NeuroPharmac Journal

Journal homepage: www.neuropharmac.com Original Article

Peganum harmala seeds: evaluation of antiasthmatic effect by using clonidine induced mast cell degranulation

Aslam Pathan [a,*], Gautam Vadnere [a]

[a] Smt. S. S. Patil College of Pharmacy, Chopda 425107, North Maharashtra University, Jalgaon, Maharashtra, India.

Email: aslamkhan_14@yahoo.com *Corresponding author

ABSTRACT

Aim of study: The aim of study was to evaluate antiasthmatic activity of various extracts of *Peganum harmala* seeds to validate its traditional use.

Materials and methods: In the present study petroleum ether, ethanol and water extract of *Peganum harmala* seeds at the doses of 25-100 mg/kg i. p. was evaluated for antiasthmatic activity using clonidine induced mast cells degranulation in mice.

Results: Treatment group of PEEE (*Peganum harmala* ethanol extract) exhibited significant mast cell stabilizing potential at 100 mg/kg i.p 68.85±1.30 as compared to control group. Percent protection offered by PHEE at 100 mg/kg was 20.28% and was of statistical significance as compared to PHPEE and PHWE.

Conclusion: It can be concluded that the ethanol extract of *Peganum harmala* (PHEE) may be useful in management of asthma.

Keywords: *Peganum harmala*, asthma, mast cell degranulation

INTRODUCTION

Harmal (*Peganum harmala*) is a plant of the family Zygophyllacea, native from the eastern Mediterranean region east to India. It is also known as Wild Rue or Syrian Rue because of its resemblance to plants of the rue family. It is a perennial plant which can grow to about 0.8 m tall. But normally it is about 0.3 m tall [1]. The roots of the plant can reach a depth of up to 6.1 m, if the soil it is growing in is very dry, It blossoms between June and August in the Northern Hemisphere [1]. The flowers are white and are about 2.5–3.8 cm in diameter. The round seed capsules measure about 1–1.5 cm in diameter have three chambers and carry more than 50 seeds [2]. *Peganum harmala* was first planted in the United States in 1928 in the state of New Mexico by a farmer wanting to manufacture the dye "Turkish Red" from its seeds [1]. Since then it has spread invasively to Arizona, California, Montana, Nevada, Oregon, Texas and Washington. Because it is so drought tolerant, African rue can displace the native saltbushes and grasses growing in the salt-desert shrub lands of the Western U.S. [2,3] .

MATERIAL AND METHOD

Plant material

Seeds of *Peganum harmala* were purchased from traditional medicinal seller Dagadu merchants from Nashik, Maharashtra, India. seeds was authenticated by Botanical Servey of India , Pune, Maharashtra, India. Woucher specimen was deposited in the herbarium for further use.

Extraction

Dried and coarsely powder of *Peganum harmala* seeds (100 g) was defatted with petroleum ether and the marc remaining was extracted successively by petroleum ether, ethanol and water in different Soxhlet extractor. Solvent was evaporated in rotary evaporator under reduced pressure.

Animals

Swiss albino mice of either sex weighing (25-30 g) were housed under standard laboratory condition of temperature (25 ± 2°C) and 12/12 h light/dark cycle. The animals had free access to food and water. The Animal Ethical Committee of the Institute approved all the protocols of the study.

Drugs and Chemicals

5% polyethylene glycol (PEG-400), saline solution, buffer medium, Sodium chromoglycate, RPMI-1640 were purchased from Himedia, India. Clonidine (Alembic), petroleum ether (60-80°C), ethanol (95%). All Chemical and reagents were of analytical grade.

Acute toxicity study

Acute oral toxicity study was performed as per OECD 423 guideline. Extract was administered up to the maximum dose of 2000 mg/kg and animals were observed for mortality. [4]

NeuroPharmac Journal

Journal homepage: www.neuropharmac.com Original Article

Clonidine induced mast cell degranulation in mice

Mice were divided into five groups, five animals in each group. A three-day drug treatment schedule was followed.

Group-I serves as control receives vehicle only (5 % PEG-400, 1ml / kg, i.p.).

Group-II receives standard drug disodium cromoglycate (DSCG, 200µg/kg, i.p.).

Group-III, IV and V was treated with test extract 25, 50 and 100 mg/kg, i.p. respectively.

On day fourth, each animal was injected with 4 ml/kg, 0.9 % saline solution, into peritoneal cavity. By gentle massage, peritoneal fluid was collected after 5 mins and transferred into siliconised test tubes containing 7-10 ml RPMI-1640 buffer medium (pH 7.2-7.4). This solution was then centrifused at 400-500 RPM. Pellet of mast cell was washed with same buffer medium twice by centrifugation, discarding supernatant. These cells were challenged with clonidine (50 µg), incubated at 37°C in a water bath for 10 mins. Followed by staining with 1% toluidine blue and observed under microscope (10X). Total 100 cells were counted from different visual area. Percent protection against degranulation was calculated. [5,6]

Statistical analysis

The data were presented as mean ± Standard error mean. The statistical significance between the groups has been tested by analysis of variance followed by Dunnett's test. A $P < 0.05$ were considered as significant.

