

Original Article

Pharmacological Effects of *Peganum Harmala L.* Root Extract on Isolated Rat small Intestine

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ABSTRACT: **Background:** *Peganum harmala L.* is a medicinal plant used in Jordan and in many countries in traditional medicine against several diseases, including gastrointestinal disorders. **Objective:** In this study, the effects of crude ethanolic root extract of *P. harmala* on the isolated smooth muscle of the rat jejunum intestine were investigated. **Materials and Methods:** The isolated organ bath method was adopted. The efficacy was recorded and analyzed by biological signal collection and analysis recording. **Results:** The ethanolic root extract inhibited the spontaneous contraction of rat jejunum and showed dose dependent inhibition of acetylcholine Ach (10^{-5} m) and potassium chloride KCl (60 mm), as well as inducing contractions of isolated rat jejunum. The extract also reduced barium chloride BaCl₂ (5 mm) induced contractions of isolated rat jejunum dose-dependently. The inhibitions were statistically significant. **Conclusion:** These results indicated that the root extract of *P. harmala* possesses antispasmodic activity and justifies its use traditionally in alleviating gastrointestinal disorders.

KEYWORDS: Gastrointestinal, Antispasmodic, Spasmolytic, Zygophyllaceae, *Peganum harmala L.*, Small intestine, Rats.

INTRODUCTION

The Gastrointestinal tract (GIT) uses the smooth muscle of the mucosal lining enriched with an enteric neural network to regulate propulsive transport and mixing of food material directionally through the digestive systems.^[1] The neural network initiates and coordinates secretion and absorption across the intestinal lumen as well.^[2]

Antispasmodics are muscular relaxants that are used to relieve cramps or spasms of the stomach, intestines and bladder. They are commonly used for the treatment of different gastrointestinal disorders, including diarrhea and irritable bowel syndrome, which affect millions of people.^[1]

Diarrhea continues to be one of the leading causes of mortality and morbidity especially in children in developing countries.^[3] Diarrhea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, accompanied by an increased intestinal transit rate and an excess loss of fluid in the faeces. In some diarrhea, the secretory component predominate whilst other diarrheas are characterized by hypermobility.^[4]

Plants are sources of many medicinal drugs. Many people nowadays turn to the use of natural product of medicine for treatment of intestinal disorders. Dependence on plants as the source of medicines is prevalent in developing countries where traditional medicine plays a major role in health care.^[5] In recent times numerous scientific studies have been performed to test the potential effect of plant extracts on intestinal contractions.^[3] However, the mechanism of action by which these plants exert their therapeutic effects has not been completely elucidated.

Peganum harmala L. Family Zygophyllaceae is a perennial herbaceous, glabrous plant, which may grow to 30–100 cm. Its normal habitat is semi-arid steppe areas and sandy soils. The plant is widely distributed in the Central Asia, North Africa and Middle East and has been introduced in America and

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Australia. It is one of the most famous medicinal plants used in traditional medicine of Jordan, commonly known as Harmal, wild Rue or Syrian Rue.^[6] The medical uses of harmal are varied and extensive. The fruits and seeds are digestive, diuretic, hallucinogenic, hypnotic, antipyretic, antispasmodic, nauseant, emetic, narcotic and are uterine stimulant.^[7]

In recent years, different pharmacological and therapeutic effects of *P. harmala* and its active alkaloids, especially harmine and harmaline have been demonstrated.^[8] These effects include spontaneous effects on isolated rat uterus^[9] anti-leishmanial,^[10] anti-tumor effect,^[11,12] insecticidal,^[13] vasorelaxant effect^[14,15] wound healing, anti-oxidant activity,^[16] leukemic healing,^[17] hypoglycemic effect, wound healing, anti-oxidant activity,^[16] leukemic healing,^[17] hypoglycemic effect,^[18] immuno-modulator properties,^[16] analgesic and anti-inflammatory properties,^[19] antibacterial, antifungal and antiviral effects.^[20,21] Recently, anti-secretory and cytoprotective effects have also been reported.^[22]

Despite the broad use of *P. harmala*, there is little scientific information to reinforce its spasmodic effects. Only a single publication^[23] reported the *in vitro* effects of an aqueous extract of *P. harmala* seeds on the smooth muscles of rabbit and guinea pig using isolated segments of intestine, trachea and aorta. These data suggested that seed extracts have anti-spasmodic, anti-cholinergic, anti-histaminic and anti-adrenergic effects.^[23]

As the previous study has focused on the bioactivity of the seeds, the present study focused on plant root extract to evaluate the possible spasmolytic activity of the ethanolic extract of *P. harmala* roots on rat intestine; since there is no data of the physiological effect of the extract on isolated rat jejunum and justifying its use traditionally in alleviating gastrointestinal disorders.

