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Evaluation of antipyretic and analgesic activity of methanolic extract of *Rivea ornata* (roxb.) Leaves

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ABSTRACT

Riveaornata is a climbing or straggling shrub, widely distributed throughout the south Indian region. The juice of this plant is used topically in haemorrhagic diseases and piles. Aerial parts of this plant possess anti-inflammatory, anti-bacterial and anti-oxidant activities. Traditionally, it is utilized to treat fever and to relieve the pain. Hence the methanolic extract of *Riveaornata* leaves (200mg/kg, *p.o.*) was examined for antipyretic activity in rats by Brewer's yeast induced hyperpyrexia method using paracetamol (150mg/kg, *p.o.*) as a reference standard and analgesic activity in mice by tail immersion and hot plate method using diclofenac (10mg/kg, *p.o.*) as a reference standard. The result showed that methanolic extract of *Riveaornata* (MERO) possess significant antipyretic and analgesic activity.

Keywords: *Riveaornata*, MERO, Anti pyretic, Analgesic, Paracetamol, Diclofenac

INTRODUCTION

Plants that possess therapeutic properties or exert beneficial pharmacological effects on the human body are generally designated as medicinal plants. Medicinal plants naturally synthesize and accumulate some secondary metabolites like alkaloids, sterols,

terpenes, flavonoids, saponins, glycosides, cyanogenics, tannins, resins, lactones, quinines, volatile oil etc. The medicinal plants have been used for the treatment of illness and diseases, since the dawn of time. [1]

Riveaornata (Roxb.) choisy from the family of Convolvulaceae. It is widely distributed throughout

the south Indian region. In traditional Tamil language, it is known as 'Machuttai' and in folklore it is named as 'Baravat' and 'Phaang'. [2]

Riveaornata is Erect shrub or scandent from woody rootstock; stems 1–2.5 m tall, whitish hairy. Leaves are orbicular to reniform, 10–15cm long and 6–20 cm broad, base cordate, apex rounded or emarginate, upper side glabrous, underside densely whitish tomentose. Flowers are sepals subequal, oblong to elliptic-oblong, 14–17 mm long and 8–9 mm wide. Fruits are subglobose, c. 20–30 mm diam., glossy brown colour. Seeds are 7–9 mm long and 4–6 mm wide, brown in colour, embedded in crumbly crust. [3]

The juice of this plant is used topically in haemorrhagic diseases and piles. [2] Aerial parts of this plant possess anti-inflammatory activity, [4] anti-bacterial activity [5] and anti-oxidant activity. [6] Traditionally, it is utilized to treat fever and to relieve the pain. Hence, the current study had been carried out with the methanolic extract of *Riveaornata* (MERO) to investigate its antipyretic activity against brewer's yeast-induced fever in rats using paracetamol as a reference standard and its analgesic activity in mice by tail immersion and hot plate method using diclofenac as a reference standard.

MATERIALS AND METHODS

Plant material

The whole plants of *Riveaornata* were collected from kolli hills during the month of April 2019. It was identified and authenticated by Prof. Dr. V. Nandagopalan, Dean of Science & Department Head, Botany, National College of Arts and Science (Autonomous), Trichy, Tamil Nadu, India.

Preparation of plant extract

The leaves of *Riveaornata* were collected then shade dried and coarsely powdered. About 500 g of the dry powder was defatted with petroleum ether and extracted with methanol continuously in soxhlet apparatus for 24 hrs, after 24 hrs, the solvent was evaporated to obtain the crude extract. The extract was then dried under vacuum and suspended in water before use.

Experimental animals

Healthy wistar albino rats weighing about 150 – 200g body weight and albino mice weighing about

20-25g body weight were procured from Venkateshwara enterprises, Bangalore. The animals were maintained at a well ventilated, temperature controlled (30°C ± 1°C) animal room for 7 days prior to the experimental period and provided with food and water *ad libitum*. The animals were acclimatized to laboratory conditions before the test. The protocol of this study was approved by the Institutional Animal Ethical Committee (IAEC) with approval number PCP/IAEC/004/2019.

Antipyretic activity

Brewer's yeast-induced hyperpyrexia in rats [7, 8]

Wistar albino rats of either sex weighing 150-200g were divided into three groups of six animals each. The animals were fasted overnight for 18h before experiment but water was provided *ad libitum*. The dosage of the drugs administered to the different groups as follows

- Group I : 12.5% w/v yeast suspended in a 0.5% w/v normal saline (10ml/kg, b.w., sc.) + Normal saline at dose of (5ml/kg, b.w., sc.)
- Group II : 12.5% w/v yeast suspended in a 0.5% w/v normal saline (10ml/kg, b.w., sc.) + Methanolic extract of *Riveaornata* (200mg/kg, b.w., p.o.)
- Group III : 12.5% w/v yeast suspended in a 0.5% w/v normal saline (10ml/kg, b.w., sc.) + Paracetamol (150mg/kg, b.w., p.o.)

