Assessment of Antidiarrheal, Antispasmodic and Antimicrobial Activities of Methanolic Seeds Extract of *Peganum harmala* L. (Nitrariaceae)

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**Author’s contribution**

The sole author designed, analysed, interpreted and prepared the manuscript.

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**ABSTRACT**

**Background:** Many plants and herbs have been shown to possess antidiarrheal, antispasmodic, and antimicrobial activities. The present study was developed to determine the possible antidiarrheal and antispasmodic effects of methanolic extract of *Peganum harmala* L. (MEPH) in diarrhea and hyperactive gut.

**Methodology:** The crude MEPH was studied using the in-vivo castor oil-induced diarrhea model in mice, while isolated rat ileum was used in the in-vitro studies. Antimicrobial efficacy of MEPH was tested against different bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*), Yeast (*Candida albicans*) and Fungus (*Aspergillus niger*) using in-vitro assays.

**Results:** In the castor oil-induced diarrhea, MEPH at 100 and 200 mg/kg showed 40% and 80% protection in mice while positive control drug, loperamide showed 100% protection. In the in-vitro experiments, MEPH dose-dependently (0.01 to 0.3 mg/mL) inhibited carbachol and high K⁺-induced contractions at comparable EC₅₀ values similar to papaverine, a phosphodiesterase (PDE) inhibitor. The PDE inhibitory like effect was further confirmed when pre-incubated ileum tissues with MEPH (0.1 and 0.3 mg/mL) shift the isoprenaline-mediated inhibitory CRCs against carbachol to the left, similar to papaverine. In antibacterial assay, MEPH showed efficacy against two Gram
positive bacteria (S. aureus, and B. subtilis) and one Gram negative bacteria (E. coli). But extract didn’t show any effect against K. pneumoniae. The MEPH also showed efficacy against fungal strain (A. niger) and yeast strain (C. albicans).

**Conclusions:** MEPH shows antidiarrheal and antispasmodic effects because of its antimotility effect which were possibly due to the inhibition of PDE enzyme. The study has shown an optimal antimicrobial activity of MEPH as all bacteria, yeast and fungal strains were found sensitive except K. pneumoniae. MEPH proved efficacy against both enteric and non-enteric pathogens causing diarrhea, thus confirming its role in both the infectious and non-infectious diarrhea.

**Keywords:** Syrian rue; hyperactive gut disorder; diarrhea; antibacterial; PDE inhibition.

### 1. INTRODUCTION

The prevalence of severe infections in humans caused by various disease causing microorganisms has been increasing worldwide and is becoming a major threat to morbidity and mortality all over the world [1]. Recently, it has been reported that resistance of pathogenic microorganisms is developing very frequently and extensively to drugs [2,3,4]. Gastroenteritis, also known as infectious diarrhea, is inflammation of the gastrointestinal tract. It is usually caused by different types of bacteria, viruses, parasites, and fungus. Plants have been used in many fields including nutrition, culinary, dyeing, cosmetics and other industrial purposes; furthermore, medicinal plants play very important role in human life. Medicinal plants have been used for treatment of various ailments as traditional medicine for centuries [5,6]. One of the most promising approaches for fighting with multidrug-resistant bacteria is the use of combinations between antibiotics and natural antimicrobial substances [7]. Hence, there is a need to explore new remedies from natural products.

*Peganum harmala* L. (Nitrariaceae) is a perennial, bushy, and wild-growing flowering plant with a long history of medicinal use [8,9]. Various parts (fruits, root, seeds, and bark) of *P. harmala* have been used as traditional medicine for a long time in many countries. Previous literatures have reported different pharmacological activities of *P. harmala* and/or its active alkaloids (particularly harmaline) [10]. Some studies reported that instead of the most important alkaloids (harmaline and harmine) there are some other alkaloids also present in *P. harmala* that are responsible for pharmacological effects of the plant [11]. *P. harmala* has also been reported to exhibit antibacterial activity, including against drug-resistant bacteria [12]. In Saudi Arabia, *P. harmala* has been commonly used for fungal infections [13]. *P. harmala* extract and powdered seeds have also been used traditionally to treat colic in different parts of the world [14].

