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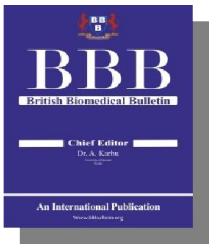

Original

Comparative Phytochemical and Pharmacological Evaluation of flowers of *Plumeria rubra* L. f. *rubra* and *Plumeria rubra* f. *lutea*

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A B S T R A C T

Shade dried flowers of *Plumeria rubra* f. *rubra* and *Plumeria rubra* f. *lutea* were subjected to successive solvent extraction by soxhlation using n-hexane, chloroform, ethyl acetate and methanol. The extracts were subjected to preliminary phytochemical screening using standard procedures and the data obtained from the flowers of both the species was comparatively evaluated. The methanolic extracts, rich in phytochemical constituents, were screened for analgesic activity (acetic acid-induced writhing & tail immersion methods) using diclofenac (10mg/kg b.w) as standard and antipyretic activity (brewer's yeast-induced pyrexia test) using paracetamol (100mg/kg; p.o.) as standard at dose levels of 250 and 500 mg/kg b.w. Oral administration of methanolic extracts of *Plumeria rubra* f. *rubra* and *Plumeria rubra* f. *lutea* (MEPR & MEPL) produced significant reduction in number of writhes induced by acetic-acid. Moreover, in tail immersion, MEPR & MEPL significantly raised the pain threshold at different time intervals, in comparison with control. There was a significant dose-dependent inhibition in both the methods. In antipyretic activity, MEPR & MEPL significantly reduced hyperthermia at either dose levels. The results obtained in all the animal models were highly significant and comparable to that of standard drugs. Hence, from the above findings, it can be concluded that *Plumeria rubra* possesses potent analgesic and moderate antipyretic properties. However the methanolic extract of flowers of *Plumeria rubra* f. *rubra* was found to be more potent than that of *Plumeria rubra* f. *lutea* flower extract.

Introduction

Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage. Pain motivates us to withdraw from potentially damaging situations, protect a damaged body part while it heals, and avoid those situations in the future[1].

Pyrexia or fever is caused as a secondary impact of infection, malignancy or other diseased states. It describes a regulated rise in body temperature after an increase in the hypothalamic set point. Under the influence of the hypothalamus, physiologic and behavioral functions favoring heat production and heat retention are stimulated until newly elevated set point temperature is reduced[2].

Natural products have proved to be a rich source of therapeutic agents. Several herbal remedies are helpful in reducing pain and speeding recovery of fever[3]. Due to the side effects caused mostly by synthetic drugs, interest in natural products is growing rapidly and research into natural products has advanced tremendously in academia and pharmaceutical companies. *Plumeria rubra* belonging to the family Apocynaceae is widely distributed throughout the Southern parts of India. In traditional medicinal system different parts of the plant have been mentioned to be useful in a variety of diseases. The plant material is widely used as a purgative, febrifuge and remedy for diarrhoea and cure for itch. The leaves were reported to have analgesic-antipyretic[4], anti-inflammatory[5], and antioxidant properties[6]. The flowers have been reported to be useful as antioxidant and hypolipidemics[7]. However, till date there are no reports on the comparative phytochemical evaluation of the flower extracts of different cultivars of *P.rubra*. Herein we report the preliminary result of studies on analgesic and antipyretic activities of the methanolic extracts of two cultivars of *P. rubra* viz., *Plumeria rubra* f. *rubra* and *Plumeria rubra* f. *lutea* (MEPR & MEPL) on experimental models.

Materials and Methods

Plant material

The flowers of the plants *Plumeria rubra L.* f. *rubra (pink flowers)* (PRRP) and *Plumeria rubra* f. *lutea (white flowers)* (PRLW) (Family: Apocynaceae) were collected from Hyderabad, Andhra Pradesh, India. The plant material was taxonomically identified by Dr. Vastavaya S. Raju, Head, Department of Botany, Kakatiya University, warangal. The voucher specimen (No: KS 04/11) has been deposited in our laboratory for future reference. The flowers were dried under shade and then powdered with a mechanical grinder and stored in airtight container.

