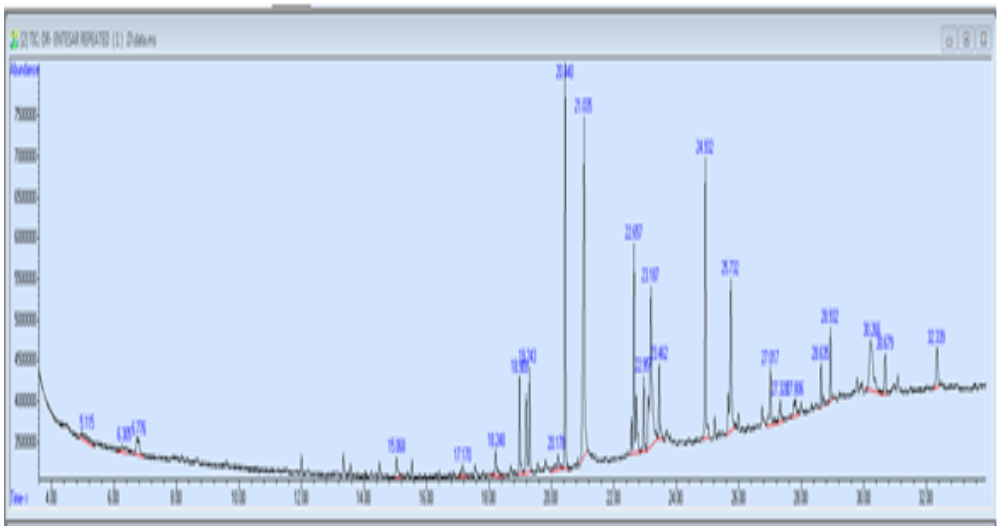

**Figure 2 :** Chromatogram GC-MS of compounds isolated from Prosopis farcta fruit.

**Table 1:** Most important compounds and peaks identified in aqueous extract of Prosopis farcta fruit using GC-mass device.

| Chemical compounds | %Percentage | (Rf) Detention time | Peaks |
|---|---|---|---|
| 1,2-Hydrazinedicarboxylic acid, di ethyl ester | 1.38 | 5.11 | 1 |
| Cathinone | 1.1 | 6.30 | 2 |
| dl-Homoserine | 1.53 | 6.77 | 3 |
| Propanamide | 1.18 | 15.07 | 4 |
| 1,3-Propanediamine, N-methyl | 0.94 | 17.16 | 5 |
| Tetraacetyl-d-xylonic nitrile | 1.45 | 18.24 | 6 |
| Benzene, (1-methylenepropyl)- | 2.94 | 18.98 | 7 |
| o-Veratramide | 5.64 | 19.244 | 8 |
| 1-Octadecanamine, N-methyl- | 1.03 | 20.17 | 9 |
| Hexadecanoic acid, methyl ester | 10.55 | 20.03 | 10 |
| n-Hexadecanoic acid | 15.48 | 20.03 | 11 |
| 9-Octadecenoic acid (Z)-, methyl e ster | 8.88 | 22.65 | 12 |
| Methyl stearate | 2.09 | 22.95 | 13 |
| cis-13-Octadecenoic acid | 10.94 | 23.19 | 14 |
| Octadecanoic acid | 2.61 | 23.45 | 15 |
| Heneicosane<br>Heptadecane | 7.77 | 24.93 | 16 |
| 1-Piperidinamine | 7.28 | 25.73 | 17 |
| Hexacosane | 2.37 | 27.32 | 18 |
| Diisooctyl phthalate<br>Dicyclohexyl phthalate<br>Phthalic acid, undec-2-en-1-yl und ecyl ester | 1.17 | 27.32 | 19 |
| Adipamide | 1.44 | 27.8 | 20 |
| Octacosanol | 1.1 | 28.63 | 21 |
| Hexacosane | 2.17 | 28.93 | 22 |
| Stigmasterol | 5.26 | 30.26 | 23 |
| Octacosyl acetate<br>1-Heptacosanol | 1.64 | 30.67 | 24 |
| 1-Piperidinamine | 2.05 | 32.34 | 25 |

### 3.1 The Effect of *Prosopis farcta* Fruit Aqueous Extract on Biochemical Parameters in Rats with Diarrhea

Treatment of rats with an aqueous extract of *Prosopis farcta* fruit resulted in a significant increase in vitamin C concentration from 24.2 ± 1.63 µmol/L to 26.41 ± 4.5 µmol/L and 30.35 ± 3.3 µmol/L over the experimental periods of 10 and 20 days, respectively, at a concentration of 200 mg/kg of body weight. Diarrhea is directly linked to oxidative stress, and *Prosopis farcta* exhibits protective effects against toxicity caused by castor oil-induced diarrhea. This protection is attributed to the antioxidant properties of *Prosopis farcta*, which mitigates oxidative stress on cells, including nerve-like cells. The plant serves as a nutritional supplement that helps mitigate oxidative damage, partly due to its vitamin C content, which is known for its anti-diarrheal properties. Diarrhea occurs through increased fluid secretion and an imbalance in intestinal physiological functions and is a type of acute gastroenteritis [20,21].

