ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Analgesic and Anti-Inflammatory Activities of *Citrus Maxima* (J.Burm) Merr in Animal Models

Shivananda A[1], Muralidhara Rao D[2], and Jayaveera KN[3]

[1] Department of Zoology, SBGNSC, Chickballapur, Karnataka, India-562 101
[2] Department of Biotechnology, S.K.University, Anantapur, Andrapradesh, India
[3] Department of Chemistry, Jawaharlal Nehru Technological University Anantapur, Anantapur, Andrapradesh, India

ABSTRACT

The present study was aimed to investigate the analgesic and anti-inflammatory activities of *Citrus maxima* in animal models. Analgesic activity was studied in acetic acid induced writhing, hot plate methods in mice and tail flick method in rats. whereas CM-LF-ETH, CM-BRK-ETH, CM-FP-ETH, CA-LF-ETH, CA-BRK-ETH, CA-FP-ETH 300 mg/kg extracts exhibits significant analgesic activity in acetic acid-induced writhing test. A dose of 300 mg/kg CM-LF-ETH, CM-BRK-ETH, CM-FP-ETH, CA-LF-ETH, CA-BRK-ETH, CA-FP-ETH extracts exhibited significant ($P<0.001$) in hot plate method. The extracted compounds exhibited analgesic activity against chemically and thermal noxious stimuli on both early and late phases of pain by the *Citrus maxima* extracts (300 mg/kg). Acute and Chronic inflammatory activities were studied in rats by formalin induced paw edema models respectively. In both models, the standard drug used was diclofenac sodium 10 mg/kg, 100 mg/kg. A dose of 300 mg/kg CM-LF-ETH, CM-BRK-ETH, CM-FP-ETH, CA-LF-ETH, CA-BRK-ETH, CA-FP-ETH exhibited significant ($P<0.001$) anti-inflammatory activity in formalin induced paw edema models in comparison to control. In conclusion *Citrus maxima* possesses anti-inflammatory and analgesic activities.

Keywords: *Citrus maxima,* Analgesic, Anti-inflammatory activity.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Pain is defined as neuralgia, an unpleasant sensory experience associated with tissue damage, such as injury, inflammation or cancer, but severe pain can arise independently of any obvious predisposing cause, or persist long after the precipitating injury has healed [1]. It can also occur as a consequence of brain or nerve injury [2]. Inflammation involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair and pain is a complex, multidimensional sensory experience that involves not only the transduction of noxious environmental stimuli but also cognitive and emotional processing by the brain [3-5]. So, Inflammation and pain has become the focus of global scientific research because of its implication in virtually all human and animal diseases.

Citrus maxima (J.Burm.) Merr (Fam. Rutaceae) commonly known as Pomelo, Chinese grapefruit, Pummelo, Pommelo, Jabong, Shaddock, a crop plant of India, China, Japan, Indonesia, United state of America, Philippine, Thailand.6 The tree has large evergreen oblong to elliptic leaves, 10.5 to 20 cm (4 to 8 in) long, with winged petioles (leaf stems). The flowers and fruits are borne singly, in contrast to grapefruits, in which they grown in clusters of 2 to 20. The hot leaf decoction is applied on swellings and ulcers. The fruit juice is taken as a febrifuge. The seeds are employed against coughs, dyspepsia and lumbago. The fruit include treatment of coughs, fevers, cardiotonic, cancer and gastrointestinal disorders [7, 8]

MATERIALS AND METHODS

Plant material:

The leaves, stem bark and fruit peels of *Citrus maxima (Pomelo* were collected from the local gardens around Devanahalli, Bangalore, Karnataka, India

Preparation of plant extract:

Ethanolic, acetone and aqueous extracts of each of the leaves, stem bark and fruit peels of *Citrus maxima* were prepared by soaking 20g of the material in various solvents for 72h and after every 24h, the mixture was stirred with a sterile glass rod. After the completion of 72h time period the extract was filtered and concentrated in water bath under reduced pressure to obtain semisolid material which was then used to obtain the crude extract.

Determination of Acute Toxicity (LD_{50})

The procedure was divided into two phases. Phase I (observation made on day one) and Phase II (observed the animals for next 14 days of drug administration). Two sets of healthy female rats (each set of 3 rats) were used for this experiment. First set of animals were divided into three groups, each of one in a group. Animals were fasted overnight with water *ad libitum.* Animals received a single dose of 2000 mg/kg, p.o. was selected for the test, as the test item was a source from herb. After administration of extract, food was withheld for 3-4 hrs [9].

