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Effect of Methanolic and Alkaloid Extracts of *Peganum harmala* on Immune System-Related Organs in Female Mice

Rima Allouni[1*], Hassina Guergour[2], Sabrina Allouni[3], Rahma Allouni[4], Abdelouhab Bouzidi[5]

Department of Basic Studies, Faculty of Natural and Life Sciences, University Ferhat Abbas, Setif 19000, Algeria[1]
Department of Biology, Faculty of Natural and Life Sciences, University Elbachir El Ibrahimi, Bordj Bou Arriridj 34000, Algeria[2]
Public Institution of Proximity Health, Setif 19000, Algeria[3]
Saadna Abdenour University Hospital Center, Setif 19000, Algeria[4]
Department of Biochemistry, Faculty of Natural and Life Sciences, University Ferhat Abbas, Setif 19000, Algeria[5]

Corresponding author email*: ryme87@yahoo.fr/ rima.allouni@univ-setif.dz

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ABSTRACT

Peganum harmala L. (Harmel) is widely employed in traditional Algerian medicine for treating various ailments. This study assessed the immunomodulatory effects of two extracts from *P. harmala* seeds, with a focus on their impact on selected immune system organs in female mice. Female NMRI white mice, *Mus musculus*, were intraperitoneally administered MCE (20.72 mg/kg) and TAE (37.75 mg/kg) for 20 days. Phytochemical analysis using thin-layer chromatography confirmed the presence of diverse alkaloids in the methanolic crude extract. The treatment induced behavioral alterations, changes in body weight, and modifications in relative organ masses. Biochemical assays revealed significant elevations in AST and ALT levels, while hematological analysis showed increased WBC, NEU, and P-LCR levels, accompanied by reductions in MCV, MCH, PLT, and MPV.

Histological examination identified no structural damage to organ architecture; however, an accumulation of immune cells was observed in the thymus and adrenal glands. Despite the known anti-inflammatory properties of *P. harmala*, this study highlights its potential to disrupt immune homeostasis, particularly through excessive neutrophil proliferation.

These findings underscore the need for cautious use of *P. harmala* to mitigate adverse effects, especially in conditions associated with immune dysregulation.

Keywords: *Peganum harmala* L, chromatography, immune system, alkaloids, mice.

1. INTRODUCTION

Today, thousands of plants are used worldwide, with a wide range of actions and varying potency. Most have specific effects on certain parts of the body and are recognized for their ability to treat various conditions [1]. Algeria has a rich and diverse plant fauna. Among the medicinal plants that make up this vegetation, the genus Peganum is widely distributed in arid and semi-arid regions [2], [3]. This is why we are interested in studying *Peganum harmala L.*, which belongs to the Zygophyllaceae family, commonly known as Harmel, and is considered one of the most well-known medicinal plants in traditional medicine [4]. Its therapeutic effects are due to the presence of β-carboline alkaloids, such as harmine, harmaline, harmol, and harmalol, as well as quinazolines, which give it anti-inflammatory, antioxidant, and immunomodulatory properties [5], [6].

A recent epidemiological survey conducted in the eastern region of Algeria has revealed that women represent the predominant demographic utilizing *Peganum harmala*, particularly for the management of rheumatic and arthritic conditions. The data suggest that these women, frequently experiencing chronic nociceptive pain and inflammatory joint disorders, utilize this plant as a therapeutic agent to mitigate symptoms and enhance their overall quality of life. This pattern underscores the ethnopharmacological significance of *Peganum harmala* within the Algerian populace, where traditional botanical knowledge is systematically transmitted across generations.

In the framework of our investigation, we have selected a murine model comprising female mice to assess the immunomodulatory effects of *Peganum harmala*. This choice is supported by the findings of our survey, which identify women as a particularly vulnerable cohort to joint pathologies. By evaluating the impact of this plant on immune system functions, we aim to not only substantiate its ethnopharmacological use but also to establish a robust scientific rationale for its potential integration into evidence-based therapeutic protocols.

2. MATERIALS AND METHODS

2.1. MATERIALS

2.1.1. Plant material

The seeds of *Peganum harmala* were collected at the peak of their maturation phase, which occurs at the end of July, August, and September in the Wilaya of Oued Souf, situated in the northeastern Algerian Sahara. This region is known for its arid and dry climate. After collection, the seeds were thoroughly cleaned to remove impurities and subsequently ground into a fine powder using an electric grinder. The resulting powder was then utilized for the extraction of the plant's active bioactive compounds.

2.1.2. Animal material

Female NMRI strain white mice (*Mus musculus*), weighing between 24 and 30 g, were procured from the Pasteur Institute (Algiers). The animals were housed in standard plastic cages under controlled environmental conditions, with unrestricted access to tap water and commercial rodent chow. They were acclimatized to the animal facility for the duration of the experiment. Bedding material was replaced three times per week to maintain optimal hygiene and ensure the well-being of the animals.

2.1.3. Reference Drug

The reference drug used to evaluate the effects of *Peganum harmala* extracts on the immune system was Prednisolone, Solupred® (20 mg), provided by the "Prodiphal Production" laboratory in Rouiba, Algiers, Algeria. Prednisolone is a synthetic corticosteroid primarily used for its anti-inflammatory properties. At high doses, it suppresses the immune response.