Additionally, animals treated with castor oil alone, suffering from diarrhea, exhibited a decline in insulin levels to 6.46 ± 0.33 ng/dL and an increase in both insulin resistance and blood glucose levels, reaching 110.01 ± 3.9 mg/dL over 20 days of diarrhea. The use of *Prosopis farcta* fruit extract resulted in a decrease, in insulin resistance and blood sugar levels while also boosting insulin levels. After 10 and 20 days of treatment with 200 mg/kg

of the extract, glucose levels decreased to 94.3 ± 7.7 mg/dL and 91 ± 4.76 mg/dL, respectively, while insulin levels increased to 7.27 ± 0.6 ng/dL and 8.17 ± 0.62 ng/dL, with insulin resistance values of 1.6 ± 0.4 and 1.46 ± 0.2, respectively. Fluid loss caused by diarrhea leads to a rise, in substances in the bloodstream; however, *Prosopis farcta* combats insulin resistance by controlling insulin function and decreasing blood sugar levels. This impact is credited to the plants fibers that limit the absorption of carbohydrates unlike fibers that promote sugar absorption. Moreover, the existence of antioxidants, like flavones and chlorogenic acid enhances glucose regulation. These components also heighten gut thickness, polyphenols. *Prosopis farcta* contains essential amino acids, including lysine and proline, which aid in water retention [22-24].

Treatment with *Prosopis farcta* fruit extract also significantly increased the activity level of glutathione peroxidase (GPX) enzyme in treated animals. Following 10 and 20 days of treatment with 200 mg/kg of the extract, GPX activity increased to 2.8 ± 1.1 U/L and 2.3 ± 0.1 U/L, respectively This was compared to untreated animals with diarrhea, where GPX activity was 1.9 ± 0.2 U/L.

Diarrhea induced oxidative stress depletes the GPx enzyme responsible, for safeguarding cell membranes against harm by converting oxygen species like $RO^{\cdot}$, $OH^{\cdot}$, $ROO^{\cdot}$.and $H_2O_2$, into water and alcohol to mitigate damage [25, 26].

In addition, to that change, a decrease in blood levels was observed in animals given castor oil and experiencing diarrhea, where the levels dropped to 5.85 ± 0. 7 ug/L.

In contrast, animals treated with *Prosopis farcta* fruit extract exhibited increased manganese concentrations of 7.31 ± 0.7 µg/L and 8.6 ± 0.62 µg/L after 10 and 20 days of treatment, respectively, at a concentration of 200 mg/kg. Manganese plays a role in mitigating diarrhea by reducing intestinal dysfunction and scavenging reactive oxygen species (ROS) generated during diarrhea. Additionally, manganese contributes to blood glucose regulation by serving as a cofactor for several enzymes, including glycolytic cycle enzymes and antioxidant enzymes such as manganese superoxide dismutase (SOD-Mn). These enzymes are inversely correlated with diabetes and oxidative stress [27-29]. Treatment with *Prosopis farcta* extract also effectively reduced the frequency of diarrhea episodes per day.

**Table 2 :**Biochemical variables in the control group, the group of animals induced with diarrhea, and the group of animals treated with *Prosopis farcta* fruit extract.

| Paramete | negative control | Animals treated with castor oil | Animals treated with castor oil + Prosopis farcta extract 200 mg/kg for 10 days | Animals treated with castor oil + Prosopis farcta extract 200 mg/kg for 20 days |
|---|---|---|---|---|
| Vit C(umol\L) | 29±1.55<br>A | 24.2±1.63<br>B | 26.41±4.05<br>B | 30.33±2.87<br>A |
| Insulin (ng\dl) | 7.23±0.32<br>A | 6.46±0.33<br>B | 7.27±0.6<br>A | 8.17±0.65<br>C |
| Glucose (mg\dl) | 88.13±5.7<br>A | 110.01±3.9<br>B | 94.3±7.7<br>A | 91±4.74<br>C |
| HOMA-IR | 1.56±0.3<br>A | 1.7±1.0<br>B | 1.6±0.4<br>BC | 1.46±0.2<br>A |
| GPX(U\L) | 2.28±0.2<br>A | 1.9±0.2<br>B | 2.3±0.1<br>A | 2.8±1.1<br>C |
| Mn(ug\L) | 7.74±0.55<br>A | 5.85±0.7<br>B | 7.31±0.7<br>A | 8.6±0.65<br>C |

