Journal of Applied Pharmaceutical Science Vol. 5 (05), pp. 052-055, May, 2015
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2015.50510
ISSN 2231-3354

Phytochemical screening and evaluation of analgesic, anti-inflammatory activities of *Peganum harmala Linn*., seeds in rodents

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ARTICLE INFO

Article history:
Received on: 26/03/2015
Revised on: 11/04/2015
Accepted on: 07/05/2015
Available online: 27/05/2015

Key words:
Peganum harmala, Zygophyllaceae, analgesic, anti-inflammatory, carrageenan.

ABSTRACT

The objective of this research work is to carryout the phytochemical screening and evaluate the analgesic, anti-inflammatory activities of *Peganum harmala* Linn., seeds. In this study different extracts of *Peganum harmala (Linn)* seeds were evaluated for analgesic and anti-inflammatory activities using glacial acetic acid induced writhing and carrageenan induced rat paw edema models respectively. For analgesic and anti-inflammatory activities aspirin and diclofenac were used as standard drugs respectively. The ethyl acetate extract showed significant analgesic and anti-inflammatory activities, thus it can be considered as a potential candidate for analgesic and antiinflammatory activities. The presence of alkaloids, steroids, flavonoids in ethyl acetate extract of *Peganum harmala (Linn)* seeds could be attributed for the claimed analgesic and anti-inflammatory activities.

INTRODUCTION

Inflammation is the response to injury of cells and body tissues through different factors such as infections, chemicals, thermal and mechanical injuries (Oyedapo *et al*., 2008). Most of the antiinflammatory drugs presently available are potential inhibitors of cyclooxygenase (COX) pathway of arachidonic acid metabolism which produces prostaglandins. Prostaglandins are the mediators of inflammation. Inflammation is accompanied by redness, swelling, pain (analgesia). Inhibition of prostaglandins synthesis is essential to treat inflammation. Hence, for treating inflammatory diseases, analgesic and anti-inflammatory agents are required (Anilkumar, 2010). Nonsteroidal anti-inflammatory drugs (NSAIDs) are among the most widely used medications due to their efficacy for a wide range of pain and inflammatory conditions (IMS Health, 2005). But, the long-term administration of NSAIDs may induce gastro-intestinal ulcers, bleeding and renal disorders due to their nonselective inhibition of both constitutive (COX-1) and inducible (COX-2) isoforms of the cyclooxygenase enzymes (Robert, 1976; Peskar, 1977; Tapiero *et al*., 2002). Therefore, there is a need to develop new anti-inflammatory and analgesic drugs with potent activity, less side effects are being searched all over the world as alternatives to NSAIDs and opiates (Dharmasiri *et al*., 2003; Kumara, 2001). Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects.

Plants and plant extracts have been used since the dawn of civilization by mankind. The uses of ethnobotanical preparations for various reasons justified or not, are still continued by various cultures all over the world. Considering structural and biological diversity of terrestrial plants, they offer a unique renewable resource for the discovery of potential new drugs and modern medicine has developed a rational strategy for drug discovery which involves the study of plants and plant materials based on their ethnobotanical usage (Cordell *et al*., 1991). Natural products are sources of active compounds that may be useful in the development of new and potent drugs.

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Peganum harmala L. belongs to the family of Zygophyllaceae (Qazan, 2009). It is a wild growing flowering plant. It is also called African rue, Syrian rue and wild rue. The plant is widely distributed in predesertic regions of south-east Morocco, North Africa and the Middle East (EL-Bahri *et al*., 1991). Literature survey revealed that *Peganum harmala* L., shows different pharmacological activities like antioxidant (Dickson *et al*., 2006), antileishmanial (Di Giorgio *et al.,* 2004), antihemosporidian (Fan *et al*., 1997), antihistaminic (Gholamreza Asghari *et al*., 2002), vasorelaxant (Berrougui *et al*., 2006), antitumor (Lamchouri *et al*., 1999), wound healing (Derakhshanfar *et al*., 2010), antiplasmodial (Astulla *et al*., 2008), MAO inhibition (Herraiz *et al*., 2010), DNA topoisomerase 1 inhibition (Sobhani *et al*., 2002), myeloperoxidase inhibition (Bensalem *et al*., 2014), antibacterial and antitubercular activity (Pradeep Kumar *et al*., 2014) etc. However, *Peganum harmala (Linn)* seeds have not been investigated for analgesic and antiinflammatory activities. Hence, this study was carried out to evaluate the potent bioactive constituents for analgesic and anti-inflammatory activities in *Peganum harmala (Linn)* seeds.