2.2. METHODS

2.2.1. Preparation of Extracts

- **Methanolic Crude Extract**

The methanolic extract was obtained following the extraction protocol described by [7] with some

modifications. A total of 200 g of seed powder was macerated in 500 ml of absolute methanol for 72 hours, filtered, and evaporated at 40 °C to obtain a semi-solid residue.

♦ **Total Alkaloid Extract**

According to [8], the alkaloid extract of *P. harmala* was obtained by liquid-liquid extraction, based on the different solubilities of alkaloids in acidic and basic phases. In brief, the first step involved grinding about 100 g of powder and continuously agitating it in 250 ml of petroleum ether. After filtration, the residue was alkalinized with 40 ml of ammonia solution. Then, 450 ml of chloroform was added, followed by filtration through paper to obtain a liquid. The liquid was washed with 150 ml of sulfuric acid solution, then alkalinized to pH 9 by adding ammonia. The solution was then extracted with 150 ml of ethyl ether, followed by filtration through filter paper containing anhydrous sodium sulfate. Finally, the solution was evaporated to obtain the total alkaloids of *P. harmala*.

2.2.2. Qualitative Analysis

Thin-layer chromatography was used to perform a physicochemical separation, involving a stationary phase using MACHEREY-NAGEL Silica gel 60 F254 plates and a mobile phase consisting of a mixture of "methanol/chloroform/ammonia: 78.5 ml/20 ml/1.5 ml (V/V/V)" for the crude and alkaloid extracts.
A necessary reagent for the appearance of colored spots was Dragendorff reagent.

2.2.3. Evaluation of the Effect on the Immune System

♦ **Treatment**

The animals were divided into four groups, each containing 10 mice. The treatment involved daily intraperitoneal administration of a specific dose volume for 20 days. The four groups were divided as follows:

- Control group: received physiological saline.
- Reference group: treated with 0.4 mg/kg of prednisolone.

• Group A: treated with a dose of 37.78 mg/kg (1/10 LD50) of TAE.

- Group M: treated with a dose of 20.72 mg/kg (1/40 LD50) of MCE.

♦ **Signs and Clinical Symptoms**

The mice were weighed every 5 days and observed daily to record any physiological and behavioral changes.

♦ **Sample Collection**

a) Blood Collection

After anesthesia, blood samples were taken via cardiac puncture. Blood was collected in EDTA tubes for the complete blood count (CBC) and in heparin tubes for biochemical parameters (AST, ALT, and liver function tests) for each animal.

b) Organ Collection

After sacrifice and dissection, the lungs, liver, kidneys, spleen, thymus, heart, and adrenal glands were collected, freed from adipose tissue, rinsed in a 0.9% sodium chloride (NaCl) solution, macroscopically examined, and weighed. The spleen, thymus, and adrenal glands were preserved in 10% formalin for histological sectioning.

2.3. Statistical Analysis

The statistical package is SPSS version 25. All experimental results were processed using ANOVA tests and expressed as mean ± SEM.

3. RESULTS

3.1. Yield

Maceration extraction of the seeds produced a dark brown methanolic crude extract with a yield of 10.1%. In

contrast, the liquid-liquid extraction of total alkaloids from *Peganum harmala* seeds resulted in a brick-red extract with a yield of 0.47%.

3.2. Qualitative Analysis

Thin-layer chromatography analysis of *Peganum harmala* L. extracts enabled chromatographic separation and clear visibility of the spots. After revelation with the Dragendorff reagent, the presence of alkaloids was confirmed. The results are shown in Figure 1.

