Research Article

Available online through
www.ijrap.net

IJRAP
ISSN 2229-3566

EVALUATION OF ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF *PUNICA GRANATUM* LINN LEAVES

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Received on: 19/04/2011 Revised on: 28/05/2011 Accepted on: 10/06/2011

ABSTRACT
The methanolic extract of dried leaves of *Punica granatum linn* was studied for the anti-inflammatory activity in rat using carrageenan induced paw edema with plethysmometer and analgesic activity on mice by Eddy's hot plate & tail immersion method. A preliminary phytochemical screening of leaves extract revealed the presence of alkaloids, tannins, flavonoids, and steroids. Among all the doses (200mg/kg, 400mg/kg, 600mg/kg, 800mg/kg) of methanolic extract 600mg/kg orally showed maximum significant anti-inflammatory and analgesic activity. The finding suggests that the leaves of *Punica granatum linn* contain flavonoids that posses anti-inflammatory and analgesic activity through inhibiting the prostaglandin biosynthesis.
KEYWORDS: *Punica granatum linn*, Anti-inflammatory, Analgesic.

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INTRODUCTION

The severe side effects of steroidal and nonsteroidal anti-inflammatory drugs evoked us to search for new anti-inflammatory and analgesic agents from natural botanical sources. The inflammatory process is the response to an injurious stimulus. It can be evoked by a wide variety of noxious agents (e.g., infections, antibodies, or physical injuries[1]. Many components are involved in the inflammation process to name few are oedema formation; leukocyte infiltration and granuloma formations are widely noticeable[2]. Pain is an unpleasant sensation localized to a part of the body. It is often described in terms of a penetrating or tissue destructive process (e.g. stabbing, burning, and twisting)[3]. Pomegranate or *Punica granatum linn* is a small tree or shrub, belonging to Punicaceae family. The tree is found to grow wild in Persia, Arabia, and parts of Western Pakistan. Leaf juice is considered to be astringent, while bark and rind of the fruits are valuable in chronic diarrhoea and early stages of dysentery[4]. Seeds are demulcent and stomachic. The fruit is a mild astringent and refrigent in some fevers and especially in biliousness[5].

MATERIALS AND METHODS

Animals and Collection of Plant Material

Albino rats and albino mice of either sex, weighing around 180-200g and 20-25g respectively were used for the study. They were kept in polypropylene cages with food and water ad libitum and maintained at a temperature of 26±2°C. All animals experiments were carried out according to CPCSEA guidelines, after getting approval of Institute's Animal Ethics Committee (Registration no.828/ac/04/CPCSEA). The leaves of punica granatum were collected from CH. Devi lal herbal garden, Yamuna Nagar. The leaves were authenticated as *Punica granatum linn*, from National Institute of science communication and Information resources (NISCAIR), Ref no. (NISCAIR/RHMD/CONSULT/- 2009-10/1347/149) .

Extraction of Plant Material

Two hundred gram of the air dried and coarsely grounding leaves of plant was defatted with petroleum ether (60-80°c) by soxhlation for three days and then the solvent free marc was extracted with the chloroform and then again the solvent free marc was extracted with methanol by soxhlation for three days and extract was concentrated in-vacuo in a rotary evaporator. The dried marc was extracted with distilled water by maceration in

a round bottom flask for 24 hr. After 24 hr it was filtered through muslin cloth followed by buchner funnel. Maceration process was repeated using marc for another two times and all the three filterates were collected and combined. The solvent was removed by in-vacuo in rotary evaporator. The dried extracts were placed in a desiccator and used for further studies.

Preliminary Phytochemical Screening

The phytochemical screening of leaves of pomegranate was conducted for the detection of alkaloids, flavonoids, steroids, and tannins.

Grouping of animals

Animals were grouped for anti-inflammatory (6 groups) and analgesic (6 groups) activity. Each group was consist six animals.