Experimental animals

Albino wistar rats weighing 150-200g and Albino mice 20-30 g was procured from Biogen, Bangalore. They were maintained in the animal house of Rural College of Pharmacy, for experimental purpose. Animals were maintained under controlled condition of temperature at $27^{o} \pm 2^{o}$ C and 12 hr light-dark cycles for one week. They were housed in polypropylene cages and containing paddy husk as bedding. They had a free access to standard pellets and water *ad libitum*. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Rural College of Pharmacy, Bangalore. According to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India.

Evaluation of Analgesic Activity:

Acetic acid Induced Writhing in Mice [10]:

Albino mice weighing 20-30 mg/kg were divided into leaven groups of six in each group. One hour after the administration of the test drug and diclofenac (10 mg/kg i.p), the mice were given intraperitoneal injection of 0.7%v/v acetic acid solution (volume of injection 0.1ml 10g), the mice were placed individually into glass beakers and 5min, were allowed to elapse. The number of writhes produced in these animals was counted for 15min. For scoring purposes, a writhe is indicated by stretching of the abdomen with simultaneous stretching of at least one hind limb.

Group-I: Distilled water will be supplied and served as control.

Group-II: Animals received a dose of 10 mg/kg of Diclofenac sodium i.p. and served as standard

Group-III to XI: Animals received a dose of 300 mg/kg of p.o. acetone, ethanol and water plant extracts of each of the leaves, stem bark and fruit peels.

Tail Flick Method in Rats [11]:

Albino wistar rats weighing 150-250 mg/kg were divided into leaven groups of six in each group. The tail flick latency was assessed by analgesiometer. A light beam is focused (exerting radiant heat) tomthe proximal third of the tail. The rat tries to pull the tail away and rotates the head this reaction is known as escape reaction. The reaction time is recorded ½, 1, 2, 3, 4, 5, 6 hours following intra peritoneal administration of the standard and oral administration of the test compounds. The strength of the current passing through the naked nichrome wire was kept constant at 6 amperes. The distance between the heat source and tail skin was 1.5c.m. The site of application of the radiant heat in the tail was maintained at2.5c.m.measured from the root of the tail. The cutoff reaction time was fixed at 10 seconds to avoid tissue damage.

Group-I: Distilled water will be supplied and served as control.

Group-II: Group-II: Animals received a dose of 10 mg/kg of Diclofenac sodium i.p. and served as standard
Group- III to XI: Animals received a dose of 300 mg/kg of p.o. acetone, ethanol and water plant extracts of each of the leaves, stem bark and fruit peels.

Hot plate Method in Mice [12]:

Albino mice weighing 20-30 mg/kg were divided into leaven groups of six in each group. The temperature is controlled for $55^{o} \pm 1^{0}$C. The animals were placed into the Perspex cylinder on the heated surface and the time (sec) to discomfort reaction (licking paws or jumping) was recorded as response latency, period to and 30,60,90,120 and 180 min following intra peritoneal administration of the standard and oral administration of the test compounds. A latency period of 15 sec was identified as complete analgesia and the measurement was terminated if it exceeded the latency period in order to avoid injury.

Group-I: Distilled water will be supplied and served as control.
Group-II: Animals received a dose of 10 mg/kg of Pentazocine i.p. and served as standard
Group- III to XI: Animals received a dose of 300 mg/kg of p.o. acetone, ethanol and water plant extracts of each of the leaves, stem bark and fruit peels.

Evaluation of Anti-Inflammatory Activity

Acute Anti inflammatory Activity

Formalin-induced Paw Oedema in Rats [13]:

Acute inflammation was induce by injecting formalin (0.1 ml of 1% suspension in 0.9% saline) in sub-plantar region and paw volume was measured 0,1,2,3,4 and 5 hours, with the help of Plethysmometer. All the treatment compounds compound were administered 30 min, perior to formalin. Acute inflammation was induced in right hind paw. A mark was put on the leg second at the leg at the mallaleous region to facilitate the dipping of the leg to the same level at the second and subsequent times. The initial reading was taken at 0 hr., i.e., immediately after injecting formalin and the procedure was repeated at 1,2,3,4 and 5 hours after formalin injection. The difference between 0 hr reading and one of the subsequent reading provides the actual edema volume at the time. The mean paw volume at different times was calculated and compared with the control.