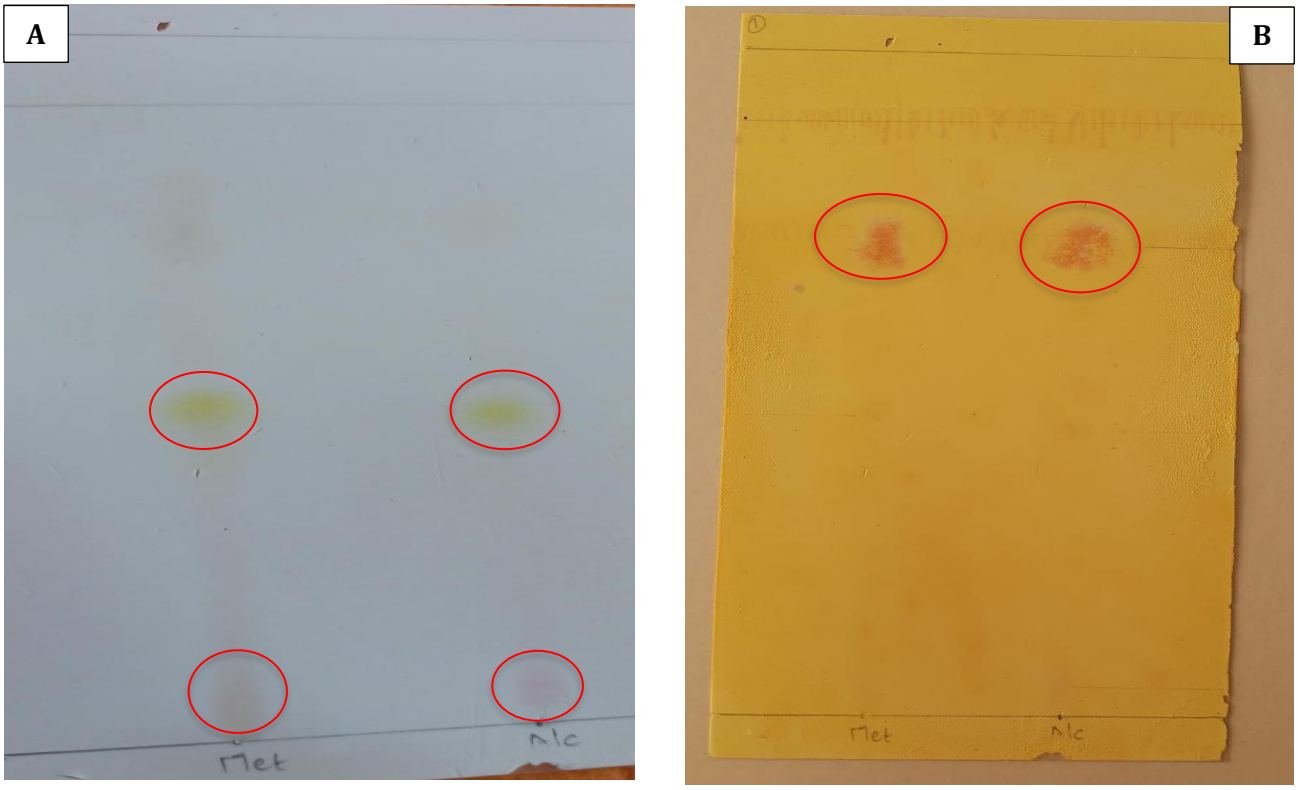

Fig. 1. Chromatogram of *Peganum harmala L.* extracts, showing the methanolic crude extract and the total alkaloid extract before revelation (A) and after revelation (B).

Thin-layer chromatography of the crude methanolic and alkaloid extracts *from Peganum harmala L.* revealed several spots, including yellow and pink ones with Rf values of 0.50 and 0.04. After visualization with the Dragendorff reagent, a brick-red spot appeared, confirming the presence of alkaloids in the crude methanolic extract, with an Rf value of 0.77.

3.3. Effect on the Immune System

3.3.1. Behavioral and Physiological Observations

Observations conducted shortly after the intraperitoneal injection of total alkaloids and crude methanolic extract of *Peganum harmala* seeds in female mice revealed a reduction in locomotor activity, ataxia, and nervous symptoms, including excitability, tremors, rapid respiration, protruding eyes, and erythema of the ears

3.3.2. Body Weight

Clinical observation of body weight in female mice, both control and those treated with MCE and TAE, was conducted following daily intraperitoneal injections for 20 days. A significant reduction in body weight was observed on day 10 in the animals treated with MCE and TAE, with a subsequent recovery by day 15 (Figure 2).

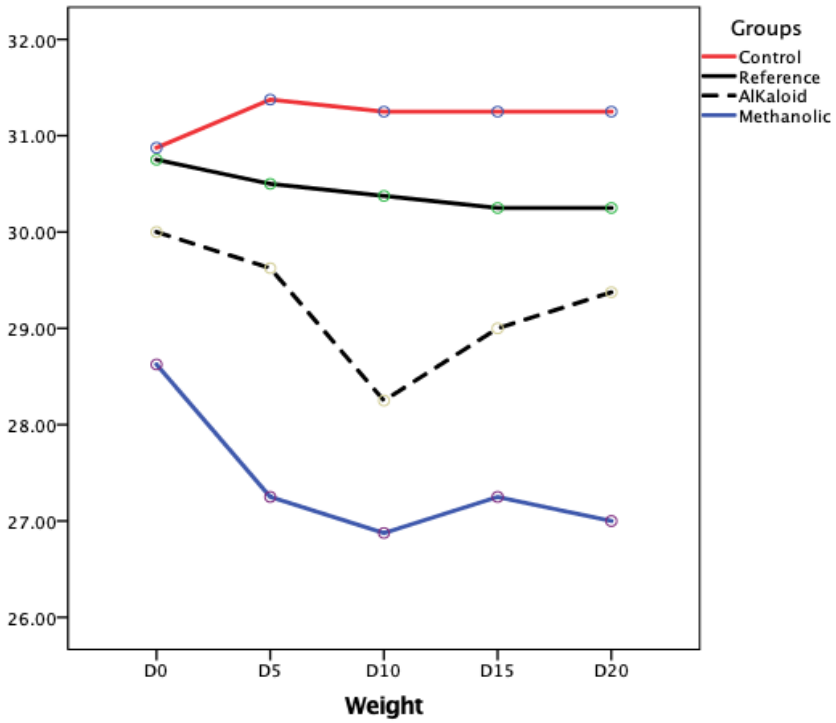

Fig. 2. Progression of body weight in female mice.