Although the previous published literature reported the antimicrobial activity of *P. harmala* and ethnobotanical claim for the different gut disorders, no detailed scientific evidence can be found on this plant concerning anti-diarrheal, antispasmodic and antimicrobial activities. Therefore, the present study was developed to assess the potential antimicrobial and gut inhibitory effects of *P. harmala* with detailed mechanism of action.

### 2. MATERIALS AND METHODS

#### 2.1 Plant Materials

The seed of *P. harmala* were procured locally from the Al-Kharj, Kingdom of Saudi Arabia and authenticated by expert taxonomist in the Department of Pharmacognosy, College of Pharmacy, Prince Sattam Bin Abdulaziz University (PSAU), KSA.

#### 2.2 Preparation of Extracts

Seeds were dried in shade and crushed into a coarse powder. Each of 100 g fine powders was extracted with 700 mL methanol by soxhlet extraction. The extracted materials were filtered (Whatman No. 1 filter paper) and evaporated to dryness using rotary evaporator. The dried extract was stored at 4°C in air tight container till further use.

#### 2.3 Chemicals

Following analytical grade chemicals procured from Sigma Company, St. Louis, MO, USA, were used; Carbamylcholine (CCh), loperamide, acetylcholine (ACH), isoprenaline, papaverine. Following reagents (salts) were used to prepare physiological salt solution (Tyrode): potassium...
2.6 Animals

Wistar rats (200-250 g) and Swiss albino mice (25-30 g) procured from the Animal Care Unit, College of Pharmacy, PSAU, KSA, were housed with standard laboratory conditions of ideal temperature (23 ± 1 °C), relative humidity (55 ± 5%) and light/dark cycle (12h/12h). During the acclimatization, animals were provided with free access to standard pellet diet and water. Rats were fasted for 24 h and then sacrificed by cervical dislocation under light ether anesthesia. All experiments were accomplished by following the instruction as provided by NRC [15].

2.5 In-vivo Antidiarrheal Activity

Twenty five mice were randomly divided into five groups (n=5). Following 24 h fasting, mice of 1st and 2nd groups were administered with normal saline (10 mL/kg, PO) and labelled as sham and negative control, respectively. Mice of 3rd and 4th groups (test groups) were orally administered with two increasing doses of 100 and 200 mg/kg of MEPH, respectively. Mice of 5th group (positive control) were administered with loperamide (10 mg/kg, PO). After all treatments, all mice were placed in separate cages (1 mice/cage) with a blotting sheet in the floor to observe the absence or presence of diarrhea. After one hour, all mice were treated with castor oil (10 mL/kg, PO), except sham control. After 4 h, blotting sheets in all cages were inspected for diarrheal droppings [16].

2.6 In-vitro Antispasmodic Activity

The rats were sacrificed to isolate ileum, the last part of small intestine [16]. Following isolation, 2-3 cm length of ileum was cleaned gently from adjacent tissues and fecal material and mounted in emkaBath (France) connected with IOX software. Fresh tyrode solution was filled in tissue bath (20 mL), supplied with carbogen and temperature was maintained at 37°c. Tension of 1 g was applied and the tissues were left for stabilization with multiple exposure of Ach (0.3 µM). After getting the stable band in the spontaneous contractions of ileum, increasing concentrations of MEPH were added to the bath solution which produced inhibition of the CCh and high K+-induced contractions.

2.7 Phosphodiesterase Inhibitory Effect

After observing the inhibitory pattern of MEPH on CCh and high K+-induced contractions with comparable potencies, indirect validation was done by dose-mediated inhibitory curves of isoprenaline against CCh in the absence and presence of MEPH. Potentiation of curves towards left is an indication of PDE inhibition, which was compared with papaverine, a known PDE-inhibitor [17].

2.8 Antimicrobial Assay

MEPH was tested on 4 bacterial, 1 fungal and 1 yeast strain as test organisms to assess the anti-microbial activity. Among the 4 bacterial strains, 2 were Gram positive and 2 Gram negative. S. aureus (ATCC 35501), B. subtilis (ATCC 10400), E. coli (ATCC 10536), K. pneumonia (NCTC 13368), Candida albicans (ATCC 66027) and Aspergillus niger (ATCC 16404), were provided by the Laboratory of Microbiology, College of Pharmacy, PSAU, KSA.