Peganum harmala Linn., Seeds

MATERIALS AND METHODS

The Seeds of *Peganum harmala (Linn)* were collected from the local areas of Dharwad in Karnataka and were authenticated by Dr. S. S. Hebbar, Department of Botany, Government Pre-university College Dharwad. A voucher specimen (No- SETCPD/Pharmacog/Herb/2013/14) has been deposited in the Herbarium of Department of Pharmacognosy, S.E.T.'s College of Pharmacy, Dharwad, Karnataka.

The Seeds of *Peganum harmala (Linn)* were shade dried and finely powdered to particle size (#) 40. About 300g of dried powder was subjected to continuous hot soxhlet exhaustive extraction with petroleum ether, chloroform, ethyl acetate and ethanol (95%). Aqueous extract was also obtained by cold maceration of the drug (300 g) with 2% chloroform water. After the extraction, the extracts were filtered and concentrated under reduced pressure using a rota evaporator. The yield of petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extract was found to be 9.45 g (3.15 % w/w), 8.4 g (2.8 % w/w), 16 g (5.33 % w/w), 22 g (7.33 % w/w) and 20 g (6.66 % w/w) respectively. All the extracts were kept in a dessicator for drying.

Pharmacological activity

The pharmacological activities of synthesized compounds were investigated in albino rats and mice. Rats were used for acute anti-inflammatory and chronic anti-inflammatory activities. Mice were used for analgesic activity. All animal experiments were approved by institutional animal ethical committee. All the animals were stabilized to laboratory conditions before starting the experiments.

Acute toxicity

The acute toxicity test was carried out according to the Organization for Economic Co- operation and Development (OECD) guidelines (OECD/ OCDC, 2000) to establish the effective dose of all the synthesized and extracted compounds.

Anti-inflammatory activity

Anti-inflammatory activity was evaluated using the well known Carageenan induced rat paw oedema model (Winter *et al*., 1962) using groups of six animals each. A freshly prepared aqueous suspension of carrageenan (1.0% w/v, 0.1 ml) was injected in the sub planter region of right hind paw of each rat. One group was kept as control and the animals of the other group were pre-treated with the test extract at a dose of 200 mg/ kg body weight of rat, 1 h before the carageenan treatment. The volume was measured before and after carageenan treatment at the 30 min. interval with the help of digital plethysmometer. Standard drug used was diclofenac at a dose of 200 mg/kg body weight of rat.

Analgesic activity

Twenty four hours prior to actual testing a large number of mice (20-25 gm) received intraperitoneally (i.p) 10 ml/kg of 0.6% glacial acetic acid. Animals were observed for writhing movements. Only those showing one or other type of writhing movements (positive responders) were chosen for the test on the next day. On the test day the responders received compounds half an hour prior to glacial acetic acid challenge (Koster *et al*., 1959). Extracts were given at a dose of 200 mg/kg orally. Standard drug used was aspirin at a dose of 30 mg/kg body weight of mice.

RESULTS AND DISCUSSION

Phytochemical screening

Phytochemical screening revealed the presence of alkaloids, flavonoids in the alcoholic extract and alkaloids, flavonoids and steroids in the ethyl acetate extract. The results are shown in Table-2. Physico chemical parameters for the *Peganum harmala* Linn., seeds are shown in Table-1.

Hence, the presence of alkaloids, flavonoids and steroids in the ethyl acetate extract could be attributed for observed significant analgesic (Jain *et al.,* 2011; Parveen *et al.,* 2007) and anti-

inflammatory (Sakat *et al.*, 2010; Nagore *et al.*, 2010) activities. However, research work is under progress to confirm the exact mechanism of action and to elucidate the structure of bioactive principle for the claimed analgesic and anti-inflammatory activities.

Table 1: Physico-chemical parameters of *Peganum harmala* Linn., seeds.