For Anti-inflammatory (Carragenan Induced Rat Hind Paw Oedema)

The carragenan induced rat paw edema test was performed according to winter et al 1962[6].1% w/v of carragenan was injected in left hind paw (plantar region). The paw volume was measured using plethysmometer (model pth-7070, sr.no.pt 070509, Medicad system). The groupI was treated with 2% gum acacia and groupII was administered with diclofenac sodium (5mg/kg) orally. The groups III-VI of mice were treated with methanolic extracts of 200mg/kg, 400mg/kg, 600mg/kg and 800mg/kg body weight orally respectively. The administered of drug and extract was done before half an hour of carrageen injection. The measurements of paw volume were done after 1hr, 2hr and 3hr. Percent inhibition of inflammation was calculated using the formula,

$$\% \text{ inhibition} = V_c - V_t / V_c \times 100$$

Where V_t is the of paw oedema volume (ml) in test/standard compound at corresponding time and V_c is paw oedema volume (ml) in control.

For Analgesic Activity

Eddy's Hot Plate

The animals were placed on Eddy's hot plate at temperature of 55±0.5ºC. A cut off period of 15 sec, was observed to avoid damage of the paw. The response in the form of withdrawal of paws or licking of the paws[7]. Eddy's hot plate of model KI 9514 used. The group-I was treated with 2% gum acacia and group-II was administered with diclofenac sodium (1mg/kg) orally. The groups III-VI of mice were treated with methanolic extracts of 200mg/kg, 400mg/kg, 600mg/kg and 800mg/kg body weight orally respectively. The latency was recorded before and after 15, 30, 60,120 min administration of drug.

Tail Immersion Method

This procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice. 1 to 2 cm of the tail of mice was immersed in warm water kept constant at 55°C temperature. Within a few seconds the rat reacts by withdrawing the tail[8]. The latent period of the tail flick response was determine before and 15, 30, 60 and120 min. after drug administration. The group-I was treated with 2% gum acacia and group-II was administered with diclofenac sodium (1mg/kg) orally. The group III-VI of mice were treated with methanolic extracts of 200mg/kg, 400mg/kg, 600mg/kg and 800mg/kg body weight orally respectively. The reaction time was recorded by a stop watch.

RESULTS AND DISCUSSION

The methanolic extract of the leaves of pomegranate has shown significant analgesic and anti inflammatory activity when compared to the control. It has been suggested that prostaglandins and bradykinin play a major role in the carrageenan induced paw edema and analgesia[9]. So it may be predicted that the methanolic extract acting by inhibiting the synthesis of these substances[10]. The phytochemical analysis has revealed the presence of Flavonoids, steroids, alkaloids, and tannins in the extract. Flavonoids have been reported to target prostaglandins, which are involved in the late phase of acute inflammation and pain[11]. The polyphenolic compounds like phenolic acids, flavonoids and tannins have been reported to posses other biological activities such as wound healing, analgesic, anti inflammatory and antioxidant[12]. It may be presumed that the analgesic and anti inflammatory activity could be due to the individual or combined synergic effects of the phytoconstituents present in the extract.

CONCLUSION

The methanolic extract of leaves of *Punica granatum linn* showed maximum significant ($P<0.05$) at dose 600mg/kg.

ACKNOWLEDGEMENT

The authors are grateful to M.M. University, Mullana for providing the facilities.

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Table 1: Effect of methanolic extract of Pomegranate leaves on carragenan induced paw edema volume (in ml) after specified time (in hr)

Groups	Dose	Change in paw volume (ml)		
		1hr	2hr	3hr
Control	2% gum acacia	2.26±0.10	3.10±0.09	3.0±0.018
Diclofenac sodium	5 mg/kg	1.03±0.77 (54%)	0.8±0.04 (74.09%)	0.68±0.04 (77.09%)
Methanol extract	200mg/kg	1.73±0.10* (22.13%)	1.68±0.10* (45.80%)	1.51±0.11* (49.66%)
Methanol extract	400mg/kg	1.80±0.09* (20.35%)	1.69±0.09* (45.48%)	1.33±0.09* (55.66%)
Methanol extract	600mg/kg	1.67±0.09* (26.1%)	1.26±0.08* (59.35%)	1.19±0.14* (65.59%)
Methanol extract	800mg/kg	1.94±0.09 (14.15%)	1.86±0.07* (40.0%)	1.79±0.07* (41.06%)