Table 1: Effect of PHPEE on Mast cells degranulation in mice

Group	Treatment	Dose	No. of Mast cell degranulation
I	Control	1 ml / kg, i.p	86.37±1.62
II	DSCG	200µg/ kg, i.p	15.10±1.12
III	PHPEE	25mg / kg, i.p	79.33±1.56
IV	PHPEE	50mg/ kg, i.p	78.68±1.48
V	PHPEE	100mg/ kg, i.p	77.58±1.05

n=5, values are expressed in mean±SEM
Control = Vehicle (5 % PEG-400, 1ml / kg, i.p)
*p< 0.05 compared with control group (ANOVA followed by Dunnett's test)

PHPEE– *Peganum harmala* Petroleum ether extract

Table 2: Effect of PHEE on Mast cells degranulation in mice

Group	Treatment	Dose	No. of Mast cell degranulation
I	Control	1 ml / kg, i.p	86.37±1.62
II	DSCG	200µg/ kg, i.p	15.10±1.12
III	PHEE	25mg / kg, i.p	74.66±1.06
IV	PHEE	50mg/ kg, i.p	72.30±1.20
V	PHEE	100mg/ kg, i.p	68.85±1.30

n=5, values are expressed in mean±SEM
Control = Vehicle (5 % PEG-400, 1ml / kg, i.p)
Statistically non significant data (ANOVA followed by Dunnett's test)

PHEE– *Peganum harmala* Ethanol extract

Table 3: Effect of PHWE on Mast cells degranulation in mice

Group	Treatment	Dose	No. of Mast cell degranulation
I	Control	1 ml / kg, i.p	86.37±1.62
II	DSCG	200µg/ kg, i.p	15.10±1.22
III	PHWE	25mg / kg, i.p	81.30±1.22
IV	PHWE	50mg/ kg, i.p	80.50±1.31
V	PHWE	100mg/ kg, i.p	79.74±1.27

n=5, values are expressed in mean±SEM
Control = Vehicle (5 % PEG-400, 1ml / kg, i.p)
Statistically non significant data (ANOVA followed by Dunnett's test)

