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Research Article

Pharmacological Screening of Ethanolic Extracts of Emblica Officinalis Gearth Plant on Animals

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ABSTRACT

The main objective of the present work is to find out good pharmacological activities in herbal source with their preliminary phytochemical study, and also it is aimed to investigate of ethanol, and aqueous extracts of dried stem of plant Emblica Officinalis Gearth against with Anti-inflammatory and in rats, Analgesic activity in mice and antioxidant in invitro methods. Normally herbal products are free from side effects/adverse effects and they are low cost medicines, which will be beneficial for human being. The main objective of this work is to find active constituents which are potent Anti-oxidant, Anti-inflammatory, analgesic having no or minimum side effects from indigenous plants for the therapeutic management.

Keywords: Analgesic, Anti-oxidant, Anti-inflammatory, Emblica Officinalis Gearth

INTRODUTION

India is a source of medicinal plants. These medicinal plants are boon for the human but we are unknown for their natural use and effect. Plant drugs are the major source for the treatment of diseases.[1,2] The medicinal plants were used in traditional medicine on the basis of experience and practice. The search for better and safer ways of relieving pain is what led us to herbology. It would seem most people agree with the importance of pain relief, for these analgesic herbs are some the best loved and most popular remedies. Some uses for analgesic herbs are: arthritis pain, headaches, toothaches, sore muscles, lower back pain and neuralgia. It is believed that bioactive compounds from plant foods may have health beneficial effects and reduce the risk of chronic inflammatory diseases. Pharmaceutical drugs may not be your only path to pain relief [3].

Natural pain treatments like herbal medicine, in which parts of a plant are used medicinally to treat health problems is an increasingly popular way to manage pain as well. Though research on herbal remedies is still in its early phases, many herbs are thought to provide pain management and decrease inflammation [3, 4]. However, it's important to exercise caution. Almost all herbs and plants are anti-inflammatory to some degree; some with a more pronounced action than others where the effect is secondary yet still an important part of the overall synergistic effect of the herb. Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness.

Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials with a limited number

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of antioxidants detected no benefit and even suggested that excess supplementation with certain putative antioxidants may be harmful. [2,5,6] Antioxidants also have many industrial uses, such as preservatives in food and cosmetics and to prevent the degradation of rubber and gasoline.

MATERIALS AND METHODS

Collection and Authentication of Plant Material: The specimen copy (Herbarium) of selected plant collected in month of July, were collected from the outfield of Nimar Region, MP, India in February-March 2013. The plant materials were identified and authenticated by Dr. Rao, HOD, Botany and Plant Anatomy, Khargone, India. Voucher specimens (NIP/13/009) of the collected plant samples were deposited in the Department of Pharmacognosy, Nimar Institute of Pharmacy, Dhamnod, India for future reference.

Preparation of extract:

The stem of *Emblica Officinalis* were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve no. 30 and stored in an airtight container for further use.

Solvent For Extraction:

- Petroleum Ether (60-80o C)
- Alcohol (95% v/v)
- Distilled water with chloroform (0.25%)

Extraction Procedure: [6,7]

The dried powders of stem of *Emblica Officinalis* were defatted with petroleum ether (60-80ºc) in a Soxhlet Apparatus by continuous hot-percolation. The defatted powder material (marc) thus obtained was Further extracted with ethanol (95% v/v) with same method and fresh powder used for aqueous extraction by Cold maceration method. The solvent was removed by distillation under low pressure and evaporation. The resulting semisolid mass was vacuum dried by using rotary flash evaporator. The resultant dried extracts were used for further study.

Procurement of Experimental Animals

Swiss albino mice (20-25 g) and albino Wister rats (150-200 g) of either sex and of approximate same age are used in the present studies. Animal were procured from Veterinary college Mahu, Indore, India. The animals were fed with standard pellet diet (Poshak Aahar ltd. Indore) and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under alternate cycle of 12 hours of darkness and light. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The animals were fasted for at least 12 hours before the onset of each activity. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC No.-NIP/13/005) after scrutinization. The animals received the drug treatments by oral gavage tube.

Acute Oral Toxicity Study [8]

The lethal dose (LD 50) of the alcoholic and aqueous extract of dried stem of *Emblica Officinalis* was determined by OECD guideline (423 guideline). The LD50 of alcoholic extract and aqueous extract was found to be 1500 mg/kg therefore the ED50 value is 200mg/kg.

Evaluation of Anti-Inflammatory Activity [8,9,10]

Ethanolic and Aqueous extract of plant *Emblica Officinalis* was tested for Anti-Inflammatory activity against carrageenan induced paw-edema in rats. Both the extracts having anti-inflammatory activity against the carragenan induce paw oedema in rats. The reductions of paw oedema of rats are compared with the standard drug i.e. indomethacin were approved by Institutional Animal Ethics Committee (IAEC No.-NIP/13/006) after scrutinization. The

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animals received the drug treatments by oral gavage tube.

Treatment Design

Animals are divided into 6 groups. Swiss albino mice (20-25 g)

- Group I:- Normal control (Carrageenan 1%w/v)
- Group II:- Positive control (indomethacin 10mg/kg, i.p.)
- Group III:- Ethanolic extract (300mg / kg)
- Group IV:- Ethanolic extract(600mg / kg)
- Group V:- Aqueous extract (300mg / kg)
- Group VI:- Aqueous extract (600mg / kg)

The paw-volume measured at 0, 30, 60, 120, 180 mins after carrageenan injection using the plathysmometer. The animals of group III, IV, were pretreated with ethanolic extracts and V, VI with aqueous extracts, 60 minutes before the administration of Carrageenan. Acute inflammation was produced by the sub plantar administration of 0.1% carrageenan in normal saline in the left paw or rats. Inhibition of swelling is compared with that of control group.(Kulkarni S.K.-2005).