MATERIALS METHODS

Plant material

P. harmala fresh roots were collected from Wadi Mujeb (123 km south of Amman, Jordan) in May/2012 and a voucher specimen has been deposited at the Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan (Herbarium No. 5 ZYGO PHA). The plant material was identified and authenticated by Professor Suleiman Al-Olimat.

Preparation of the extract

Finely crushed and coarsely dried powdered roots (100 g) were extracted by refluxing for 1 hr using 1L of 96% ethanol. The extract was kept overnight at room temperature, filtered and vacuum dried under reduced pressure to yield a solid ethanolic residue. For the *in vitro* experiment, 0.1

g of the completely dried ethanolic extract was dissolved in 5 ml 70% ethanol and used for the preparation of the desired concentrations by further dilution in 70% ethanol immediately prior to use.

Animals

Male Wister rats weighing (250–350 g each) were obtained from Faculty of medicine, University of Jordan. They were housed at 22–25°C with 12 h light-dark cycle. The animals had free access to food and water. Rats were fasted approximately 15 hours before sacrifice. All animal experiments were conducted in concordance with the University of Jordan's "Regulations and Ethical Guidelines for the Care and Use of Laboratory Animals."

Test substances

P. harmala root ethanolic extract was re-suspended and diluted in Krebs solution before adding to the organ bath to get (0.33, 1, 1.5 mg/ml concentrations). Acetylcholine chloride (ACh), barium chloride, potassium chloride were all purchased from Sigma (St. Louis, MO, USA). The drugs were freshly prepared on the day of the experiments.

Tissue preparation

On the day of experiment, fully ether anesthetized rats were sacrificed by opening of the chest cavity. The whole intestine was removed and selected portion of the jejunum (approximately 10 cm), without considering the 10 cm nearest to the ileocecal valve, was surgically excised and placed in Krebs bicarbonate solution maintained at 37°C and bubbled with gas mixture of (95% O₂ and 5% CO₂).

Intra-luminal content was flushed out with freshly prepared Krebs solution (pH 7.4) and cut to several sections of approximately (1.5 cm). The composition of Krebs-Henseleit solution was (mm): NaCl 118.1, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2, NaHCO₃ 25 and glucose 5.6. The solution was kept at 37°C, (pH 7.4) and gassed with carbogen (a 95% O₂/5% CO₂ mixture). The pre-dissected segments were then mounted vertically into a 15 ml organ-bath filled with Krebs solution (pH 7.4) warmed by circulating water jacket at 37°C and constantly aerated with the gas mixture of (95% O₂ and 5% CO₂). One end of preparation was hooked to the bottom of the bath and the other end was connected to on force transducer. Specimens were allowed to equilibrate at 2g resting tension for 60 minutes and bubbled with (95% O₂ and 5% CO₂) to maintain the pH 7.4. The mechanical responses of the isolated preparation were recorded using Radnoti, 159901A, isometric force transducers and a computerized data acquisition system. The equilibration time for the jejunum incubation was 30 min. After the tissues had been pretreated with the extract or compounds cumulative concentration response

curves using the agonists ACh-chloride, potassium chloride KCl (60 mM) or BaCl₂ (5 mM) were recorded isotonicly in the organ bath, and the effect was allowed to reach a steady state at each concentration.

Evaluation of Antispasmodic activity

After stabilization for 30 min, the antispasmodic effect of the *P. harmala* root ethanolic extract was evaluated by the following procedures:

Isolated jejunum pre-contracted with KCl

For the extract-induced relaxation of preparations pre-contracted with (60 mM) of KCl solutions, only those with an at least 3-min lasting plateau contraction were used. The percentage inhibition of contraction induced by KCl in the presence of each concentration of the extract was calculated.

Isolated jejunum pre-contracted with (ACh)

Preliminary experiments were conducted to determine contractile response curves of intestinal preparation to different concentrations of (ACh). An increase in ACh concentration caused increasing smooth muscle contraction and the submaximal contractile response was obtained for (ACh) at (3×10^{-5} M). This concentration was selected in our experimental protocols out of a series of cumulative concentration response-curves. The proposed dose of (ACh) were applied to preparation in the absence of the *P. harmala* root extract and the control data were recorded. Thereafter, the tissue was washed out and incubated in ethanolic extract of *P. harmala* root extract (0.33 mg/ml) for 20 min and then exposed to a second single application of (ACh) (3×10^{-5} M). The tissue was washed twice with Krebs buffer and incubated in extract of *P. harmala* root extract (1 mg/ml) for 20 min and exposed to the third single application of (ACh) (3×10^{-5} M). Finally, the tissue was washed twice with Krebs buffer and incubated in extract of *P. harmala* root extract (1.5 mg/ml) for 20 min.