3.3.3. Relative Organ Mass

No significant pathological changes were observed in the color, shape, or texture of the organs (liver, kidneys, lungs, heart, thymus, and spleen) of treated female mice. However, statistical analysis presented in Table 1 revealed notable differences: the reference group showed significant increases in the relative mass of the spleen, liver, and thymus (0.92%, 1.80%, and 0.12%, respectively). The alkaloid extract group exhibited a 0.4% decrease in spleen mass, while the methanolic extract group showed a 0.10% increase in thymus mass, alongside reductions in liver and spleen mass by 1.21% and 0.48%, respectively.

Table 1. Relative organ mass in female mice treated with a dose of 37.78 mg/kg (≈ 1/10 DL50) of TAE and a dose of 20.72 mg/kg (≈ 1/40 DL50) of MCE from *Peganum harmala* L

Organs	Control Group	Reference Group	Alkaloid Group	Methanolic Group

Lungs	0.7825± 0.0523	0.6713 ± 0.0512	0.595±0.0725	0.9075± 0.0454
Kidneys	0.9413 ± 0.0477	1.0488± 0.0397	0.9213± 0.0433	1.095± 0.0803
Liver	1.5213± 0.1002	**1.8020± 0.0417***	1.5349± 0.0766	**1.2113± 0.0378***
Spleen	0.6763± 0.054	**0.9263± 0.0209****	**0.4337± 0.0231****	**0.485± 0.0088****
Thymus	0.0619± 0.0064	**0.1279± 0.0133****	0.0389± 0.0063	**0.1079± 0.0125***
Heart	0.45± 0.0500	0.475± 0.0152	0.3925± 0.0178	0.5063±0.0126

The results are expressed as mean ± SEM (* P < 0.05; ** P < 0.01).

3.3.4. Hematological Parameters

The hematological parameters of female mice treated daily via intraperitoneal injection with prednisolone, MCE, and TAE of *Peganum harmala* at doses of 75 mg/kg, 20.75 mg/kg, and 37.75 mg/kg, respectively, are shown in Table 2.

Table 2. Hematological parameters of female mice treated with prednisolone, methanolic, and alkaloid extracts from *Peganum harmala* seeds.

Groups	Control	Reference	Alkaloid	Methanolic
WBC (103 /µL)	14.040 ±0.4523	15.264 ± 0.9149	**17.120 ± 0.5004 ***	**8.280 ± 0.7964 ****
RBC (106/µL)	7.808 ± 0.1658	8.914 ± 0.2683	9.042 ± 0.2477	8.550 ± 0.5557
HGB (g/dL)	12.960 ± 0.2785	13.7 ± 0.5224	14.18 ± 0.2615	14.22 ± 0.1157
HCT (%)	44.42 ± 1.2101	44.98 ± 1.5190	46.66 ± 0.8262	46.92 ± 0.4329
MCV (fL)	54.92 ± 0.6044	52.86 ± 0.6249	54.06 ± 0.6454	**51.94 ± 0.5173***
MCH (pg)	16.62 ± 0.3742	16.08 ± 0.2905	16.42 ± 0.2817	**15.72 ± 0.1067***
MCHC (g/dL)	30.34 ± 0.3075	30.46 ± 0.2657	30.38 ± 0.2010	30.18 ± 0.1624
PLT (103 /µL)	1110± 16.5981	**827.6 ± 42.9890****	1280± 64.8865	**752.2 ± 56.1964****
LYM (%)	62.42 ± 1.5711	70.52 ± 2.6554	69.18 ± 1.5916	66.5 ± 3.8505
NEU (%)	30.18 ± 0.9243	**42.32 ± 0.5285****	**38.28 ± 1.7313****	**40.78 ± 1.9257****
RDW (fL)	30.6 ± 0.5839	31.70 ± 0.5263	29.83 ± 0.4397	34.60 ± 3.1061
PDW (fL)	8.64 ± 0.1661	7.96 ± 0.3155	9.42 ± 1.0408	7.58 ± 0.3673
MPV (fL)	7.28 ± 0.0860	6.66 ± 0.1326	7.00 ± 0.2258	**6.40 ± 0.1923****
P-LCR (%)	9.02 ± 0.8656	7.00 ± 0.9690	**12.94 ± 0.7858***	7.36 ± 0.8565

The values are expressed as M ± SEM; *P < 0.05 and **P < 0.01.

The table shows a significant reduction in platelet count (PLT) and an increase in neutrophil count (NEU) in the reference group compared to the control group. In the TAE-treated group, there is a notable increase in white blood cell count (WBC), NEU, and platelet-large cell ratio (P-LCR). In contrast, the MCE-treated group exhibits a significant rise in NEU, along with a significant decrease in WBC, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), PLT, and mean platelet volume (MPV) compared to the control group. No significant changes are observed in lymphocyte count (LYM) across all groups, although NEU levels are notably elevated in all experimental conditions.