The antimicrobial activity of MEPH was assessed by the diffusion method in an agar environment [18,19]. It is famous in-vitro assay for primary screening of compound which may exhibit any antimicrobial activity. It is a qualitative or quantitative assay specifying the sensitivity or resistance of the microorganism to the test materials. The first step is to prepare the microbial strains followed by an antibiogram. This method is highly flexible in selecting the tested antibiotics, and to be applied on a large number of bacterial species [20].

2.9 Statistics Analysis

Results were expressed as mean ± standard error of mean (SEM) and the number of experiments repeated are represented by “n”. Median effective concentrations (EC50) were analyzed with 95% confidence intervals (CI). The statistical parameters applied were Student’s t-test or two-way ANOVA followed by Bonferroni’s post-test for multiple comparisons of concentration-response curves (CRCs) with control. Chi-square (χ2) test was used to assess diarrhea protection by comparing all groups with control group. p<0.05 was considered as statistically significant. Graph Pad prism (version 4) was used for regression analysis of CRCs.
3. RESULTS

3.1 Protection in Castor Oil-Induced Diarrhea

Treatment of MEPH in both doses exhibit dose-dependent protection of diarrhea in mice whereas no protection was observed in saline group. The lower dose of MEPH (100 mg/kg) showed 40% protection while higher dose (200 mg/kg) showed 80% protection whereas loperamide showed 100% protection in mice (Table 1).

3.2 Antispasmodic Effect

When tested against CCh and high K+-mediated spasm in rat ileum preparations, MEPH caused dose-dependent (0.01-0.3 mg/mL) inhibition with resultant EC\textsubscript{50} values of 0.86 mg/mL (0.64-1.2, 95% CI, n=4) and 0.72 mg/mL (0.68-0.98, 95% CI, n=4), respectively as shown Fig. 1A. Similarly, papaverine showed comparable relaxant effects against spontaneous and high K\textsuperscript+ with resultant EC\textsubscript{50} values of 6.98 µM (5.84-7.52, 95% CI, n=4) and 6.44 µM (6.12-8.14, 95% CI, n=4), (Fig. 1B).

MEPH dose dependent (0.1 and 0.3 mg/mL) pre-incubation of ileum preparations deflected the isoprenaline-induced inhibitory CRCs towards left (Fig. 2A), similar to papaverine (0.3-1 µM) as shown in Fig. 2B.

3.3 Antimicrobial Activity

To assess antimicrobial activity, the inhibition zones of different strains were measured (Fig. 3). Through Table 2, it is remarked that MEPH have inhibitory actions on all bacterial strains, yeasts and fungus except K. pneumoniae, but with different degrees.

4. DISCUSSION

Keeping in view the medicinal use of P. harmala in hyperactive gut related issues, its methanolic extract was tested for possible gut inhibitory effect(s) using in-vivo and in-vitro assays.

<table>
<thead>
<tr>
<th>Treatment (p.o.), dose (mg/kg)</th>
<th>No. of mice with diarrhea</th>
<th>% Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (10 mL/kg) + Castor oil</td>
<td>5/5</td>
<td>0</td>
</tr>
<tr>
<td>MEPH + Castor oil</td>
<td>100 (mg/kg) + 10 (mL/kg)</td>
<td>3*/5</td>
</tr>
<tr>
<td>200 (mg/kg) + 10 (mL/kg)</td>
<td>1*/5</td>
<td>80</td>
</tr>
<tr>
<td>Loperamide (10 mg/kg) + Castor oil</td>
<td>0**/5</td>
<td>100</td>
</tr>
</tbody>
</table>

* p < 0.05 and **p < 0.01 vs. Saline + Castor oil treated group (χ\textsuperscript{2}-test).