Animals

Male Wistar albino rats weighing 180-200g and Swiss albino mice, aged 1 month weighing 25 ± 5g were used for the present study. They were procured from Mahaveer enterprises, Medipalli, Hyderabad, India. The animals were housed in poly acrylic cages (38cm × 23cm × 10cm) at an ambient temperature of 18±2^{0}C with 12/12 hrs dark–light cycle. They had free access to standard feed pellet diet, under good management conditions and water *ad libitum*. Maintenance and handling of animals were performed according to the rules and regulations of Institutional Animal Ethical Committee (VCOP/2011/10/4/14).

Chemicals

Paracetamol, diclofenac sodium were used as the standard drugs. They were a kind gift from Farmson Pharmaceutical Pvt. Ltd. Gujarat. Brewer's yeast was used to induce pyrexia. All other reagents used are of analytical grade.

Preparation of solvent extracts

The coarsely powdered PRRP and PRLW flowers (750gm) were extracted successively using n-hexane, ethyl acetate, chloroform (60-80^{o}C) and methanol by soxhlation. The solvent was removed by distillation, the extracts thus obtained were weighed. The color, nature, percentage yields were calculated.

Phytochemical analysis

The obtained extracts of PRRP and PRLW flowers were subjected to preliminary phytochemical screening by performing standard qualitative analysis[8]. Various standard chemical tests were used to detect the presence of alkaloids, glycosides, flavanoids, saponins, tannins, steroids, terpenoids, phenolic compounds, proteins, amino acids and carbohydrates and chromatographic analysis was done by using Thin Layer Chromatography by visual observation under UV lamp at 254 nm, developed in an iodine chamber and spots were located[9,10].

Acute toxicity study

Acute toxicity study was carried out in albino mice by acute toxic class method of OECD guideline no. 423. This method is a step wise procedure with three animals of a single sex per each step and used defined doses (5, 50, 300, 2000mg/kg) of MEPR & MEPL. Depending on the mortality of the animals, 2-4 steps may be necessary to allow judgment on the toxicity of the test substance[11,12].

Analgesic activity

Evaluation of analgesic activity of methanolic extracts of the flowers was carried out by the chemical, mechanical and thermal noxious stimuli.

Acetic acid-induced writhing

Writhing is induced by chemical substances due to sensitization of nociceptors by prostaglandins. Male albino mice, weighing 18–25g, were randomly divided into six groups, six animals each. In this method, acetic acid is administered intraperitoneally (i. p.) to the experimental animals to create pain sensation. Diclofenac sodium was used as standard (10mg/kg b. w). The *P. rubra* extracts (MEPR & MEPL) were administered orally in two different doses (250 and 500mg/kg) to the mice after an overnight fast. The animals were administered control, standard and test extracts. In the present study, test samples and vehicle were administered orally 60 min prior to i.p. administration of 0.75% v/v acetic acid solution (0.1ml/10g) but diclofenac sodium was administered 15 min prior to acetic acid injection. Then the animals were placed on an observation table. Each mouse of all groups were observed individually for counting the number of writhes made by the animals in 15 min commencing just 5 min after the i.p. administration of acetic acid solution. Full writhing was not always accomplished by the animal, because sometimes the animals gave incomplete writhes which were considered as half-writhing. Accordingly, two half-writhings were taken as one full writhing. The number of writhes in each treated group was compared to that of a control group[4,13,14].

Tail immersion method

Animals were randomly divided into six groups, six animals each. Prior to the experiment, all animals were screened for sensitivity test by immersing the tail of mice gently in water maintained at 55^{0}C. The animal dislodging the tail from hot water within 5 sec was selected for study. The reaction time was recorded by a stop watch. After the determination of reaction time, the tail was carefully dried. In the present study the reaction time was determined before oral

feeding of the drug and various extracts, which was recorded as zero min reading. After oral administration of two doses (250 and 500mg/kg) of MEPR, MEPL and standard diclofenac (10mg/kg), animal's tail was gently immersed in hot water and reaction time was noted for every 30 min upto 3 hrs[4,13,14].