Isolated jejunum pre-contracted with barium chloride

For the extract-induced relaxation of preparations pre-contracted with (5 mM) BaCl₂, only those with at least 3-min lasting plateau contraction was used. The percentage inhibition of contraction induced by BaCl₂ in the presence of each concentration of the extract were also calculated.

Statistical analysis

Results are expressed as the mean \pm SEM of 4–6 repetitions as indicated in the figure legends. Values were analyzed using ANOVA followed by Dunnett's test. A value of $P < 0.05$ was considered as a significant difference. Statistical analysis was performed using GraphPad Prism

version 5.02 for Windows (GraphPad Software, San Diego, CA, USA).

RESULTS

Antispasmodic effects of ethanolic extract of the roots of *P. harmala* on the KCl-induced jejunum contractions

Harmal extract caused a concentration-dependent relaxant effect on the contraction of rat's jejunum. Figure 1 illustrates the concentration-dependent inhibition obtained using KCl (60 mM) as an inducer of jejunum muscle contractility in the presence of three different concentrations of harmal extract. Inhibition of KCl induced contraction by $68 \pm 11\%$, $P < 0.001$ and by $86 \pm 6\%$, $P < 0.001$ (ANOVA followed by Dunnett's test) ($n=4$) at extract concentrations of 1 mg/ml and 1.5 mg/ml respec-

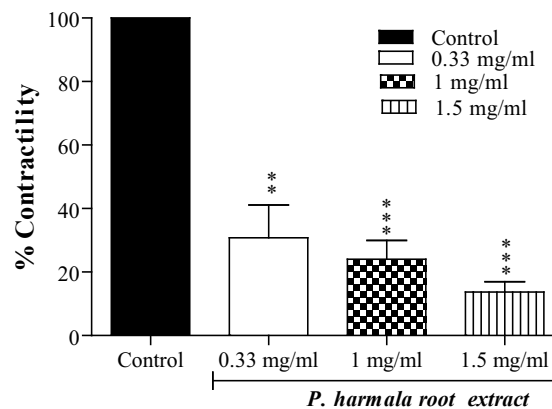


Figure 1. Effect of *P. harmala* root extract (0.33, 1 and 1.5 mg/ml) on rat isolated jejunum precontracted with KCl (60 mM). Each bar represents the mean \pm SEM of 4 repetitions. ** $P < 0.01$, *** $P < 0.001$ ANOVA followed by Dunnett's test.

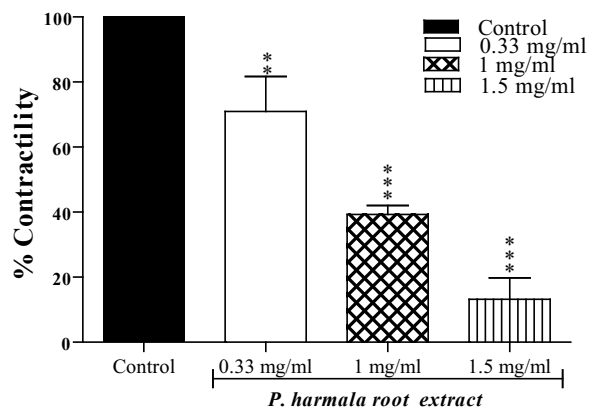


Figure 2. Effect of *P. harmala* root extract (0.33, 1 and 1.5 mg/ml) on rat isolated jejunum precontracted with ACh (3×10^{-5} M). Each bar represents the mean \pm SEM of 4 repetitions. ** $P < 0.01$, *** $P < 0.001$ ANOVA followed by Dunnett's test.

tively were obtained. The relaxant effect was reversible and the spontaneous activity returned to normal after washing the preparation.

Antispasmodic effects of ethanolic extract of the roots of *P. harmala* on the ACh-induced jejunum contraction

A single application of ACh (3×10^{-5} M) evoked a contraction of (100%) ($n=4$) (Figure 2). These contractile responses were inhibited in a concentration-dependent manner in the presence of *P. harmala* root extract (0.33, 1 and 1.5 mg/ml). The extract inhibited the (ACh) induced contractile responses by $30 \pm 10\%$ ($P < 0.01$), $61 \pm 3\%$ ($P < 0.001$) and by $87 \pm 6\%$ ($P < 0.001$) (ANOVA followed by Dunnett's test) at extract concentrations of (0.33, 1 and 1.5 mg/ml) respectively.