3.3.5. Biochemical Parameters

The analysis of serum biochemical parameters assessing liver function in mice treated with prednisolone, methanolic extract, and alkaloid extract of *Peganum harmala* revealed significant alterations. Notable increases in aspartate aminotransferase (AST, TGO) levels were observed in the prednisolone and methanolic

extract-treated groups (146.20% and 90.6%, respectively), with a significant increase also observed in the alkaloid extract-treated group (51.4%) compared to the control group. Additionally, a significant elevation in alanine aminotransferase (ALT, TGP) levels was noted in the prednisolone and methanolic extract-treated groups (132% and 98.6%, respectively) compared to the control group (Figure 3). These findings indicate a significant increase in both TGO and TGP levels in the prednisolone and methanolic extract-treated groups relative to the control group.

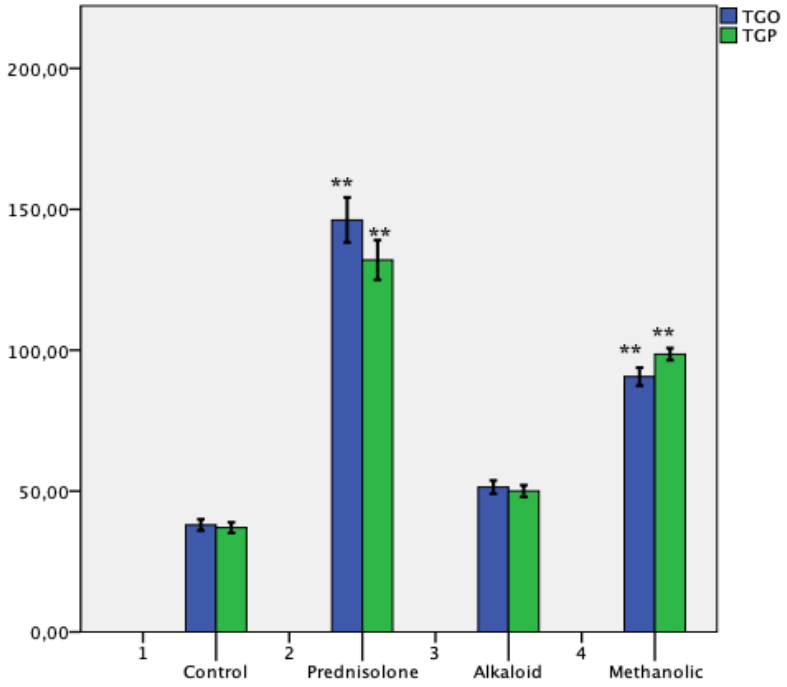

Fig. 3. Some biochemical parameters of female mice treated with MCE and TAE from *Peganum harmala L.* seeds.

3.3.6. Histological Study

Figures 4-9 present histopathological observations of the spleen, thymus, and adrenal glands from mice treated with daily intraperitoneal injections of prednisolone, CSE, and TAE from *Peganum harmala* L. No significant pathological changes were observed in the spleen architecture across all groups (Figures 4, 5). Histological analysis of the thymus revealed neutrophil infiltration in the alkaloid extract-treated group, eosinophils in the methanolic extract-treated group, and neutrophils, mast cells, and lymphoplasmacytic cells in the prednisolone-treated group (Figures 6, 7). Examination of the adrenal glands showed inflammatory disruptions in the MCE group characterized by lymphocytes and neutrophils, while the TAE group exhibited an infiltrate of eosinophils, neutrophils, and lymphocytes. The reference group also displayed hemorrhagic changes with inflammatory cells, including lymphocytes and neutrophils (Figures 8, 9).

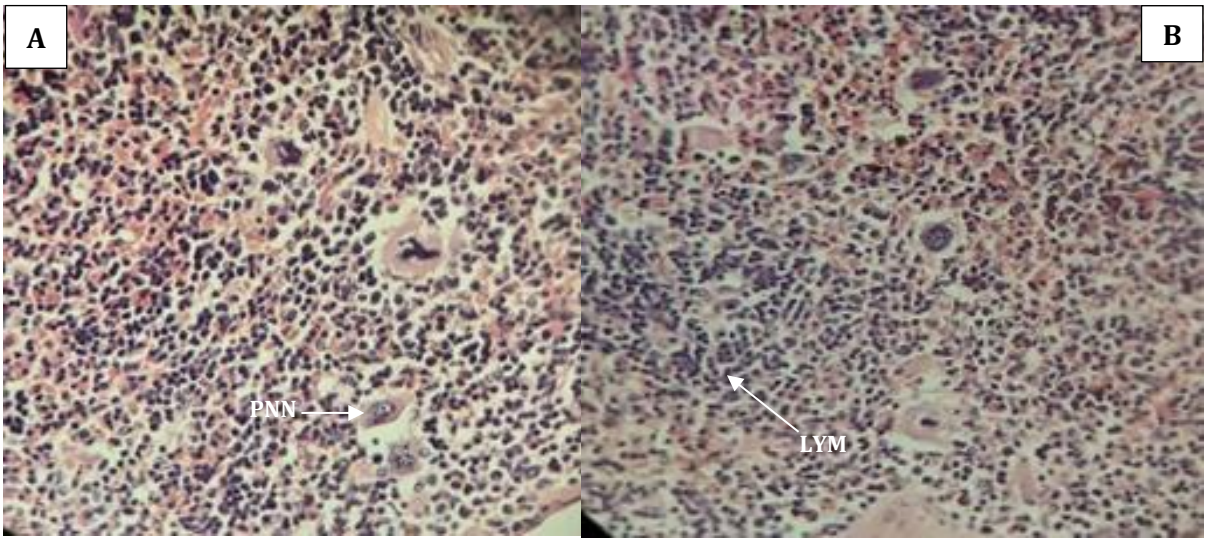

Fig. 4. Histological sections of spleen tissue: (A) Control group; (B) Group treated with the methanolic extract (PNN: polymorphonuclear neutrophils, LYM: lymphocytes).