Antipyretic activity:Brewer's yeast induced pyrexia in rats

In the present study albino rats were randomly distributed in six groups of six animals each. After 1 hr starvation, the rectal temperature of rats was recorded. Hyperthermia was induced by giving 1ml/kg of 15% suspension of brewer's yeast subcutaneously (s.c.) in the nape of the neck. Initial temperature was recorded. After 18 hrs animals that showed an increase in rectal temperature by 0.5^0C were selected. The thermometer was inserted about 2cm into the rectum of each rat for measuring the temperature. Control (0.5ml CMC solution), standard (paracetamol 100mg/kg, p.o.) and test extract MEPR and MEPL each (250, 500mg/kg) were administered to six groups respectively. Rectal temperature was determined after 18 hrs of treatment at 30, 60, 90 and 120 min[4,14-16].

Results

Extraction

Various extracts (n-hexane, ethyl acetate, chloroform and methanol extracts) of *Plumeria rubra* cultivar flowers were prepared by successive solvent extraction. The colour, nature and percentage yields of all the extracts are depicted in the Tables 1 and 2 which indicate that all the extracts of PRRP and PRLW flowers were similar in their colour and consistency but differed in their yields. The percentage yields of all the four extracts of PRRP flowers were found to be higher than that of PRLW flowers.

Phytochemical screening

Phytochemical analysis of the extracts revealed the presence of alkaloids (+ve test result for wagners), flavanoids (+ve result for Schinoda test), glycosides (+ve result for Borntrager's test), saponins (+ve result for Foam test), steroids (+ve result for Liebermann-Burchard's test), carbohydrates (+ve result for Molisch's test), phenolics/tannins (+ve result for $FeCl_3$ test) and absence of proteins (-ve result for Millons test) as shown in Table 3. The methanolic extracts (MEPR & MEPL), rich in phytochemical constituents, were screened for analgesic and antipyretic activities. They were analyzed by TLC using precoated plates using the solvent system n-hexane: ethyl acetate (4.7: 0.3). Five spots were identified for both PRRP and PRLW flowers whose R_f values were found to be 0.8, 0.7, 0.4, 0.3. 0.2 and 0.8, 0.6, 0.5, 0.3, 0.1 respectively. Plates were visualized by spraying with 50% H_2SO_4 and appeared yellow in colour with white fluorescence under UV light.

Acute oral toxicity study

All experimental animals were observed for 14 days for any toxic signs. No mortality and toxic signs were observed during period of study from which it was clearly understood that both MEPR and MEPL were found to be safe even at dose 2000mg/kg (Table 4). Further, there was no change in body weight and gross behavioral characters of the tested animals and since LD_{50} was found to be > 2000mg/kg, MEPR and MEPL were selected for the study at dose of 250mg/kg and 500mg/kg.

Tail immersion method

In tail immersion method the extracts considerably increased the animal's reaction to heat stimulus (Fig. 1). The extracts showed a significant difference to that of control animals. The effects were dose-dependent at the concentrations tested for

MEPR & MEPL (250mg/kg & 500mg/kg). The analgesic activity for both the extracts at 500mg/kg was comparable to that of the standard diclofenac (10mg/kg). However, MEPR was found to be more potent than MEPL.

Acetic acid induced writhing test

The MEPR & MEPL extracts strongly reduced the abdominal constrict-tions induced by the i.p. administration of acetic acid solution (Fig. 2). The effects produced by MEPR &MEPL were dose dependent and the values were found to be significant ($p<0.001$) at the dose tested, compared to the control. MEPR at 500mg/kg dose exhibited significant inhibition (58.6%), comparable to that of the standard diclofenac at 10mg/kg (68.6%).

Antipyretic activity

The s.c. injection of yeast suspension markedly elevated the rectal temperature after 18 hrs of administration. MEPR & MEPL extracts at the doses of 250 and 500mg/kg showed significant decrease in yeast-induced fever in rats in a dose dependent manner compared to the standard paracetamol (100mg/kg; p.o.) (Table 5). The activity started at 1hr and was maintained for 4hrs after administration.