Experimental Design

The body temperature of each Wistar albino rat was recorded by measuring rectal temperature at predetermined intervals. The rats are trained to remain quiet in a restraint cage. A thermister probe is inserted 3 to 4 cm into the rectum and fastened to the tail by adhesive tape. Temperature is recorded on a thermometer. After measuring the basal rectal temperature, animals were given subcutaneous injections of 10 ml/kg of 12.5% w/v yeast suspended in a 0.5% w/v normal saline (10ml/kg, b.w., sc.). At the 19th hour after yeast injection the rectal temperature of the rats were recorded then Normal saline at a dose of 5ml/kg was injected to the group I. Methanolic extract of *Riveaornata* was administered with dose 200mg/kg to group II animals. The group III rats were received the standard drug paracetamol

at the dose of 150 mg/kg. Subcutaneous injection of yeast suspension markedly increased the rectal temperature. Rectal temperature of all the rats was recorded again on the 20th, 21st and 22nd hour after yeast injection.

ANALGESIC ACTIVITY

Tail Immersion Test in Mice [9, 10]

Wistar albino mice of either sex weighing 20-25g were divided into three groups of six animals each. The animals were fasted overnight for 18h. The dosage of the drugs administered to the different groups was as follows

- Group I : Control - Normal Saline (5ml/kg, b.w., p.o.)
- Group II : Standard (Diclofenac sodium 10mg/kg in 10mL of normal saline, p.o.)
- Group III : Test (Methanolic extract of *Riveaornata* 200mg/kg, p.o.)

$$\text{MPA} = \frac{\text{Reaction time for treatment} - \text{Reaction time for saline}}{15 - \text{Reaction time for saline}} \times 100$$

Hot plate method [11,12]

Wistar albino mice of either sex weighing 20-25g were divided into three groups of six animals each. The animals were fasted overnight for 18h. The dosage of the drugs administered to the different groups was as follows

- Group I : Control - Normal Saline (5ml/kg, b.w., p.o.)
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- Group III : Test (Methanolic extract of *Riveaornata* 200mg/kg, p.o.)

$$\text{MPA} = \frac{\text{Reaction time for treatment} - \text{Reaction time for saline}}{15 - \text{Reaction time for saline}} \times 100$$

Statistical analysis

The results were expressed as Mean ± SEM (n=6) two way ANOVA using a Graph pad and PRISM software version 8.2.1. (441). *** P<0.001, ** P<0.01 and *P<0.05 were considered as statistically significant.

Experimental Design

The procedure is based on the observation that NSAID drugs are selectively capable of prolonging the reaction time of the typical tail-withdrawal reflex in mice induced by immersing the end of the tail in warm water of 55°C.

The lower 5 cm portion of the tail is marked. This part of the tail was immersed in to the water bath of exactly 55 °C. Within a few seconds the rat reacts by withdrawing the tail. After each determination the tail was carefully dried. The reaction time was determined before and periodically after oral administration of the test and standard substance. The cut off time is 15 seconds. If the reading exceeds 15 sec, it would be considered as maximum analgesia. The maximum possible analgesia (MPA) was calculated as follows:

The mice were placed on a hot plate maintained at 55°C within the restrainer. The reaction time (in seconds) or latency period was determined as the time taken for the mice to react to the thermal pain by licking their paws or jumping. The reaction time was recorded before and at 30, 45, 60 and 90 minutes after the administration of the treatments. The maximum reaction time was fixed at 15 sec to prevent any injury to the tissues of the paws. If the reading exceeds 15 sec, it would be considered as maximum analgesia. The maximum possible analgesia (MPA) was calculated as follows:

RESULTS

Antipyretic activity

Yeast-induced hyperpyrexia in rats

Subcutaneous injection of yeast suspension markedly elevated the rectal temperature after 19 hours of administration. Treatment with the methanolic extract of *R. ornata* at the dose of

200mg/kg significantly decreased the rectal temperature of the rats. The antipyretic effect started from the first hour and the effect was maintained for 5 hours, after administration of the extract. The result

obtained from both the standard (paracetamol) and MERO treated rats were compared with that of control.