PHWE– *Peganum harmala* Water extract

NeuroPharmac Journal

Journal homepage: www.neuropharmac.com

Original Article

Figure 1: Effect of PHPEE on Mast cells degranulation in mice

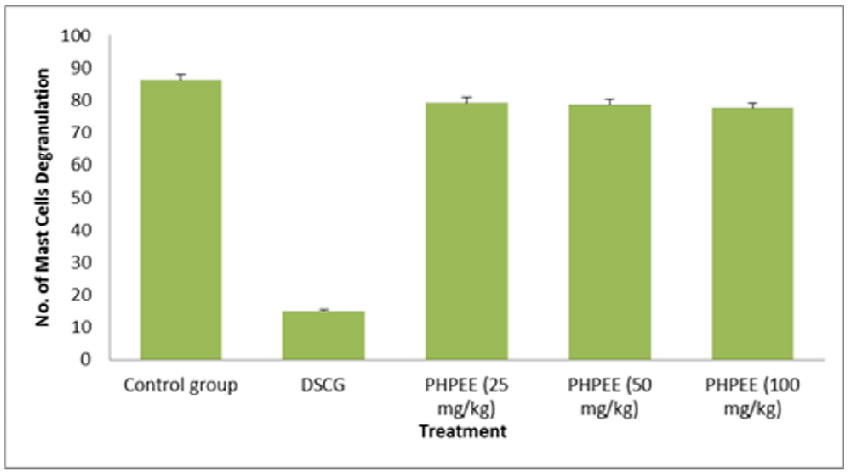


Figure 2: Effect of PHEE on Mast cells degranulation in mice

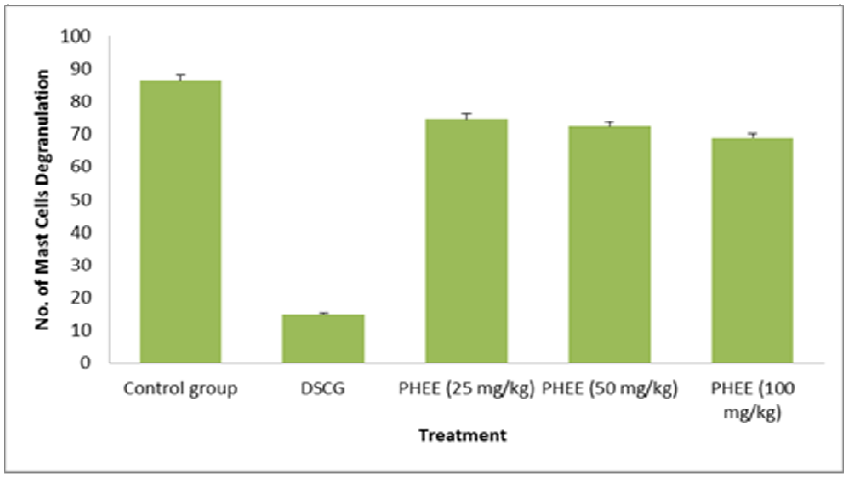
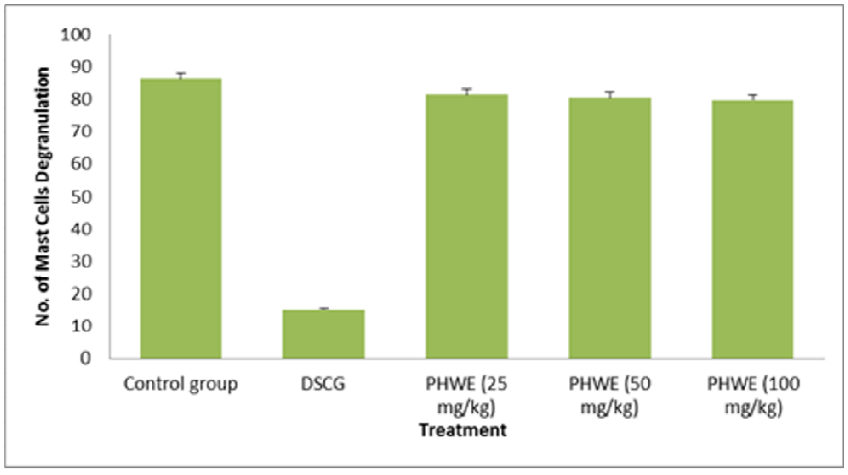


Figure 3: Effect of PHWE on Mast cells degranulation in mice



RESULTS

Acute toxicity test

The LD50 value of *Peganum harmala* seeds extracts when given intraperitoneally and tested in albino mice was found to be more than 2000 mg/kg body weight.

Clonidine induced mast cell degranulation

Treatment group of PHEE (*Peganum harmala* seeds ethanol extract) exhibited significant mast cell stabilizing potential at all three doses (100 mg/kg i.p) 68.85±1.30 (Table 2 and figure 2) as compared to ethanol and water extracts as well as control group. Percent protection offered by PHEE at 100 mg/kg was 20.28 % and was of statistical significance as compared to *Peganum harmala* Petroleum ether extract (PHPEE) and *Peganum harmala* water extract (PHWE). PHPEE at 100 mg/kg shows 77.58 ± 1.05 mast cells degranulation (Table 1 and Figure 1) while PHWE at 100 mg/kg shows 79.74 ± 1.27 mast cells degranulation (Table 3 and Figure 3) which was not significant.

DISCUSSION

Mast cells are the principal store of histamine and other many chemical mediators responsible for the pathogenesis of asthma. Mast cells are necessary for the development of allergic reactions, through cross liking of their surface receptor for IgE, leading to degranulation and the release of vasoactive, pro-inflammatory and nociceptive mediators. Released mediators from mast cells, each having more than one potent effect on airway inflammation.[7] present study revealed that ethanolic extract of *Peganum harmala* seeds shows significant role in mast cell protection against clonidine induced degranulation, while petroleum ether and water extract of *Peganum harmala* seeds was non-significant.

CONCLUSION

Ethaniolic extract of *Peganum harmala* seeds offers mast cell protection against degranulation as compared to petroleum ether and water extracts. It leads to conclusion that PHEE may be useful in the management of asthma.

REFERENCES

1. Peganum genus: recognition and control of African rue in Nevada [02]. 2008 Feb Available from:http://www.cdfa.ca.gov /phpps/ipc/ weedinfo / peganum.htm.
2. Erowid Syrian Rue Vaults: Smoking Rue Extract / Harmala. [09] 2008 Dec. available from: http://www.erowid.org/ plants / syrian_rue/syrian_rue_info9.shtml.
3. Pathan Aslam R, et al. *Peganum harmala*: A Phyto-pharmacological Review. Inventi Rapid: Planta Activa, 2012(4):1-2, 2012.
4. Organization of Economic Co-operation and Development (OECD). The OECD Guidelines for Testing of Chemical: 423 Acute Oral Toxicity, France, 2001
5. Gupta P, Srimal R. Passive cutaneous anaphylactic inhibitory and mast cell stabilizing activity of Coleonol and its derivatives. *Indian J of Pharmacol* 1994; 26: 150 – 152.
6. Lakdawala AD, Dadkar NK, Dohadwalla AN. Action of clonidine on the mast cells of rats. *J. Pharm. Pharmacol.* 1980; 32: 790-791.
7. Theoharis C, David E. Critical role of mast cells in inflammatory disease and the effect of acute stress. *J Neuropharmacology* 2004; 146: 1-12.