Discussion

This study has shown the highest yield in methanolic extract. The phytochemical analysis of the methanolic extract revealed that it contains alkaloids, flavanoids, saponins, steroids, tannins, glycosides and carbohydrates. Flavanoids and saponins are well known inhibitors of pain perception[17]. Administration of MEPR and MEPL to the experimental animals produced a significant effect in all the treated models. Both the extracts (MEPR and MEPL) protected mice against both thermal and chemical induced noxious stimuli, which were evidenced from both the tail immersion and acetic acid-induced writhing tests. Variation in order of activity for MEPR in acetic acid-induced writhing and tail immersion tests indicated that the phytoconstituents (flavonoids and phenolic compounds) present in MEPR may be responsible for central and peripheral analgesia. Acetic acid, which is used as an inducer for writhing syndromes, causes algesia by releasing endogenous substances, which then excite the pain nerve endings; the abdominal constriction is related to the sensitization of nociceptive receptors to prostaglandins. It is possible that *Plumeria rubra* flower exerts an analgesic effect probably by inhibiting the synthesis of prostaglandins.

Plumeria rubra also exhibited significant antipyretic activity when compared to that of control and it was found that methanolic extracts of *Plumeria rubra* flowers at the doses of 250 and 500mg/kg showed significant decrease in yeast-induced fever in rats. This result seems to support the view that *Plumeria rubra* extracts have some influence on prostaglandin biosynthesis because prostaglandin is believed to be a regulator of body temperature. Hence the possible mechanism of action of *Plumeria rubra* extract may be due to inhibition of COX-2.

Conclusion

The results obtained in this study indicate that the methanolic extract possesses potent antipyretic and antinociceptive properties, which are mediated via peripheral and central inhibitory mechanisms. The analgesic and antipyretic activities of MEPR and MEPL were good in their higher dose i.e., 500 mg/kg. Further MEPR was found to be more potent than MEPL; the presence of flavonoids and phenolic compounds were

thought to be responsible for the above activities.

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Table 1. Colour, nature and percentage yields of PRRP flowers extracts

S.NO	EXTRACT	%YIELD	COLOUR	CONSISTENCY
1	n-Hexane extract	6.2% w/v	Yellowish black	Resinous
2	Ethyl acetate extract	2.4% w/v	Brownish black	Resinous
3	Chloroform extract	3.7% w/v	Greenish black	Resinous
4	Methanol extract	11.6% w/v	Pinkish black	Resinous

Table 2. Colour, nature and percentage yields of PRLW flowers extracts

S.NO	EXTRACT	%YIELD	COLOUR	CONSISTENCY
1	n-hexane extract	4.2% w/v	Yellowish black	Resinous
2	Ethyl acetate extract	0.72% w/v	Brownish black	Resinous
3	Chloroform extract	2.7% w/v	Greenish black	Resinous
4	Methanol extract	9.6 % w/v	Pinkish black	Resinous

Table 3. Phytochemical screening of different type of extracts of flowers

Name of Test	*Plumeria rubra* f. *rubra* [pink flowers] extract				*Plumeria rubra* f. *lutea* [white flowers] extract			
	n-hexane	Ethyl acetate	Chloroform	Methanol	n-Hexane	Ethyl acetate	Chloroform	Methanol
Alkaloids	+	+	+	+	+	+	+	+
Flavanoids	-	+	-	+	-	+	-	+
Glycosides	-	+	+	+	-	+	+	+
Saponins	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+
Carbohydrates	+	+	-	+	+	+	-	+
Phenolics/tannins	-	+	-	+	-	+	-	+
Proteins	-	-	-	-	-	-	-	-

Table 4. Acute toxicity studies on *Plumeria rubra* flower extract

Groups(n=3)	Dose (mg/kg)	Lethality
I	50	No
II	100	No
III	200	No
IV	500	No
V	1000	No
VI	2000	No

Table 5. Antipyretic activity of *Plumeria rubra* on brewer's yeast induced pyrexia in rats

Group	Temperature of pyretic rats (^{o}F)				
	0 hr	1 hr	2 hr	3 hr	4 hr
Control	100.1±0.62	101.1±0.5	100.4±0.7	100.5±0.6	101.1±0.4
Standard	99.9±0.62	97.1±0.22***	96.9±0.43***	97.0±0.19***	96.5±0.12***
MEPL250mg/kg	99.9±0.37	99.1±0.25**	98.5±0.34**	98.7±0.29**	97.2±0.30***
MEPL500mg/kg	99.7±0.40	99.0±0.32**	98.4±0.46**	98.3±0.30***	96.7±0.37***
MEPR250mg/kg	99.7±0.29	99.0±0.24**	98.3±0.30***	98.2±0.23***	96.9±0.15***
MEPR500mg/kg	99.8±0.27	98.7±0.46***	97.3±0.43***	97.5±0.10***	96.5±0.28***

n=6, all values were expressed as mean ± S.D., **p<0.01 and ***p<0.001 in response to control.