Group-I: Distilled water will be supplied and served as control.
Group-II: Animals received a dose of 10 mg/kg of Diclofenac sodium i.p. and served as standard
Group- III to XI: Animals received a dose of 300 mg/kg of p.o. acetone, ethanol and water plant extracts of each of the leaves, stem bark and fruit peels.

Chronic Anti inflammatory Activity

Formalin Induced Paw Oedema [14]:

Albino wistar rats weighing 170-250 mg/kg were divided into leaven groups of six in each group. All these animals were fasted for 18 hrs before the beginning of the experiment and water was given ad libitum. In animals of all the groups chronic inflammation was produced by sub plantar injection of 20µ of freshly prepared 2% suspension of formalin in normal saline in right hind paw of rat was used as the oedematogenic agent. Animals were treated with drugs for 6 consecutive days. The paw volume was measured using a plethysmometer before and 6 days after formalin challenge in each group. The increase in paw volume and percent of inhibition was calculated.

Group-I: Distilled water will be supplied and served as control.
Group-II: Animals received a dose of 100 mg/kg of Diclofenac sodium i.p. and served as standard
Group- III to XI: Animals received a dose of 300 mg/kg of p.o. acetone, ethanol and water plant extracts of each of the leaves, stem bark and fruit peels.

Statistical analysis

The values are expressed as Mean ± SEM. The data was analysed by using one way ANOVA followed by Dunnett's test using Graph pad prism software. Statistical significance was set at $P \leq 0.05$.

RESULTS

Analgesic Activity

Effect of *Citrus maxima* Plant Extracts on Acetic acid Induced Writhing in Mice

Control and various treated groups were tested for analgesic activity against acetic acid induced writhing, which is nothing but the painful reaction. Thirty minutes after the treatment, each mouse was injected with 0.1 ml 0.7% v/v aqueous solution of acetic acid i.p. The number of abdominal constrictions was cumulatively counted from 0 - 10 minutes. The % reduction of writhing in standard diclofenac sodium 10 mg/kg treated group was found to be 60.02% against control. The mean response of control and standard was 41.50 ± 1.25 and 16.59 ± 0.92 respectively. The respective test compounds CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK- WATE, CM-FP-ETH, CM-FP-ACET and CM-FP- WATE in its 300 mg/kg dose, showed mean writhing responses as 23.00 ± 1.06, 26.33 ± 1.38, 26.17 ± 1.49, 23.83 ± 1.30, 28.33 ± 1.66, 25.59 ± 1.43, 22.67 ± 1.17, 27.83 ± 1.30 and 27.83 ± 1.30. In terms of percentage inhibition of writhing by diclofenac sodium was 60.02% while with the test compound it was CM-LF-ETH 44.57%, CM-BRK-ETH 42.57% and CM-FP-ETH 45.37% respectively.

ISSN: 0975-8585

Table 1: Effect of *Citrus maxima* Plant Extracts on Acetic acid Induced Writhing in Mice

Groups	Treatment	Mean no of writhing ±SEM	% Inhibition of writhes
Group-I	Saline	41.50 ± 1.25	-
Group-II	Diclofenac (10mg/kg)	16.59 ± 0.92***	60.02%
Group-III	CM-LF-ETH (300mg/kg)	23.00 ± 1.06***	44.57%
Group-IV	CM-LF-ACET (300mg/kg)	26.33 ± 1.38***	36.55%
Group-V	CM-LF-WATE (300mg/kg)	26.17 ± 1.49***	36.93%
Group-VI	CM-BRK-ETH (300mg/kg)	23.83 ± 1.30***	42.57%
Group-VII	CM-BRK-ACET (300mg/kg)	28.33 ± 1.66***	31.73%
Group-VIII	CM-BRK- WATE (300mg/kg)	25.59 ± 1.43***	38.33%
Group-IX	CM-FP-ETH (300mg/kg)	22.67 ± 1.17***	45.37%
Group-X	CM-FP-ACET (300mg/kg)	27.83 ± 1.30***	32.93%
Group-XI	CM-FP- WATE (300mg/kg)	27.33 ± 0.98***	34.86%

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. CM-*Citrus maxima,* LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone.