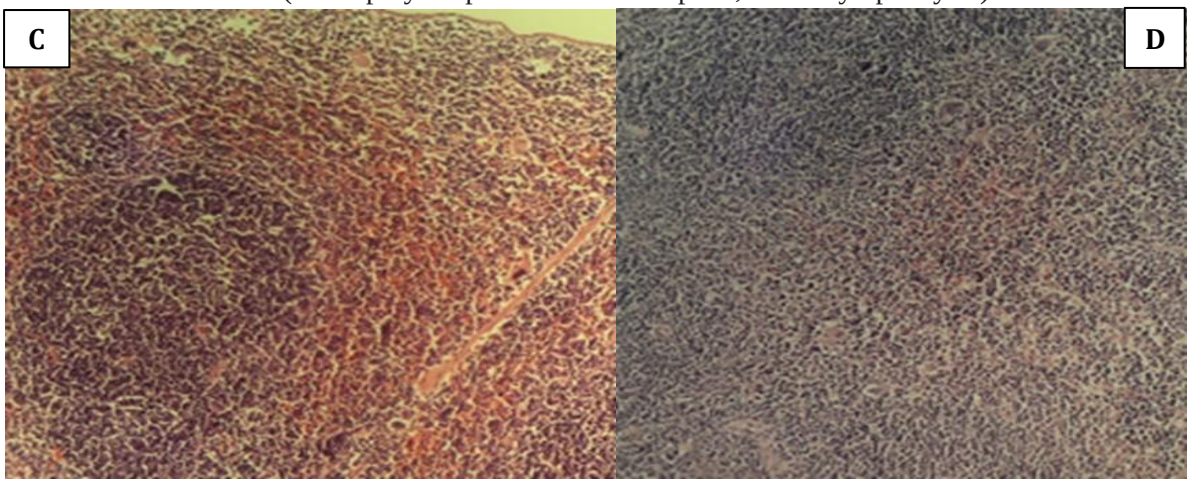

Fig. 5. Histological sections of spleen tissue: (C) Group treated with the alkaloid extract; (D) Reference (Prednisolone).

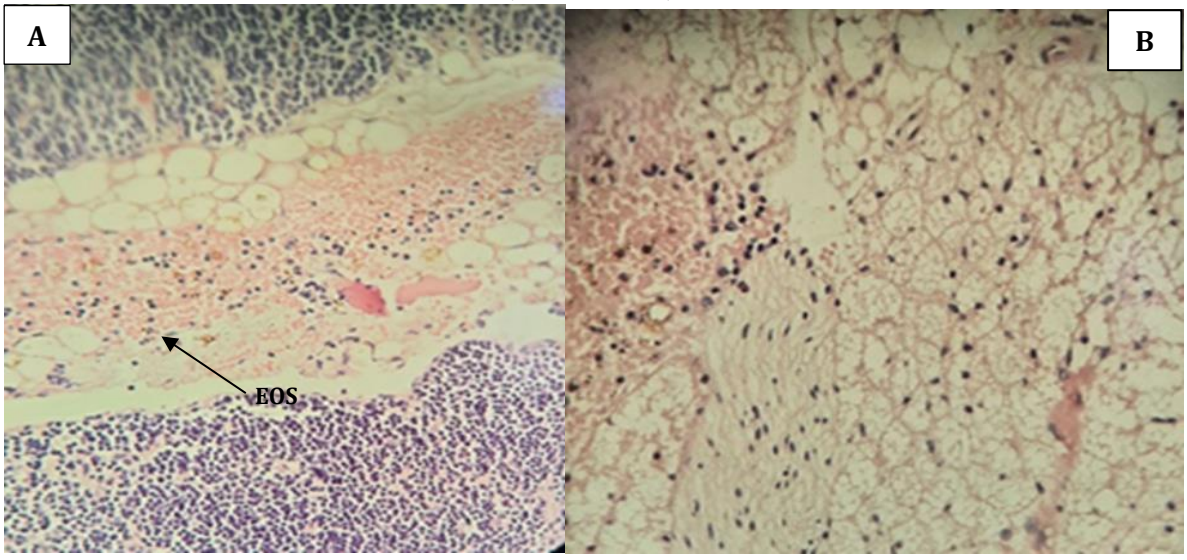

Fig. 6. Histological sections of thymus tissue: (A) control group, (B) group treated with the methanolic extract (EOS: eosinophils).