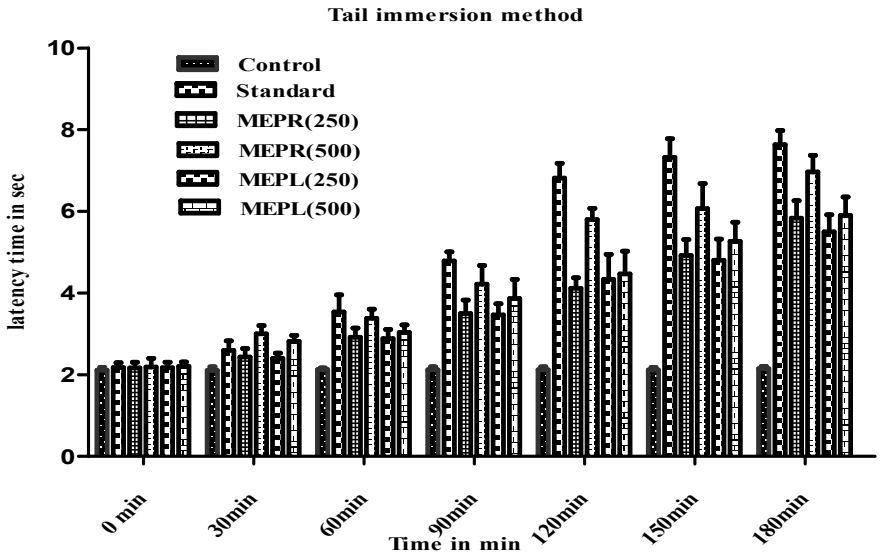

Fig. 1. Antinociceptive activity of *Plumeria rubra* by Tail immersion method

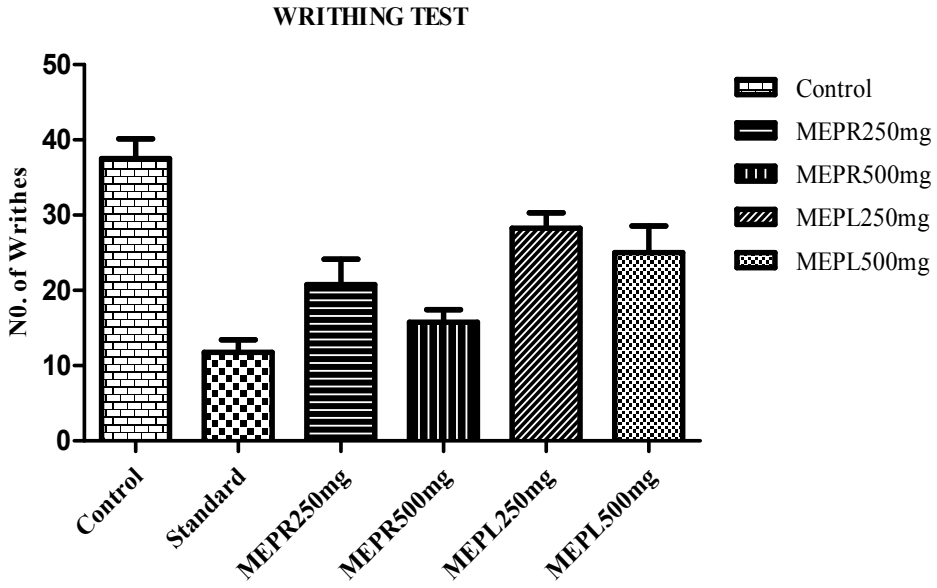

Fig. 2. Antinociceptive activity of *Plumeria rubra* by acetic acid induced writhing test

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