Table No. 1: Effect of MERO in Brewer’s yeast induced pyrexia

S. No.	Group	Basal Temperature (before yeast administration) (F)	Pre-Drug control (before drug administration) (F)	Rectal temperature after drug administration (F)				
				1 Hour	2 Hours	3 Hours	4 Hours	5 Hours
1	Control Normal Saline (5ml/kg)	97.54 ± 0.19	102.44 ± 0.05	102.42 ± 0.06	102.42 ± 0.10	102.39 ± 0.09	102.37 ± 0.08	102.34 ± 0.10
2	Standard Paracetamol (150mg/kg)	97.49 ± 0.14	102.21 ± 0.08	99.66 ± 0.04	99.15 ± 0.03	98.57 ± 0.03	97.82 ± 0.03	97.48 ± 0.03
3	Test MERO (200mg/kg)	97.56 ± 0.13	102.06 ± 0.19	99.81 ± 0.03	99.27 ± 0.05	98.8 ± 0.04	98.19 ± 0.03	97.77 ± 0.03

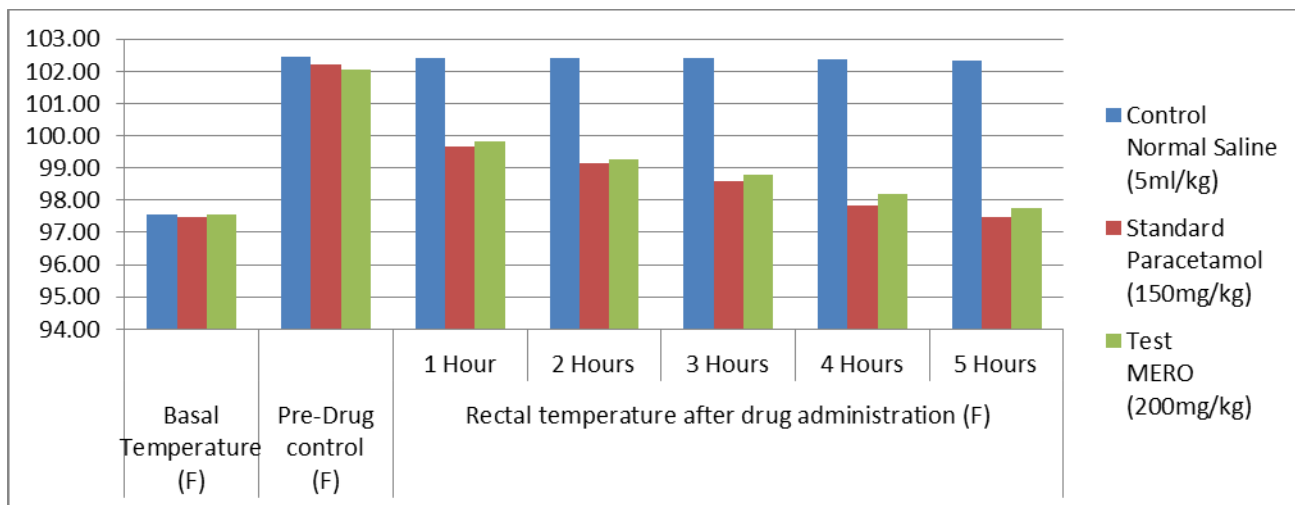


Figure No. 1: Effect of MERO in Brewer’s yeast induced pyrexia

Analgesic Activity

Tail immersion Method

Treatment with the methanolic extract of *R.ornata* at the dose of 200mg/kg significantly increases the

pain threshold during the observation period of 90 minutes as compared to the standard drug diclofenac 10mg/kg.

Table No. 2: Analgesic activity of MERO by Tail Immersion Method

S.No.	Drug/Dose	Reaction Time in Seconds				
		0 minute	30 minutes	45 minutes	60 minutes	90 minutes
1	Control Normal saline (5ml/kg)	1.72 ± 0.06	1.81 ± 0.04	1.8 ± 0.02	1.85 ± 0.04	1.81 ± 0.02
2	Standard Diclofenac (10mg/kg)	1.74 ± 0.02	3.1 ± 0.02	5.7 ± 0.02	7.6 ± 0.01	9.49 ± 0.02
3	Test MERO (200mg/kg)	1.81 ± 0.04	2.93 ± 0.02	4.61 ± 0.02	6.95 ± 0.02	8.37 ± 0.02