Effect of *Citrus maxima* Plant Extracts on Tail Flick method in Rats

In the tail flick method, the increase in latency period at different time points significantly differed (P<0.001) compared to baseline values within the same drug treated groups. The CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK-WATE, CM-FP-ETH, CM-FP-ACET and CM-FP- WATE and diclofenac sodium caused significant increase (P<0.001) in the percentage reaction time whilst the control and dose of extracts (300 mg/kg). At all the specified time intervals, the percentage of tail flick elongation time differed significantly (P<0.001) between the extracts and diclofenac sodium at the doses of plant extracts, being greater for diclofenac sodium. At the peak of activity, CM-LF-ETH, CM-BRK-ETH and CM-FP-ETH extracts showed (P<0.001) and significantly of tail flick elongation time respectively, whilst diclofenac sodium gave (P<0.001) elongation of tail flicking time.

Effect of *Citrus maxima* Plant Extracts on Hot Plate Method in Mice

The standard pentazocine lactate (10 mg/kg) was given i.p**.,** CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK- WATE, CM-FP-ETH, CM-FP-ACET and CM-FP- WATE extracts given orally, in a dose of 300 mg/kg, elicited a significant analgesic activity in the hot plate method as evidenced by increase in latency time in seconds as compared with vehicle control. The increase in latency tine was dose dependant. Latency time was noted 30, 60, 90, 120 and 180 minutes after administration of vehicle, standard and plant extracts.

ISSN: 0975-8585

Table 2: Effect of *Citrus maxima* Plant Extracts on Tail Flick method in Rats

Groups	Treatment	Reaction Time (Sec)							
		0 min	30 min	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
Group-I	Saline	4.51 ± 0.29	4.48 ± 0.23	5.05 ± 0.34	5.30 ± 0.28	5.70 ± 0.38	5.76 ± 0.36	4.86 ± 0.57	5.65 ± 0.51
Group-II	Diclofenac (10mg/kg)	4.71 ± 0.31	8.86 ± 0.39***	10.05 ± 0.45***	11.52 ± 0.98***	12.32 ± 0.77***	13.17 ± 1.19***	14.28 ±0.90***	13.62 ± 0.63***
Group-III	CM-LF-ETH (300mg/kg)	5.25 ± 0.48	8.23 ± 0.66**	9.38 ± 0.58***	11.38 ± 0.65***	11.80 ± 0.75***	12.23 ± 0.65***	13.08 ±0.72***	11.82 ± 0.83 ***
Group-IV	CM-LF-ACET (300mg/kg)	4.56 ± 0.48	6.13 ± 0.35^{ns}	7.48 ± 0.44 *	8.63 ± 0.65**	9.16 ± 0.73**	9.60 ± 0.60**	9.90 ± 0.45***	8.16 ± 0.57*
Group-V	CM-LF-WATE (300mg/kg)	4.98 ± 0.38	4.93 ± 0.86^{ns}	6.73 ± 0.43 *	8.15 ± 0.75*	8.15 ± 0.44*	9.63 ± 0.42**	10.70 ± 0.78***	9.58 ± 1.35*
Group-VI	CM-BRK-ETH (300mg/kg)	4.21 ± 0.55	7.25 ± 0.49**	8.10 ± 0.54***	9.96 ± 0.70**	10.18 ± 0.64***	11.23 ± 0.57***	12.32 ±0.39***	11.53 ± 0.86 ***
Group-VII	CM-BRK-ACET (300mg/kg)	4.71 ± 0.48	6.95 ± 0.86^{ns}	7.71 ± 0.74^{ns}	8.61 ± 1.01*	8.71 ± 0.63*	9.80 ± 0.75**	10.90 ± 0.90***	8.86 ± 0.82*
Group-VIII	CM-BRK-WATE (300mg/kg)	4.90 ± 0.69	6.93 ± 0.47 *	7.66 ± 0.54**	9.06 ± 0.72**	9.50 ± 0.30**	10.07 ± 0.69***	11.07 ± 0.51***	8.68 ± 0.51**
Group-IX	CM-FP-ETH (300mg/kg)	4.81 ± 0.59	7.78 ± 0.43**	9.03 ± 0.56***	10.80 ± 0.81**	11.32 ± 0.57***	12.08 ± 0.69***	13.12 ± 0.87***	12.07 ± 0.85***
Group-X	CM-FP-ACET (300mg/kg)	4.86 ± 0.61	6.60 ± 0.46^{ns}	7.50 ± 0.81*	8.53 ± 0.61**	8.71 ± 0.77**	10.37 ± 0.99***	11.25 ± 0.51***	10.37 ± 0.57**
Group-XI	CM-FP- WATE (300mg/kg)	4.51 ± 0.48	7.26 ± 0.88*	8.31 ± 0.74***	10.97 ± 0.71***	11.35 ± 0.42***	11.55 ± 0.44***	12.17 ± 0.68***	10.95 ± 0.50***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. CM-*Citrus maxima,* LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone.

