

Full Length Research Paper

Antibacterial, muscle relaxant, and hypnotic effects of seeds of *Peganum harmala* on mice

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***Peganum harmala* seed extract has been frequently reported to possess antibacterial potential through *in vivo* studies. *P. harmala* L. (Zygophyllaceae) is one of the most famous medicinal plants used in traditional medicine of Iraq. The harmaline, harmalol and harmine exerted many pharmacodynamic effects on the central nervous system: stimulation and depression depending on the dose. *P. harmala* indicates a great variety of pharmacological activities such as antimicrobial, antitumor, antinociceptive and monoamine oxidase (MNO) inhibitory activities. The most important components of *P. harmala* seeds are harmaline, vasicinone and deoxyrsinone. The antibacterial effect of *P. harmala* was studied. The antibacterial activity of aqueous extracts was determined by agar well diffusion method. It inhibited the growth of *Escherichia coli* and *Staphylococcus aureus*. All animals injected with 100 mg/kg b. w of aqueous extract of *P. harmala* show myorelaxation or incoordination; so the animals dropped down from the wire 3 consecutive times in 60 s. Aqueous extract of *P. harmala* also induced muscle relaxation and prolonged the sleeping time induced by pentoparpitol. These data suggest that *P. harmala* extract could inhibit the growth of *S. aureus* and *E. coli* strain *in vitro* and this activity may contribute to its chemopreventive effect.**

Key words: Antibacterial, muscle relaxant, hypnotic, *Peganum harmala*.

INTRODUCTION

Antibiotics at the present time are produced either synthetically or through microbial fermentation. The development of microbial resistance is one of the greatest public health problems. This problem has promoted a continual search for a new source of antimicrobial agents. Medicinal plants were the first medicines and have been used since ancient time (Seyyednejad and Motamedi, 2010) and they continue to be used by various cultures around the world (Mahmoudian et al., 2002). All drugs from plants contain substance

such as alkaloids, essential oils, phenols, unsaturated long chain aldehydes, peptides, ethanol, methanol, and butanol-soluble compounds with specific therapeutic activities (Servention et al., 1999). The antibacterial activities of several species of plant have been reported by many researchers (Cam, 2001; Sagdic and Ozcan; 2003). *Peganum harmala* is a perennial herbaceous, glabrous plant that can grow up to 30-100 cm and is distributed throughout the Middle East, North of Africa. This plant is famous for its antimicrobial effect, and is

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traditionally used as disinfectant. Also alkaloid from *P. harmala* has vasorelaxant, antihemosporidian, anticancer, antinociceptive, antitumor and antineoplastic and antiprtozol effects (Arshad et al., 2008; Moghadam et al., 2010; Prashanth and John, 1999).

P. harmala is traditional medicinal plant that is used for many purposes, particularly in treating gastrointestinal problems. This plant has different varieties with different chemical constitutes, some which have antimicrobial activity. Previous extraction and purification of *Peganum harmala* showed that this plant contained harmaline, harmol, harmine, banisterines, peganine, vasicinone and roscinone alkaloids as well as harmala or turkey resin and fatty oils (Hashim and Jamel, 1988). It is used as sedative in restless and agitated patients. Its seeds are known to possess hypothermic and anti-oxidative properties (Rezvan et al., 2016; Moloudizargari et al., 2013).

Harmaline and harmine alkaloids exert antibacterial activity against a wide spectrum of bacteria. They have antifungal activity against many fungal species. They cause shrinkages of the protoplasm of the fungal cell (Al-Janabi, 1988; El-Kady et al., 1993). This result is in disagreement with Amin et al. (2014) who reported that ethanol extract of *P. harmala* has no effect on *E. coli*, only n-butanol; chloroform of *P. harmala* seed showed good antibacterial activity. On the other hand, harmaline, harmalol and harmine exert many pharmacodynamic effects on the central nervous system, ranging between stimulation and depression depending on the dose.

Aqueous extract of *P. harmala* causes motor dysfunction and is manifested by sluggish movement and unstable walking or loss of balance (Al Maliki and Elisha, 1985). Alkaloids of *P. harmala* are valued for their interesting chemistry, and pharmacological potential. They possess antitumor, antileishmanial, ant-HIV, antibacterial MAO- inhibition (Ramadhan et al., 2013). This study was designed to investigate the antibacterial and central nervous effects of *P. harmala* on mice.