*Different letters( A.B.C.D) (Horizontally) indicate a significant difference at the probability level $p \leq 0.05$

The histological examination of liver tissues from animals suffering from diarrhea revealed the beneficial effects of *Prosopis farcta* fruit extract. Treatment with the extract led to the restoration of normal hepatic architecture by addressing several pathological changes associated with diarrhea. Specifically, the extract ameliorated necrotic degeneration, characterized by cellular swelling of hepatic cells, and reduced the narrowing and congestion of sinusoids. Furthermore, the congestion of the portal vein was alleviated, and the normal shape of the hepatic cells was restored. The extract also contributed to a reduction in congestion and infiltration of inflammatory cells, as illustrated in Figures 3-5. These findings underscore the therapeutic potential of *Prosopis farcta* in protecting liver tissue from damage induced by diarrhea [30-33].

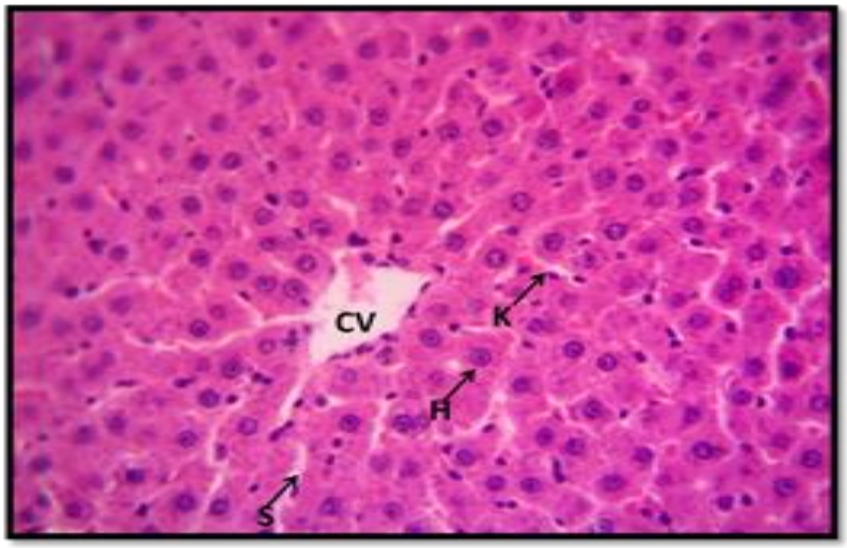


**Figure 3:** histological section of the liver of a rat from the control group showing the normal histological features represented by hepatocytes (H), sinusoids (S), Cover cells (K), and central vein (CV). Hematoxylin and eosin stain, 400X

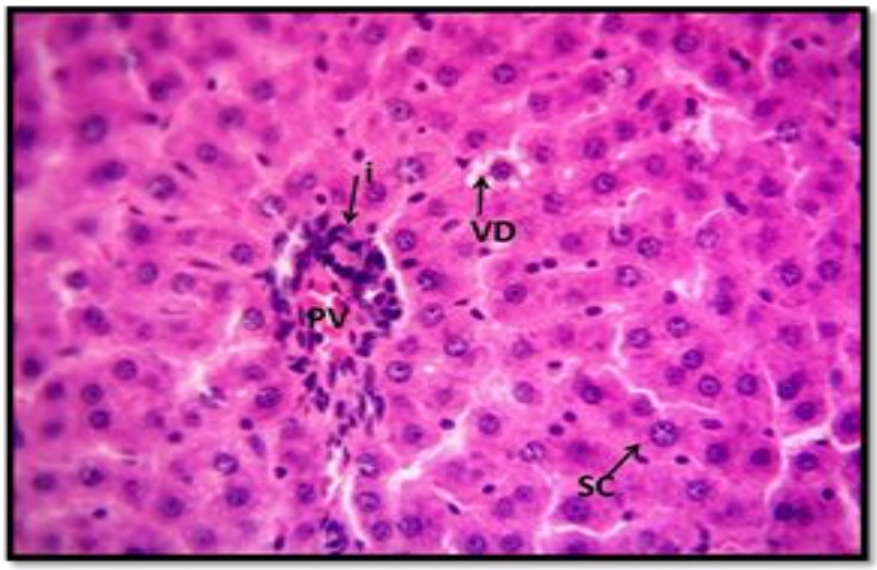


**Figure 4:** Histological section of the liver of a rat from the group treated with castor oil and has diarrhea showing channel degeneration (cellular swelling), vacuolar degeneration (SC) of hepatocytes (VD), infiltration of inflammatory cells (i), and congestion of the portal vein (PV). Hematoxylin and eosin stain, 400X.