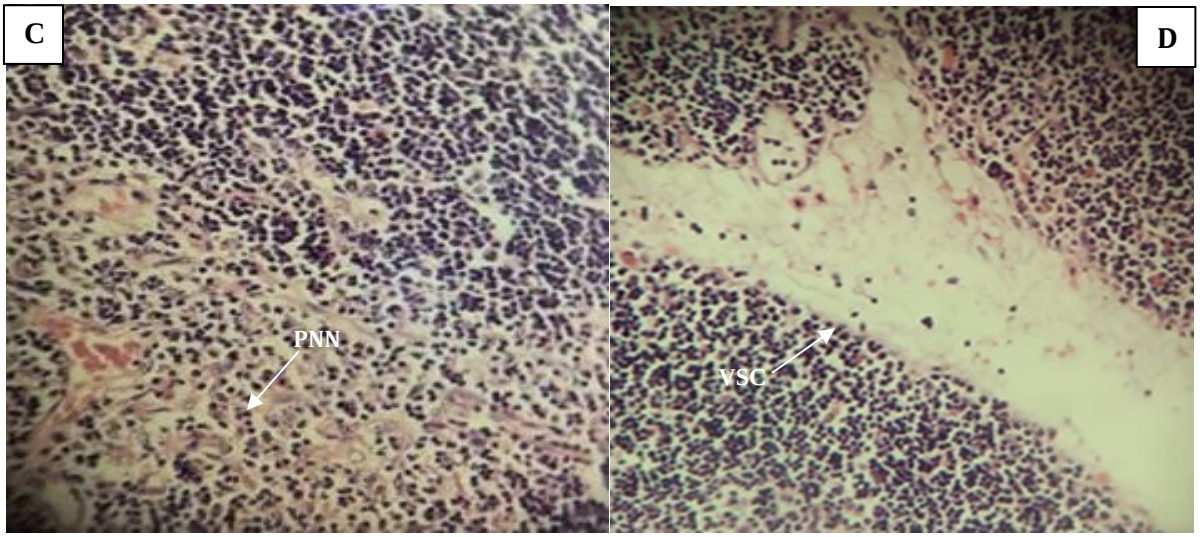

Fig. 7. Histological sections of thymus tissue: (C) Group treated with the alkaloid extract; (D) Reference (Prednisolone) (VSC: connective blood vessels).

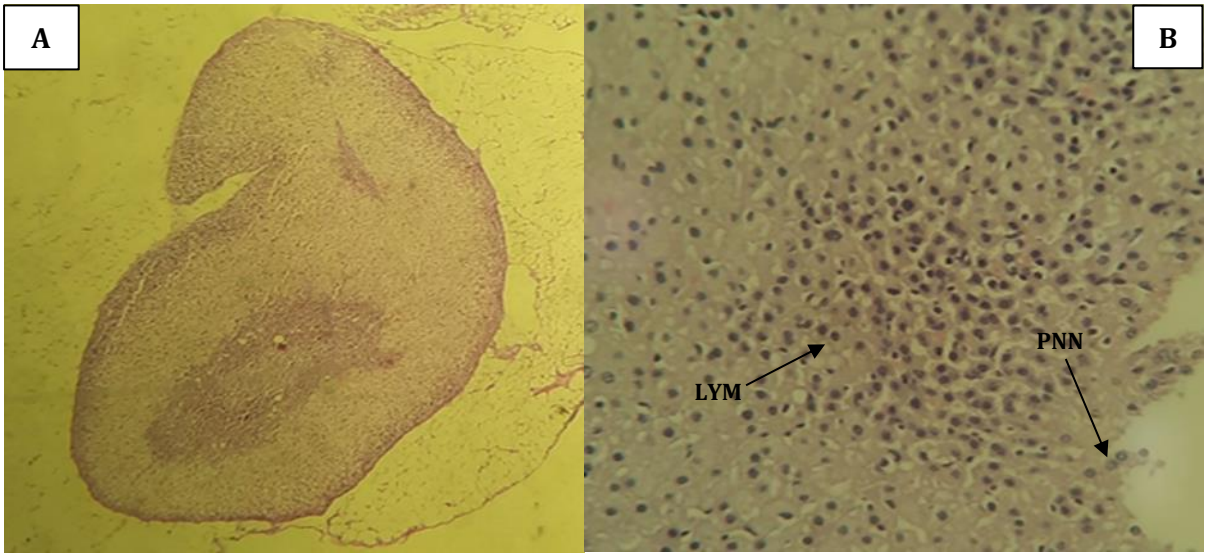

Fig. 8. Histological sections of adrenal gland tissue: (A) Control group; (B) Group treated with the methanolic extract.