At lower concentrations of *P. harmala* root extract the contractile responses were not significantly attenuated. However at higher extract concentrations, the responses were significantly reduced (e.g. at 1.5 mg/ml by $0.3 \pm 13\%$ g) (85%) $***P < 0.001$ (ANOVA followed by Dunnett's test). The relaxant effect was reversible and the spontaneous activity returned to normal after washing the preparation.

Antispasmodic effects of ethanolic roots of the *P. harmala* on the BaCl₂-induced jejunum contraction

The contractile responses obtained by a single application of BaCl₂ (5 mM) was (100%), ($n=6$) (Figure 3) with *P. harmala* root extract (0.33, 1 and 1.5 mg/ml) resulted in a decrease in the contractile responses evoked by BaCl₂. No significant decrease was observed when the preparation was pretreated with 0.33 mg/ml of *P. harmala* root extract. However, significant inhibition of BaCl₂ induced contraction by $74 \pm 15\%$, $P < 0.05$ and by $83.5 \pm 18\%$, $P < 0.05$ ($n = 6$) (ANOVA followed by Dunnett's test) (Figure 3) at extract concentrations of 1 mg/ml and 1.5 mg/ml respectively were obtained.

Solvent effect on rat jejunum contraction

The plant extracts were dissolved in 70% ethanol, control preparations of ethanol up to 100 μ l were added to the organ bath to determine whether the solvent alone was able to induce inhibition of contractions. Spontaneous contraction rhythmic contractions were not affected in the presence of ethanol (Figure 4).

DISCUSSION

Gastrointestinal motor tone is modulated through multiple physiological mediators which include neurotransmit-

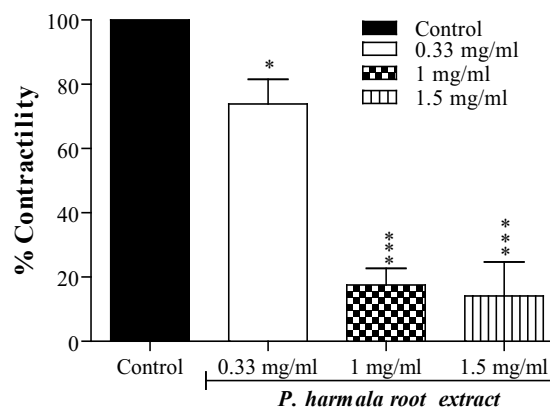


Figure 3. Effect of *P. harmala* root extract (0.33, 1 and 1.5 mg/ml) on rat isolated jejunum precontracted with BaCl₂ (5 mM). Each bar represents the mean \pm SEM of 6 repetitions. * $P < 0.01$, *** $P < 0.001$ ANOVA followed by Dunnett's test.

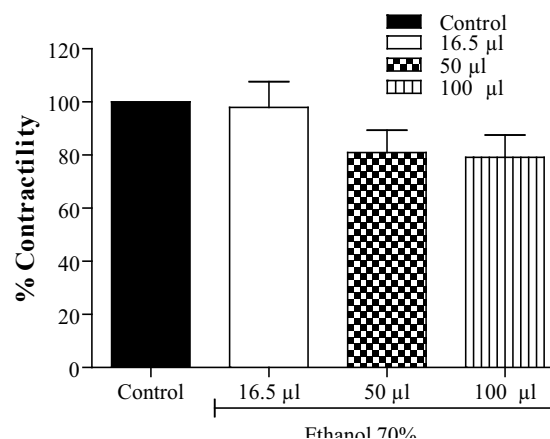


Figure 4. Effect of 70% ethanol on rat isolated jejunum compared with control. Each bar represents the mean \pm SEM of 4 repetitions.

ters, inflammatory mediators and oxidative metabolites.^[24] The release of these chemical modulators in GIT causes stimulatory effect mediated through an ultimate increase in cytosolic Ca²⁺.^[25] Drug substances with the ability to block or alter any of these pathways, or with non-receptor specific inhibitory action (such as Ca²⁺ antagonists) could be considered to be effective as therapeutic agent in hyperactive or hypoactive GIT disorders. This study showed that that *P. harmala* root extract is a potent relaxation-producing mixture, which reduced contractions induced by a variety of agents known to produce contractions under experimental conditions in rat intestine. It reduced KCl-induced contractions, a calcium channel mediated spasmogens, as well as those produced by acetylcholine, a receptor mediated agent and BaCl₂ a non-selective smooth muscle agonist. This experimental design demonstrates a dose-dependent spectrum of anti-spasmodic activity of this plant species.