Table 3: Effect of *Citrus maxima* Plant Extracts on Hot Plate Method in Mice

Groups	Treatment	Reaction time (Sec)					
		0 min	30 min	60 min	90 min	120 min	180 min
Group-I	Saline	2.66 ± 0.33	2.33 ± 0.21	2.83 ± 0.30	3.66 ± 0.49	4.16 ± 0.60	3.16 ± 0.30
Group-II	Pentazocine (10mg/kg)	2.50 ± 0.22	5.50 ± 0.42***	7.16 ± 0.60***	9.33 ± 0.66 ***	12.17 ± 0.30***	14.33 ± 0.33***
Group-III	CM-LF-ETH (300mg/kg)	2.83 ± 0.40	5.00± 0.44***	6.33 ± 0.71***	9.00 ± 0.57***	11.67 ± 0.55***	13.17 ± 0.30***
Group-IV	CM-LF-ACET (300mg/kg)	2.50 ± 0.34	3.50 ± 0.42^{ns}	4.66 ± 0.33^{ns}	6.33 ± 0.42**	6.83 ± 0.30**	7.83 ± 0.60***
Group-V	CM-LF-WATE (300mg/kg)	3.33 ± 0.49	4.33 ± 0.42**	5.83 ± 0.30***	8.50 ± 0.50***	10.67 ± 0.49***	11.83 ± 0.60***
Group-VI	CM-BRK-ETH (300mg/kg)	2.83 ± 0.30	4.50 ± 0.42**	6.00 ± 0.63**	8.33 ± 0.49***	10.67 ± 0.40***	12.50 ± 0.42***
Group-VII	CM-BRK-ACET (300mg/kg)	3.83 ± 0.30	3.50 ± 0.42^{ns}	4.66 ± 0.49*	5.66 ± 0.33*	6.66 ± 0.42**	8.83 ± 0.47***
Group-VIII	CM-BRK-WATE	2.83 ± 0.30	4.00 ± 0.36*	4.83 ± 0.30*	6.66 ± 0.33**	7.50 ± 0.42***	10.17 ± 0.47***

ISSN: 0975-8585

	(300mg/kg)						
Group-IX	CM-FP-ETH (300mg/kg)	3.33 ± 0.33	5.16 ± 0.47***	6.63 ± 0.60***	6.83 ± 0.60***	11.33 ± 0.49***	12.50 ± 0.56***
Group-X	CM-FP-ACET (300mg/kg)	2.16 ± 0.16	3.83 ± 0.47*	5.00 ± 0.36**	6.50 ± 0.42**	7.16 ± 0.30***	9.83 ± 0.47***
Group-XI	CM-FP- WATE (300mg/kg)	2.66 ± 0.33	3.83 ± 0.30*	5.00 ± 0.57*	7.50 ± 0.42***	7.33± 0.49***	8.50 ± 0.42***

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. CM-*Citrus maxima,* LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone.

Table 4: Effect of *Citrus maxima* Plant Extracts on Formalin-induced paw Oedema in Rats