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Zone of inhibition [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MEPH 25 mg/ml</td>
</tr>
<tr>
<td>Gram positive bacteria</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>35.0</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>38.0</td>
</tr>
<tr>
<td>Gram negative bacteria</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>38.0</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>-</td>
</tr>
<tr>
<td>Yeast</td>
<td></td>
</tr>
<tr>
<td>C. albicans</td>
<td>28.0</td>
</tr>
<tr>
<td>Fungus</td>
<td></td>
</tr>
<tr>
<td>A. niger</td>
<td>40.0</td>
</tr>
</tbody>
</table>
Fig. 1. Concentration-response curves showing comparison of the (A) crude extract of *Peganum harmala* (*P. harmala*) and (B) papaverine, for the inhibitory effect against carbachol (CCh, 1 µM) and high K+ induced contractions in isolated rat ileum preparations. Values shown are mean ± SEM, n=4-5

Fig. 2. Inhibitory concentration-response curves of isoprenaline against carbachol (CCh)-induced contractions in the absence and presence of different concentrations of (A) crude extract of *Peganum harmala* (*P. harmala*) and (B) papaverine, in isolated rat ileum preparations. Values shown are mean ± SEM, n=4-5

In the in-vivo study, experiments were planned to test the medicinal claim of the crude extract of this plant in diarrhea using castor oil-induced diarrhea model in mice while isolated rat ileum preparations were used in the in-vitro experiments for the elucidation of the detailed mechanism(s) of antispasmodic activities [21]. Castor oil hydrolyze into ricinoleic acid which evoked spasms in the gut and produces diarrhea in normal mice [22]. Treatments of MEPH show dose-dependently protection in mice from diarrhea, comparable to the loperamide, a standard antidiarrheal drug [23]. After observing the antidiarrheal response, the second objective of this study was to evaluate the possible mechanism involved in this effect. The method of Palla et al. [24] was followed by testing *P. harmala* cumulative concentrations in isolated rat intestine with spasm induced by CCh and high K+. Interestingly, *P. harmala* exhibited
Fig. 3. Effect of *Peganum harmala* L. (Nitrariaceae) seeds extract on sensitivity of bacterial, yeast and fungal strain
complete inhibition of both type of contractions dose-dependently. The critical analysis of the pattern of the inhibitory CRCs of *P. harmala* against CCh and high K⁺-induced contractions show that no statistically significant (p>0.05) difference was found in the inhibitory effect observed, similar to papaverine, an inhibitor of PDE [25]. The potentiation of the inhibitory CRCs of isoprenaline towards left in the pre-incubated ileum tissues with increasing doses of *P. harmala* further confirm its PDE-inhibitory character. Papaverine, a standard PDE-inhibitor used also deflected isoprenaline curves towards left [26]. It is previously reported that in smooth muscles, PDE-inhibitors reverses CCh-mediated spasm [27].

In antimicrobial assay, MEPH showed 35.0 and 38.0 mm in diameter of zone of inhibition against two Gram positive bacteria like *S. aureus*, and *B. subtilis*, respectively and 37.0 mm in diameter of zone of inhibition was observed against Gram negative bacteria like *E. coli* but no activity was observed against *K. pneumoniae*. The results of the antifungal activity reveal the efficiency of extract against the tested strain. Consequently, the results show inhibition zones of MEPH against *C. albicans*. The tested standard antibiotics, erythromycin (15 μg/disc) and ampicillin (10 μg/disc) showed significant activity against all tested bacteria and yeast (Table 1). The observed antibacterial activity of MEPH might also be due to the presence of high quantity of polyphenols [28]. The harmal seeds are also rich in alkaloids (harmaline, and harmine) which exhibit antimicrobial activity [29]. These alkaloids are also reported to have antifungal activity [12].

5. CONCLUSION

The observed results of the present study confirmed that harmal seeds extract possess moderate antidiarrheal, antispasmodic, and antimicrobial activity against all Gram positive and Gram negative bacteria, yeast and fungus and the most sensitive strain was *A. niger* with a diameter of 40.0 mm of the inhibitory zone for the MEPH. Whereas, the most resistant strain reported was *K. pneumonia* with no effect.

CONSENT

It is not applicable

ETHICAL APPROVAL

The study has been approved by Bio Ethical Research Committee (BERC) at Prince Sattam Bin Abdulaziz University with reference number BERC-004-12-19.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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