Sl No	Parameter	Determined values in %w/w
1	Alcohol soluble extractives	7.88
2	Hydro-alcoholic extractives	14.11
3	Water soluble extractives	18.73
4	Ether soluble extractives	6.98
5	Total ash value	7.64
6	Acid insoluble ash	1.52
7	Water soluble ash	3.59
8	Sulfated ash	8.33
9	Moisture content	2.4

Table 2: Preliminary phytochemical analysis of various extracts of *Peganum harmala* Linn., Seeds.

Phytoconstituent	Petroleum ether	Chloroform	Ethyl acetate	Alcohol	Aqueous
Alkaloids	-ve	+ve	+ve	+ve	-ve
Steroids	-ve	-ve	-ve	+ve	-ve
Carbohydrates	-ve	-ve	+ve	+ve	+ve
Phenolic	-ve	-ve	-ve	-ve	-ve
Flavonoid	-ve	-ve	+ve	+ve	-ve
Glycoside	-ve	-ve	-ve	-ve	-ve
Tannins	-ve	-ve	-ve	-ve	-ve

+ve= Present -ve= Absent

Table 3: Anti-inflammatory activity screening of *Peganum harmala* Linn seeds.

Extract/ Compound	Percentage inhibition of Paw oedema		
	1hr	3hr	5hr
Control	-	-	-
Aqueous Extract	20.08	31.43	22.42
Alcohol Extract	36.10	64.38**	67.95**
Chloroform Extract	25.40	48.53*	42.63*
Pet-ether Extract	19.89	32.28	36.44
Ethyl acetate Extract	38.13	70.34**	69.78**
Diclofenac	42.12	79.53	76.68

Animals were dosed 200 mg/kg. Data were analysed by ANOVA. Followed by Dunnet's test. **P<0.001. P < 0.05. Values are mean ± SEM, of six animals in each group.

Table 4: Analgesic activity screening of *Peganum harmala* Linn seeds.

Extract/ Compound	No. of wriths in 15 min.	± SEM	% Protection
Control	44	1.713	--
Aspirin	13	1.317	70.45
Aqueous Extract	36	1.163	18.19
Alcohol Extract	20	1.392	54.55**
Chloroform Extract	27	1.065	38.64*
Pet-ether Extract	30	1.447	31.82
Ethyl acetate Extract	17	1.287	61.37**

Animals were dosed 5 mg/kg. Data were analyzed by ANOVA. Followed by Dunnet's test. **P<0.001. P<0.05. Values are mean ± SEM, of six animals in each group.

CONCLUSION

The present study provides an evidence for the analgesic and anti-inflammatory activities of *Peganum harmala (Linn)* seeds. Aspirin and diclofenac were used as standard drugs for screening the analgesic and antiinflammatory activities respectively. Aspirin used as standard drug for analgesic activity acts by obtunding of peripheral pain receptors and prevention of PG- mediated sensitization of nerve endings (Tripathi, 2013) and the diclofenac used as standard drug for screening the anti-inflammatory activity act by inhibiting the prostaglandin synthesis and specially it is COX-2 selective (Tripathi, 2013). As the analgesic and anti-inflammatory activities results of the ethyl acetate extract studied are close to those of aspirin and diclofenac, the bioactive principles present in the extract may be having the mechanism of action similar to that of the tested standard drugs. However research is under progress to confirm the exact mechanism of action and to elucidate the structure of bioactive principles for the claimed analgesic and anti-inflammatory activities.

The present study may form the basis for the selection of plant species for further investigation in potent bioactive compounds for analgesic and anti-inflammatory activities.

ACKNOWLEDGEMENT

The authors are thankful to Shri. H. V. Dambal, President, Dr. T. M. Aminabhavi, Research Director, S. E. T's College of Pharmacy for providing the facilities to carry out this research work. The authors are grateful to Mr. Ravi Nadiger and Mr. Vijaybhaskar Joshi for their technical assistance**.**

CONFLICT OF INTEREST

Conflict of interest declared none.

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How to cite this article:

Pradeep Kumar M.R., Joshi S.D., Kulkarni V.H., Chetan Savant. Phytochemical screening and evaluation of analgesic, anti-inflammatory activities of *Peganum harmala Linn*. seeds in rodents. J App Pharm Sci, 2015; 5 (05): 052-055.