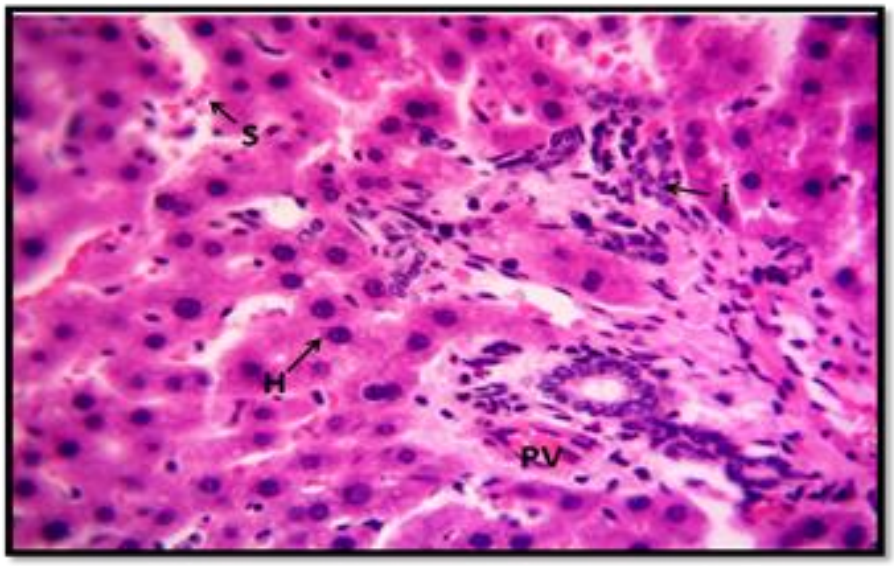


**Figure 5:** histological section of the liver of a rat from the group treated with aqueous extract of Prosopis farcta fruit for 10 days showing the normal appearance of hepatocytes (H), expansion and congestion of the sinusoids (S), the central vein (CV), and the portal vein (PV), and infiltration of inflammatory cells (I). Hematoxylin and eosin stain, 400X.

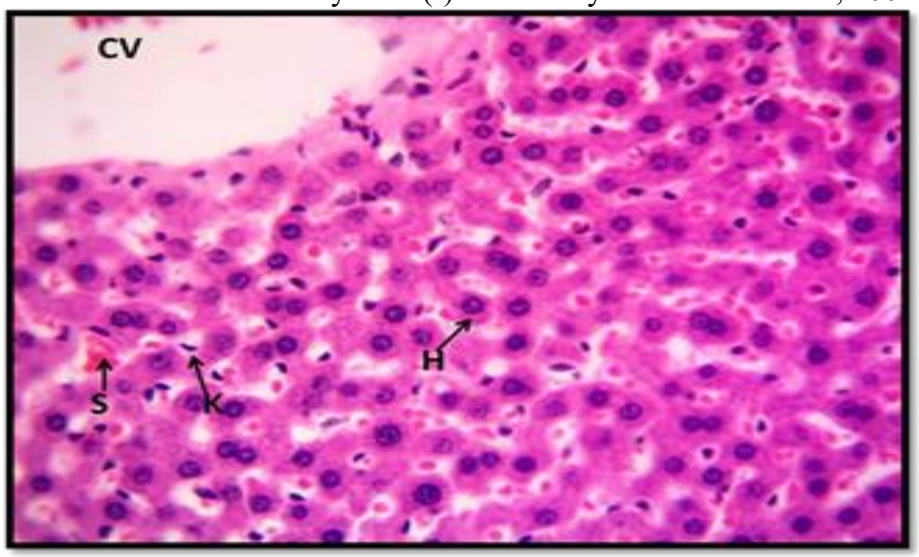


**Figure 6:** A histological section of the liver of a rat from the group treated with aqueous extract of *Prosopis farcta* fruit for 20 days showing the normal appearance of hepatic cells (H), slight congestion of the sinusoids (S) and central vein (CV), and an increase in Cover cells (K). Hematoxylin and eosin stain, 400X.

## Conclusion

The study demonstrated that the aqueous extract of *Prosopis farcta* significantly reduced the frequency of diarrhea episodes and alleviated symptoms. Enriched with anti-inflammatory and antibacterial compounds, the extract lowered blood glucose levels and insulin resistance, while also increasing insulin, manganese, and GPX levels in the blood of treated animals. These findings suggest that *Prosopis farcta* extract possesses therapeutic properties that may aid in managing diarrhea, improving metabolic parameters, and enhancing overall health.

**5. Acknowledgements**

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**6. Conflict of interest**: None

**7. Article highlights:**

The effect of treatment with aqueous extract *Prosopis farcta* on biochemical variables and liver tissue in rats treated with castor oil and Diarrhea sufferer.

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