Groups	Treatment	Reaction Time						
		0 hr	1hr	2hr	3hr	4 hr	5 hr	% Inhibition
Group-I	Saline	0.16 ± 0.01	0.78 ± 0.05	0.99 ± 0.05	1.20 ± 0.06	1.25 ± 0.07	1.40 ± 0.05	-
Group-II	Diclofenac (10mg/kg)	0.15 ± 0.01	0.37 ± 0.02***	0.55 ± 0.04***	0.36 ± 0.02***	0.30 ± 0.03***	0.18 ± 0.01***	87.14 %
Group-III	CM-LF-ETH (300mg/kg)	0.17 ± 0.02	0.54 ± 0.02**	0.63 ± 0.03***	0.50 ± 0.02***	0.40 ± 0.01***	0.20 ± 0.01***	85.71%
Group-IV	CM-LF-ACET (300mg/kg)	0.17 ± 0.02	0.63 ± 0.04*	0.76 ± 0.03**	0.65 ± 0.03***	0.49 ± 0.03***	0.42 ± 0.02 ***	70.00 %
Group-V	CM-LF-WATE (300mg/kg)	0.17 ± 0.02	0.59 ± 0.03**	0.66 ± 0.01***	0.54 ± 0.01***	0.45 ± 0.01***	0.30 ± 0.01***	77.14%
Group-VI	CM-BRK-ETH (300mg/kg)	0.18 ± 0.02	0.56 ± 0.04**	0.67 ± 0.02***	0.59 ± 0.03***	0.42 ± 0.02***	0.24 ± 0.03***	82.85%
Group-VII	CM-BRK-ACET (300mg/kg)	0.13 ± 0.01	0.60 ± 0.04*	0.76 ± 0.05**	0.63 ± 0.03***	0.48 ± 0.02***	0.44 ± 0.05***	68.57%
Group-VIII	CM-BRK- WATE (300mg/kg)	0.15 ± 0.01	0.63 ± 0.03*	0.72 ± 0.02***	0.59 ± 0.01***	0.50 ± 0.02***	0.39 ± 0.04 ***	72.14 %
Group-IX	CM-FP-ETH (300mg/kg)	0.15 ± 0.02	0.46 ± 0.023**	0.60 ± 0.02***	0.44 ± 0.02***	0.38 ± 0.02***	0.19 ±0.01***	86.42%
Group-X	CM-FP-ACET (300mg/kg)	0.14 ± 0.02	0.67 ± 0.06ns	0.79 ± 0.03*	0.61 ± 0.02***	0.51 ± 0.02***	0.46 ± 0.01 ***	67.60 %
Group-XI	CM-FP- WATE (300mg/kg)	0.13 ± 0.01	0.56 ± 0.03**	0.65 ± 0.03***	0.50 ± 0.02***	0.37 ± 0.02***	0.29 ± 0.03***	79.57%

Values are Mean ± SEM (n=6) one way ANOVA followed by Dunnett's test. Where, *** P<0.001, ** P<0.01, * P<0.05 and ns represents Not significant. CM-*Citrus maxima,* LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone.

Anti inflammatory Activity

Acute Anti inflammatory Activity

Effect of *Citrus maxima* Plant Extracts on Formalin-induced paw Oedema in Rats

ISSN: 0975-8585

All the test compounds were tested with the diclofenac sodium as a standard drug in the dose of 10 mg/kg for the anti-inflammatory activity. Presently diclofenac showed significant 87.14 % inhibition of inflammation at 5th hour (0.18 ± 0.01) when compared with control (1.40 ± 0.05) respectively. The test compounds showed maximum percentage of inhibition of oedema at 5th hour significantly in respective dose level i.e., at 300 mg/kg the test compounds CM-LF-ETH , CM-BRK-ETH and CM-FP-ETH showed 85.71%, 82.85% and 86.42%.

Chronic Anti inflammatory Activity

Effect of *Citrus maxima* Plant Extracts on Formalin-induced Paw Oedema in Rats

Formalin induced paw oedema is one of the most suitable test procedure to screen chronic anti-inflammatory agents. The results obtained as mean increase in paw volume (ml) and % inhibition are represented in table 5. The mean response of standard was 82.40% inhibition of increase in paw thickness after 6 days respectively. In this model at 300 mg/kg dose level of CM-LF-ETH, CM-LF-ACET, CM-LF-WATE, CM-BRK-ETH, CM-BRK-ACET, CM-BRK-WATE, CM-FP-ETH, CM-FP-ACET and CM-FP- WATE extracts showed 65.66%, 20.60%, 54.07%, 59.22%, 28.75%, 20.60%, 60.94%, 27.03% and 39.48% inhibition of increase in paw thickness after 6 days, However, at CM-LF-ETH, CM-BRK-ETH and CM-FP-ETH extracts showed 65.66%, 59.22% and 60.94% inhibition of increase in paw thickness after 6 days. All the results were compared with solvent control and diclofenac sodium reference drug control.