All values are Mean±SEM; (n=6) $P<0.05$ vs standard

Table 2: Analgesic activity by eddy's hot plate

Groups	Dose	Before treatment (sec)	After treatment (sec)			
			15min.	**30min.**	**60min.**	**120min.**
Control	2% gum acacia	8.03±0.02	8.1±0.30	8.20±0.20	8.32±0.20	8.23±0.20
Diclofenac sodium	1mg/kg	8.41±0.33	10.58±0.48	11.83±0.57	14.45±0.71	13.23±0.52
Methanol extract	200mg/kg	8.20±0.26	9.56±0.34*	10.24±0.40*	10.55±0.29*	10.40±0.25*
Methanol extract	400mg/kg	7.76±0.23	9.80±0.23*	10.42±0.27*	11.23±0.12*	11.10±0.13*
Methanol extract	600mg/kg	7.71±0.18	9.90±0.31*	10.50±0.25*	11.60±0.24*	11.40±0.23*
Methanol extract	800mg/kg	8.36±0.26	9.20±0.32*	9.60±0.23*	10.22±0.26*	10.08±0.21*

n=6, values are Mean±SEM; $P<0.05$ vs standard

Table 3: Analgesic activity by tail immersion.

Groups	Dose	Before treatment (sec)	After treatment (sec)			
			15min.	**30min.**	**60min.**	**120min.**
Control	**2% gum acacia**	2.5±0.2	2.51±0.21	2.78±0.18	2.89±0.16	2.7±0.15
Diclofenac sodium	**1mg/kg**	2.89±0.21	4.03±0.09	4.65±0.18	6.08±0.10	5.2±0.12
Methanol extract	**200mg/kg**	2.85±0.27	3.56±0.28*	4.06±0.17*	4.45±0.17*	4.13±0.09*
Methanol extract	**400mg/kg**	2.88±0.18	3.63±0.17*	4.2±0.18*	4.8±0.18*	4.5±0.15*
Methanol extract	**600mg/kg**	2.87±0.24	3.9±0.26*	4.5±0.29*	5.5±0.19*	5±0.10*
Methanol extract	**800mg/kg**	2.81±0.10	3.28±0.05*	3.58±0.07*	3.88±0.06*	3.7±0.22*

n=6, values are Mean±SEM; *$P<0.05$ vs standard

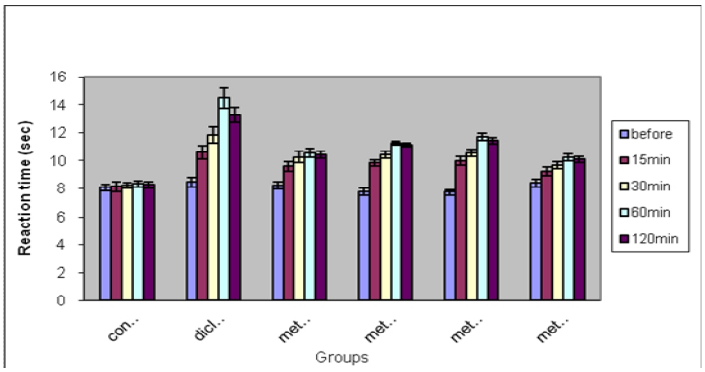

Fig.1 Eddy's hot plate

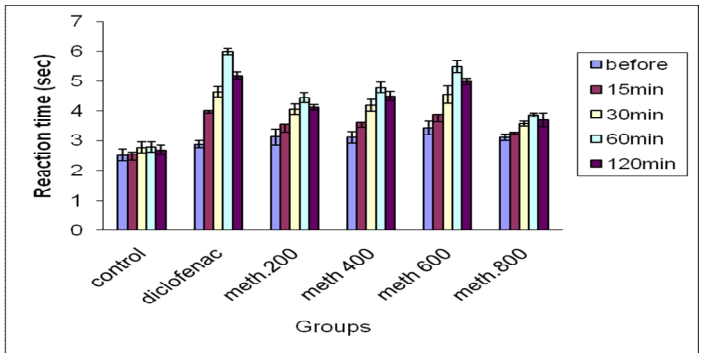

Fig.2 Tail immersion

Source of support: Nil, Conflict of interest: None Declared