MATERIALS AND METHODS

Plant materials

Fresh *P. harmala* were purchased from local market in Iraq and botanically authenticated by National Herbarium Botany Directorate.

Extraction of *P. harmala*

Dry seeds of *P. harmala* were grounded in coffee machines for 2-3 min. The powder was mixed with sufficient amount of distilled water, and shaken overnight at room temperature. The mixture was filtered, and the solvent was removed by incubation at 37°C. Distilled water was used to dissolve the dried residue to give the required concentration.

Microbial strains

Organisms were received from Department of Laboratory Clinical

Science, College of Pharmacy. Al Mustansiriya University reconfirmed by gram staining and subculturing in appropriate selective media. The Gram positive bacterium was *Staphylococcus aureus* and Gram negative was *Escherichia coli*.

Preparation of standard culture inoculation of test organisms

Three or four isolated colonies were inoculated in the 2 ml nutrient broth and incubated till the growth in the broth was equivalent to Mac-Farland standard (0.5%).

Experimental no 1 antibacterial assay

Kirby- Bouar method was performed by Muller Hinton agar (Oxoid) (Nair et al., 2005) poured on disposable plates. Holes of 5 mm in diameter were made after solidification of the agar. *E. coli* and *S. aureus* were uniformly distributed on the surface of the agar. 0.4 ml of 5 and 10 mg/ml of *P. harmala* extract were placed in the holes. The plates were incubated at 37°C, and examined after 24 h for the presence of growth inhibition zones. Ampicillin of 10 mg was used as positive control, while distilled water was used as negative control.

Experimental design and procedure

The animals were randomly separated into two groups of Swiss albino mice weighing 21-23 g. They were housed in polypropylene cages and maintained under controlled temperature conditions in a 12 h light- dark cycle. They were given ad libitum access to commercially available mouse chow and water.

Studying muscle relaxant effect

Two groups of mice (6 mice each) were used. The first group was given 100 mg extract/ kg.b.w intraperitoneally, while the second group was given distilled water by the same route as control. Muscle relaxation was determined by test delatraction. By this test, the mouse was hung by its forepaws on a thin wire placed over a bench. Normal mice pull themselves on the wire almost immediately with the aid of their hind paws. Failure of the mice to pull themselves or drop down three consecutive times in sixty second means they have myorelaxation and/ or motor incoordination (Elisha et al., 1988).

Studying the effect of potentiation of pentobarbital sleeping

Two groups of mice zone varying between 2-15 were given 100 mg of *P. harmala* extract/kg and distilled water, respectively. Thirty minutes later, sodium pentobarbital of 50 mg/kg was administrated intraperitoneally. The animals were observed at intervals of minutes. They were placed on their backs and touched slightly with a glass rod. The period of losing righting reflex was taken as sleeping time (Al Maliki and Elisha, 1985; Clark, 1989).

Determination of the minimal inhibitory concentration

The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined by the macro broth dilution assay method (Motamedi et al., 2010). A five-fold serial dilution of Mueller Hinton agar broth (50, 25, 12.5, 6.25, 3. 125) was used. The test organisms were incubated for 4 h to obtain concentration of 5 colony forming unit (CFU)/ml. Later, 50 micro of the inoculated

Table 1. Diameter of inhibition zone produced by the extract of *P. harmala*.

Treatment	Con.mg	Diameter of inhibition zone mm	
<i>Peganum harmala</i>		<i>S. aureus</i>	<i>E. coli</i>
	5	0	0
	10	3-15	3-12
Ampicillin	10	14	14

Table 2. Minimum inhibitory concentration (MIC) of *P. harmala* extract against *S. aureus*.

	0.3	0.62	1.25	2.5	5
<i>P. harmala</i> concentration mg/ml	0	11.66	29.10	20.1	23.50
	0	5.87	16.63	41	0.1

Table 3. Minimum inhibitory concentration (MIC) of *P. harmala* against *E. coli*

	0.3	0.62	1.25	2.5	5
<i>P. harmala</i> concentration mg / ml	0	9.75	28.10	20.1	1.01
	0	5.84	6.70	27.01	6.5.10

Table 4. Muscle relaxant effect of aqueous extracts of *P. harmala*.