Table 5: Effect of *Citrus maxima* Plant Extracts on Formalin-induced Paw Oedema in Rats

Groups	Treatment	Initial Paw Volume	Paw Volume After 6 Days	Increase in Paw Volume	% of Inhibition
Group-I	Saline	1.28 ± 0.07	3.61 ± 0.12	2.33 ± 0.06	-
Group-II	Diclofenac (100mg/kg)	1.23 ± 0.04	1.65 ± 0.05	0.41 ± 0.07	82.40%
Group-III	CM-LF-ETH (300mg/kg)	1.26 ± 0.03	2.00 ± 0.06	0.80 ± 0.12	65.66%
Group-IV	CM-LF-ACET (300mg/kg)	1.21 ± 0.06	3.21 ± 0.24	1.85 ± 0.16	20.60%
Group-V	CM-LF-WATE (300mg/kg)	1.25 ± 0.06	2.25 ± 0.16	1.07 ± 0.14	54.07%
Group-VI	CM-BRK-ETH (300mg/kg)	1.31 ± 0.08	2.26 ± 0.10	0.95 ± 0.14	59.22%
Group-VII	CM-BRK-ACET (300mg/kg)	1.23 ± 0.06	2.90 ± 0.14	1.66 ± 0.17	28.75%
Group-VIII	CM-BRK- WATE (300mg/kg)	1.26 ± 0.06	3.11 ± 0.08	1.85 ± 0.11	20.60%
Group-IX	CM-FP-ETH (300mg/kg)	1.28 ± 0.08	2.23 ± 0.17	0.91 ± 0.14	60.94%
Group-X	CM-FP-ACET (300mg/kg)	1.28 ± 0.05	2.71 ± 0.23	1.70 ± 0.08	27.03%
Group-XI	CM-FP- WATE (300mg/kg)	1.30 ± 0.07	2.86 ± 0.14	1.41 ± 0.19	39.48%

Results are expressed on mean + SEM from four observations Paw Volume was measured after 6 days. CM-*Citrus maxima,* LF-leaf, BRK-bark, FP-fruit peel, ETH-ethanol, ACET-acetone.

DISCUSSION

Acetic acid-acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Such pain stimulus leads to the release of free arachidonic acid from tissue phospholipids [15]. The acetic acid induced writhing response is a sensitive

procedure to evaluate peripherally acting analgesics. The response is thought to be mediated by peritoneal mast cells acid sensing ion channels and the prostaglandin pathway [16-18]. The CM-LF-ETH, CM-BRK-ETH, CM-FP-ETH, CA-LF-ETH, CA-BRK-ETH, CA-FP-ETH 300 mg/kg b.w. p.o., showed significant decrease writhes when compared to control group.

In the tail flick method, the increase in latency period at different time points significantly CM-LF-ETH, CM-BRK-ETH, CM-FP-ETH, CA-LF-ETH, CA-BRK-ETH, CA-FP-ETH 300 mg/kg b.w. p.o., extracts showed ($P<0.001$) and significantly of tail flick elongation time respectively, whilst diclofenac sodium gave ($P<0.001$) elongation of tail flicking time.

To evaluate the analgesic activity, hot plate method was chosen. In this method pentazocine (10 mg/kg i.p) was used as reference standard. The ethanol, acetone and water extracts of each of the leaves, stem bark and fruit peels of *Citrus maxima (Pomelo)* and *Citrus aurantium (Bitter orange)* produced antinociception against thermal induced pain stimuli in mice at various time points of post treatment. The hot plate test is considered to be selective for opioid like compounds, which are centrally acting analgesic in several animal species. The hot plate method has been found to be suitable for evaluation of centrally acting analgesic [19, 20]. The ethanol, acetone and water extracts of each of the leaves, stem bark and fruit peels of *Citrus maxima (Pomelo)* and *Citrus aurantium (Bitter orange)* (300 mg/kg b.w. p.o.) increase the reaction time to the thermal stimulus.

It is well known that inhibition of formalin-induced pedal oedema in rats is one of the most suitable test procedures to screen anti-arthritic and anti-inflammatory agents as it closely resembles human arthritis [21]. Injection of formalin subcutaneously into hind paw of rats produces localized inflammation and pain. The nociceptive effect of formalin is biphasic, an early neurogenic component followed by a later tissue mediated response[22]. Thus formalin-induced arthritis is a model used for the evaluation of an agent with probable anti-proliferative activity. This experiment is associated with the proliferative phase of inflammation. Results with Ethanol, acetone and water extracts of each of the leaves, stem bark and fruit peels of *Citrus maxima (Pomelo)* and *Citrus aurantium (Bitter orange)* showed quite compatible with those of the standard drug diclofenac sodium. Therefore, the drug appears to be effective against formalin-induced arthritis.