The (Ach) or KCl and BaCl₂ induced contraction *in vitro* are experimental models commonly used to analyze the spasmolytic activity and/or mechanism of action of drugs and plant extracts.^[26–28] The KCl-induced contraction is based on the depolarization of the muscle fibers causing an increase of K⁺, which leads to the opening of L-type Ca²⁺ voltage-dependent channels. Therefore, the intracellular ion concentration increases to induce contraction.^[29] The (Ach) induced contraction is produced by an increase in the frequency of the action potentials and the depolarization of the smooth muscle cells, which results in an increase in the intracellular calcium and in the activation of the myosin light chain causing smooth muscle contraction.^[30] BaCl₂ contracted intestinal smooth muscle through combining calmodulin (CaM) to increase an influx of Ca²⁺.^[31]

Using (Ach) *in vitro* on intestine tissue to induce contraction serves as a model where excessive enteric nervous system activation would be responsible for GIT spasms. Agents which counteract these spasms are considered antispasmodic drugs which exert their effects by either blocking muscarinic receptors, relaxing smooth muscles, or blocking calcium-channels.^[32]

In our study, *P. harmala* root extract at concentrations of 1 and 1.5 mg/ml has significantly reduced contractile responses induced by (Ach). This demonstrates clear interference of the extract with the signal transduction mechanisms induced by (Ach). The results obtained in this study indicate that harmal extract reduced the (Ach) induced contractions in rat jejunum in concentration dependent manner. Rat jejunum smooth muscle contractions induced by KCl were also antagonized by the extract. Agents which inhibit contractions induced by KCl are considered calcium channel blockers, where exposure of smooth muscle cells to +K stimulates contractions through the opening of voltage-dependent L-type Ca²⁺ channels and consequent influx of extra cellular Ca²⁺.^[33] Our data suggests that the extract used in this study may have at least partially conveyed its effects in this manner.

BaCl₂ depolarizes the smooth muscle membrane and opens the voltage-dependent Ca²⁺ channels resulting in a Ca²⁺ influx. Harmal extract produced a statistically significant inhibition of the contractions induced by BaCl₂. Karaki et al.,^[34] have researched contractions of intestinal smooth muscle induced by barium and high concentration of KCl. They concluded that both concentrations are due to influx of Ca²⁺.

The extract of harmal caused relaxation of the KCl and BaCl₂ induced contractions suggesting that the spasmolytic effect is possibly mediated through calcium channel

blockage.

In a previous study, a hydro-alcoholic extract from *P. harmala* was found to increase spontaneous uterine muscle contractions at low concentrations and decrease contractile activities at high concentration of 400 ug/ml^[11]. In our study, we have not found any increase in spontaneous contractile activities of rat intestinal preparation at the low dose used. The lower contractile response obtained at high dose of extract in the previous study^[11] may be attributed to increasing concentration of inhibitory constituents in the extract that have masked the effect of excitation obtained at low extract concentration. From the results obtained in this study we conclude that harmal root extract reduced smooth muscle contractility in GIT and could be is a useful herbal remedy for treating intestinal spasms. The mechanism of action is presumed to be achieved through interfering with calcium channel natural responses.

The medicinal properties of the plant *P. harmala* are mainly attributed to harmaline, harmine, harmalol, harmol and tetrahydroharmine, previously identified and quantified as the main beta-carboline alkaloids in *P. harmala* extracts.^[10–22] Based on this report, the spasmolytic activity of the ethanol extract of *P. harmala* studied here may be attributed to these alkaloids.^[10–35]

In summary, the current study indicated promising antispasmodic activity of *P. harmal* as a medicinal plant. Our results are in agreement with other studies which have reported the spasmolytic activity of an aqueous extract of *P. harmala* seeds on the smooth muscles of rabbit and guinea pig.^[23] However, the components responsible for these activities are currently unclear. Therefore, further phytochemical investigations are needed to isolate and identify the valuable bioactive substances present in this plant extract. Similar studies with the purified active constituents of *P. harmal* will be the continuation of the current work to detect the active constituents responsible for the observed biological activity. The therapeutic effectiveness in the treatment of gastrointestinal disorders and use in traditional medicine of this plant could be due to its spasmolytic effect.

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