Group	Muscle relaxation	% of effect
The isolation control	0/6	0
<i>P. harmala</i>	0/6	100

both was transferred into each dilution tube. Other dilution tubes were incubated at 37°C. The MIC was determined as the lowest concentration of *P. harmala*. It completely inhibited the growth of test organisms. The MBC was determined by culturing one standard drop of the tubes with no growth on Mueller Hinton broth and incubated at 37°C for 24 h. The control tubes were prepared with Gentamicin and free sterile solution of Mueller Hinton Broth.

Statistical analysis

Values reported are means \pm SD. Results were statistically analyzed using the t-test, with P value less than 0.05 considered significant (Sorlie, 1995).

RESULTS

It was revealed that the extract of *P. harmala* (10 mg /ml) exhibited border spectrum as well as greater activity against *E. coli* and *S. aureus* with inhibition zone varying between 2-15 mm. Table 1 shows that the water extract of *P. harmala* exerted antibacterial activity against *E. coli* at 10 mg/ml; the mean of the inhibitory zone is 3-12 mm at 20 mg/ml. It exerted also antibacterial activity against *S. aureus*. The mean of the inhibitory zone is 2-9 mm. Table 2 shows the minimal inhibitory concentration of *P. harmala* against *S. aureus*. Table 3 shows the minimal

inhibitory concentration of *P. harmala* extract against *E. coli*. Table 4 shows that all animals injected with the 100 mg/kg.b. w of aqueous extract of seeds of *P. harmala* showed myorelaxation or incoordination; so all the animals dropped down from the wire 3 consecutive times in 60 s. Table 5 shows that *P. harmala* prolonged the sleeping effect of pentobarbitol sodium.

DISCUSSION

The antibacterial activity of *P. harmala* may contribute to the flavonoid of dichloro. A biochemical analysis of *P. harmala* showed that this plant contains isoflavonoids with different side chains; flavonoids possess antibacterial activity against many bacterial species (Seyyednejad and Motamedi, 2010; Mahmoudian et al., 2002). *P. harmala* showed high antibacterial activity. This result is agreement with Arshad et al. (2008) and Moloudizargari et al. (2013) who found that *P. harmala* inhibits the growth of the tested bacteria. The inhibition produced by the plant extract against bacteria depends upon various extrinsic and intrinsic *S. aureus* and assigned as a source of antibacterial compounds against *S. aureus* and *E. coli*. Harmine has previously been identified as possessing antibacterial activities against several species of bacteria.

Table 5. Hypnotic effects of aqueous extracts of *P. harmala* 100 mg/kg 30sec after 50 mg/kg of pentobarbital sodium.

Group	Time of sleep/minute
Control	7.33±1.067
<i>P. harmala</i>	111.15±3.017*

*Significant P<0.05.

Shahverdi et al. (2005) who reported the significant activity of smoked dichloromethane extract of *P. harmala* seed against several species of Gram positive bacteria including *Bacillus subtilis* and harmine found it very effective against the Gram negative bacterium, *Proteus vulgaris*. This result is in agreement with Benbott et al. (2012) who report *P. harmala* is a potential source of antibacterial drug against various pathogenic bacteria, and disagreement with Mohamedeen et al. (2015) who report ethanol extract of *P. harmala* did not affect *E. coli* and n-butanol; and chloroform of *P. harmala* seed showed good antibacterial activity. This result is also in agreement with Fatma et al. (2016) who report that the flavonoids extract of *P. harmala* is useful to treat *S. aureus*. Muscle relaxant effect or incoordination caused by *P. harmala* are related to its motor dysfunction effect previously described by Al Maliki and Elisha (1985). The authors show that mice injected with aqueous extraction of *P. harmala* show sluggish movement, unstable walking or loss of balance. While hypnotic effect of *P. harmala* may be related to its activities on central neuro-transmission via its interference with ionic exchange (Laks and Pruner, 1989). This result is in agreement with Mina et al. (2015) who report that *P. harmala* possesses various pharmacological activities such as analgesic. These results will encourage us to undertake further studies regarding the isolation and characterization of the active principle present in the active extract. Moreover clinical studies are required to understand the mechanisms along with the actual efficacy of these herbal extracts in treating various infectious.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

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