Formalin induced paw oedema is one of the most suitable test procedure to screen chronic anti-inflammatory agents. The effect of CM-LF-ETH, CM-BRK-ETH, CM-FP-ETH, CA-LF-ETH, CA-BRK-ETH, CA-FP-ETH 300 mg/kg b.w. p.o., showed significant increase in paw thickness after 6 days.

CONCLUSION

From these investigations, it may be concluded that *Citrus maxima* showed both anti-inflammatory and analgesic effects, It is also suggested that the mechanism of action of ethanol, acetone and water extracts of each of the leaves, stem bark and fruit peels extracts of *Citrus maxima (Pomelo)* might be associated with the inhibition of prostaglandin synthesis,

ISSN: 0975-8585

Further studies will be necessary to establish the probable mechanism of action of anti-inflammatory activities of different extracts of *Citrus maxima* linn.

REFERENCES

[1] Rao YK, Fang SH, Tzeng YM. Evaluation of the anti-inflammatory and anti- Rang HP, Dale MM, Ritter JM, Flower RJ. Rang and Dale's Pharmacology. Churchill Livingstone, International Edition, USA. 2003:56.
[2] Laurence DR, Bennett PN. Clinical Pharmacology. Longman Singapore Publishers (P) Ltd.1992: 229-256.
[3] Vane JR. Nat New Biol 1971; 23(1): 232-236.
[4] Perianayagam JB, Sharma SK, Pillai KK. J Ethnopharmacol 2006; 104: 410-414.
[5] Kasper DL, Braunwald E, Hauser S, Longo D. Harrison's. Principles of Internal Medicine, 16 Ed. New York: Mc Graw-Hill Professional publisher 2005.
[6] Bailey LH, Bailey EZ, Bailey LH. Hortatorium. Hortus Third: A concise dictionary of plants cultivated in the United States and Canada. New York: Macmillan 1976:276.
[7] Morton J. Pummelo. *Citrus maxima* In: Fruits of warm climates. 1987; 147–151. Availableonlinefrom http://www.hort.purdue.edu/newcrop/morton/pummelo.html.
[8] Van Wyk BE. Timber Press 2005:141.
[9] OECD Guidelines for the Testing of Chemical. Acute Oral Toxicity – Up and Down Procedure (UDP) [Internet]. 2008 [Cited 2011 September 25]. Available from: http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OEC Dtg425.pdf.
[10] Mokarram Hossain MD, Israt Jahan Biva, et al. Afr J Pharm Pharmacol 2009; 3(5):282-286.
[11] Anar Patel, Timir Patel, et al. JPSR 2010; 2(12): 349-355.
[12] Sunetra Patwardhan, Ghansham Sakhare. Pharmacologyonline 2009; 2: 887-893.
[13] Yogesh Baravalia, Yogeshkumar Vaghasiya, Sumitra Chanda. Iranian J Pharm Res 2012; 11(3):851-861.
[14] Bikash Kumar Nanda, Jyotirmoyee Jena, et al. Der Pharmacia Lettre 2010; 2 (1) 181-188.
[15] Ahmed F, Hossain MH, Rahman AA, Shahid IZ Ori Pharm Exp Med 2006; 6: 344-348.
[16] Ronaldo AR, Mariana LV, Sara MT, Adriana BPP, Steve P, Ferreira S, Fernando QC. Eur J Pharmacol 2000; 387: 111-118.
[17] Voilley N. *Curr* Drug Targets- Inflam Aller 2004; 3: 71-79.
[18] Hossain MM, Ali MS, Saha A, Alimuzzaman M. Dhaka Univ. J Pharm Sci 2006; 5: 67-69.
[19] Ahamed KN, Kumar V, Raja S, Mukherjee PK. IJPT 2005; 105-9.
[20] Hosseinzadeh H, Younesi H. BMC Pharmacol 2002; 2: 7-13.
[21] Margolius H. Annu Rev Pharmacol Toxicol 1989; 29:343-8.
[22] Greenwald RA. Met Fin Exp Clin Pharmacol 1991; 13: 75–83.