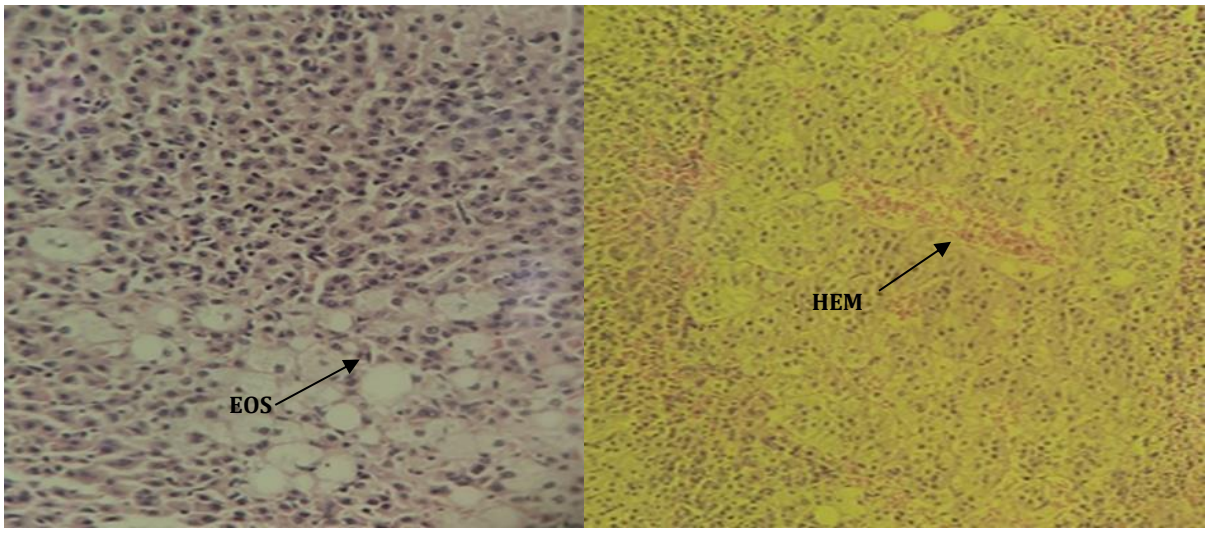

Fig. 9. Histological sections of adrenal gland tissue: (C) Group treated with the alkaloid extract; (D) Reference (Prednisolone) (HEM: hemorrhage).

4. DISCUSSION

***Peganum harmala* L.**, commonly known as "Harmel," has been identified based on its morphological features described by [9], [10], [11], and [12]. It holds significant importance in traditional medicine for ritualistic, magical, prophylactic, and therapeutic purposes [13]. This plant is rich in β-carboline alkaloids such as harmine, harmaline, harmol, and harmalol, along with quinazoline derivatives, which are responsible for its pharmacological and toxicological effects [14], [15], [16].

The yield of crude methanolic extract in our study was 10.1%, which is lower than the 13.44% reported by [17]. Variations in yield are influenced by species-specific traits and environmental factors such as soil composition and temperature. The alkaloid extract yield was 0.47%, significantly lower than the 10.1% from methanol extraction, with [17] reporting a higher alkaloid yield of 2.11%. Factors like plant growth stage, environmental conditions, drying methods, and extraction techniques contribute to yield variations [18]. Extraction methods and solvents, including maceration time and particle size, further affect the efficiency [19]. Thin-layer chromatography of the methanolic and alkaloid extracts revealed three spots with Rf values of 0.50, 0.04, and 0.77, corresponding to β-carboline alkaloids [14]. The presence of these alkaloids confirms the bioactive potential of the plant.

P. Harmala contains fixed oils, saponins, polyphenols, and alkaloids, constituting up to 10% of dry seed weight, which contribute to its pharmacological and toxicological effects [20]. Clinical signs observed in female mice treated with the methanolic and alkaloid extracts included paralysis, tachypnea, exophthalmia, auricular redness, diarrhea, frequent urination, and weight loss, consistent with previous studies [21], [22]. These effects may be attributed to the inhibition of monoamine oxidase A (MAO-A) by harmine and harmaline, leading to increased neurotransmitter levels and subsequent neurological effects [20], as well as acetylcholinesterase inhibition by quinazoline alkaloids, impacting neurotransmission [23].

The significant weight loss observed by day 10 in treated mice aligns with [24], though our study noted a more pronounced reduction, likely due to toxicity, dose, and treatment duration. The changes in relative organ weights, particularly in the liver, spleen, kidneys, and heart, indicate metabolic responses to toxicity. An increase in organ mass suggests congestion or hyperplasia, while a decrease points to atrophy or degeneration [25].

Hematological analysis revealed a decrease in MCV and PLT, coupled with an increase in MPV and P-LCR,

suggesting disrupted platelet production and increased inflammatory activity. Elevated Neu and WBC counts, along with altered transaminase levels (TGO and TGP), point to hepatocellular damage likely caused by harmine and harmaline. Histological analysis confirmed these findings, with increased inflammatory cell infiltration in the thymus and adrenal glands, suggesting immunomodulatory and pro-inflammatory effects of *P. harmala*, like those of prednisolone.

5. CONCLUSION

In conclusion, this study demonstrates the dual pharmacological and toxicological potential of *P. harmala*. Its alkaloid content offers therapeutic benefits but also poses risks of hepatotoxicity, immunosuppression, and systemic inflammation. Further research on dose optimization and the bioactive constituent of the plant is needed to balance its therapeutic effects and potential risks.

6. ACKNOWLEDGMENT

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