The % inhibition of paw-edema is calculated by:

$$\% \text{ inhibition of paw edema} = \frac{C - T}{C} \times 100$$

Where,

C = increase in paw-volume of control group

T = increase in paw-volume after administration of extracts.

Evaluation of Analgesic Activity [11,12,13]

Alcoholic and aqueous extract of plant *Emblica Officinalis* was evaluated for analgesic activity against acetic acid-induced writhing in mice.

Treatment design

Animals are divided into 6 groups. Albino mice weighing between 150-200 gm.

- Group I :- Normal control (Acetic acid 3%v/v)
- Group II:- Positive control (Pentazocine 5mg / kg)
- Group III:- Ethanolic extract (300mg / kg)
- Group IV:- Ethanolic extract(600mg / kg)
- Group V:- Aqueous extract (300mg / kg)
- Group VI:- Aqueous extract (600mg / kg)

Acetic acid is administrated in the dose of 30mg/kg or 0.3 ml to the first group (normal control) and number of writhing responses (constriction of abdomen, twisting of trunk and extension of hind limbs) are recorded for a period of 10 mins. The animals of group III, IV, were pretreated with ethanolic extracts and V, VI with aqueous extracts, 15 minutes before the administration of Acetic acid. Reduction in number of writhe is taken as analgesic activity and compared with that of control group.(Kulkarni S.K.-2005)

Evaluation of Antioxidant Activity

Ferric Thiocyanate (FTC) method [14]

The standard method as described by Kikuzaki and Nakatani (12) was used. A mixture of 4.0 mg of plant extract in 4 ml of absolute ethanol, 4.1 ml of 2.52% linolenic acid in absolute ethanol, 8.0 ml of 0.05 M phosphate buffer (pH 7.0), and 3.9 ml of water was placed in a vial with a screw cap and then placed in a dark oven at 40 ¡C. To 0.1 ml of this solution were added 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after the addition of 0.1 ml of 0.02 M ferrous chloride in 3.5% HCl to the reaction mixture, the absorbance of red color was measured at 500 nm every 24 h until one day after the absorbance of control reached its maximum. Butylated hydroxy toluene (BHT) and α-tocopherol were used as positive controls, while a mixture without a plant sample was used as the negative control.

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RESULTS

Table 1. Anti-inflammatory activity

Group	Treatment Design	Dose	Paw volume in ml as measured by mercury displacement At				
			0 min	30 min	60 min	120 min	180 min
I	Normal control+ (Carrageenan)	0.1ml	0.45 ± 0.01	0.452 ± 0.02	0.63 ± 0.02	0.66 ± 0.02	0.9 ± 0.08
II	Standard (indomethacin) +carrageenan	10mg/kg + 0.1 ml	0.25 ± 0.02	0.28 ± 0.03	0.35 ± 0.02	0.35 ± 0.02	0.28 ±0.01
III	Ethanolic extract	(300mg/kg)	0.25 ± 0.02	0.3 ± 0.04	0.35* ± 0.02	0.41* ± 0.03	0.51 ± 0.06
IV	Ethanolic extract	(600mg/kg)	0.18 ± 0.03	0.28 ± 0.04	0.40* ± 0.04	0.5* ± 0.02	0.38* ± 0.05
V	Aqueous extract	(300mg/kg)	0.21 ± 0.03	0.33 ± 0.02	0.45 ± 0.02	0.56 0 ±.03	0.45 ± 0.04
VI	Aqueous extract	(300mg/kg)	0.16 ± 0.03	0.25 ± 0.0244	0.366* ± 0.02	0.35* ± 0.04	0.34* ± 0.03

P values: * * P< 0.01; * P <0.05.
Values are expressed in mean ±SEM, n=6 animals in each group.
One way ANOVA followed by DUNNETT'S, multiple comparison tests

Table 2. Analgesic activity

Group	Treatment design	Dose	Mean No. of writhes (In 10 mins.)
I	Normal control (Acetic acid)	1%v/v	18.75±0.75
II	Positive control (Pentazocine)+ acetic acid	5mg/kg +1%v/v	2.25 ± 0.25* *
III	Ethanolic extract + Acetic acid	300 mg/kg + 1%v/v	8.5 ± 0.86* *
IV	Ethanolic extract + Acetic acid	600 mg/kg + 1%v/v	5.5 ± 0.64* *
V	Aqueous extract + Acetic acid	300 mg/kg + 1%v/v	7.25 ± 1.10* *
VI	Aqueous extract + Acetic acid	600 mg/kg + 1%v/v	3.75 ± 0.62* *

*P values: *
* P< 0.01, * P <0.05
Values are expressed in mean ±SEM, n=6 animals in each group.

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One way ANOVA followed by DUNNETT'S, multiple comparison test

Table 3. Antioxidant Activity
Absorbance at 532 nm on the final day of the FTC method.

Treatment design	Absorbance 532 nm
BHT	0.026
A-tocopherol	0.086
Water	1.030

CONCLUSION

Both the extract of dried stem of *Emblica Officinalis* having analgesic activity against the acetic acid-induced writhing in mice. Both the extract of dried stem of *Emblica Officinalis* having anti-inflammatory activity against the carragenan -induce paw edema in rats.

The antioxidant activities of the plant extracts were measured by the FTC method. The plant extracts tested showed low absorbance values, which indicated a high level of antioxidant activity. None of the plant extracts showed absorbance values greater than the negative controls (without plant extracts) at the end point of methods, indicating the presence of antioxidant activity. However, all the plant extracts exhibited strong antioxidant activity as determined by the FTC methods, surpassing the activity of the standard commercial antioxidants, alpha-tocopherol, and butylated hydroxyl toluene (Table 3).

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