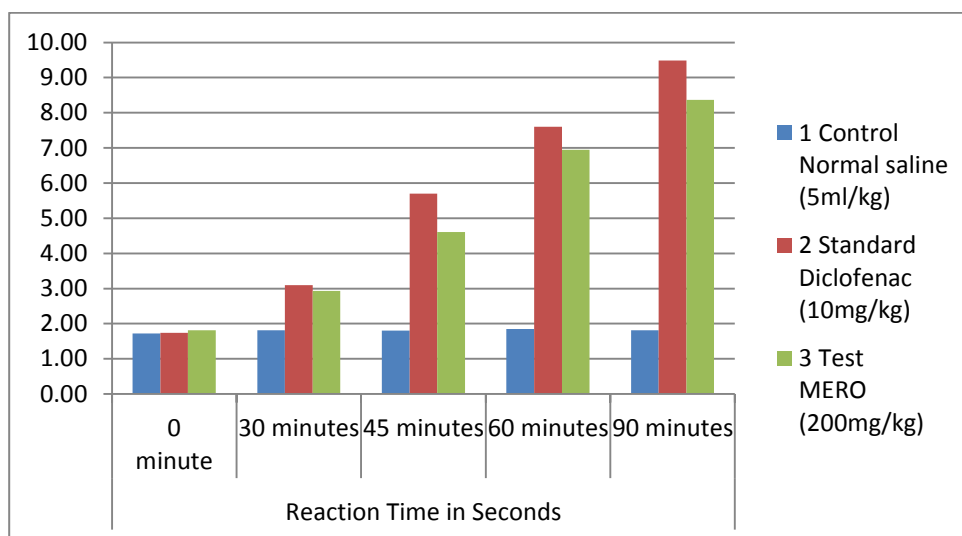


Figure No. 2: Analgesic activity of MERO by Tail Immersion Method

Hot plate Method

Treatment with the methanolic extract of *R.ornata* at the dose of 200mg/kg significantly increases the

pain threshold during the observation period of 90 minutes as compared to the standard drug diclofenac 10mg/kg.

Table No. 3: Analgesic activity of MERO by Hot Plate Method

S.No.	Drug/Dose	Reaction Time in Seconds				
		0 minute	30 minutes	45 minutes	60 minutes	90 minutes
1	Control Normal saline (5ml/kg)	4.18 ± 0.06	4.1 ± 0.05	4.16 ± 0.04	4.15 ± 0.02	4.12 ± 0.04
2	Standard Diclofenac (10mg/kg)	4.22 ± 0.06	10.19 ± 0.04	11.55 ± 0.02	12.15 ± 0.05	12.21 ± 0.03
3	Test MERO (200mg/kg)	4.04 ± 0.04	8.77 ± 0.02	10.01 ± 0.02	10.14 ± 0.02	10.27 ± 0.02

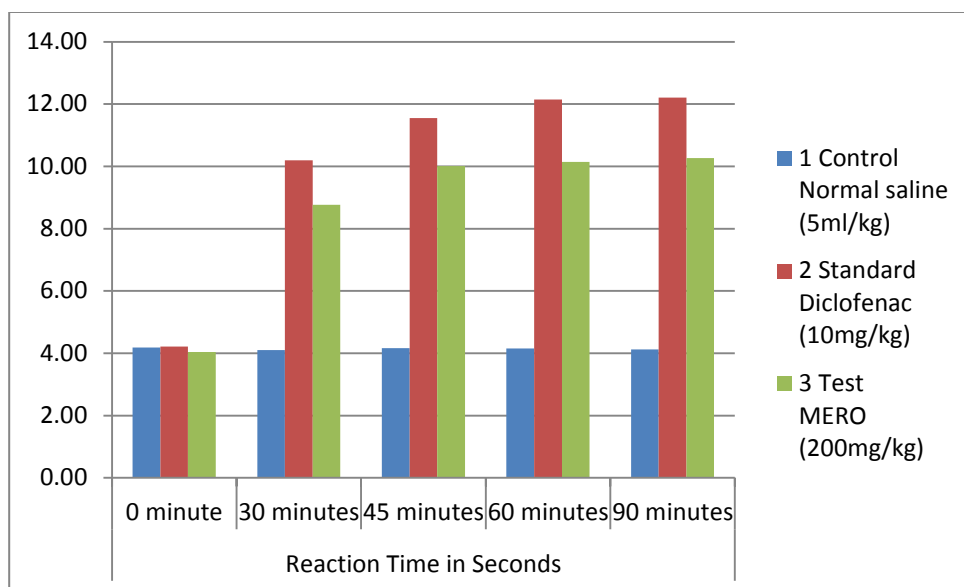


Figure No. 3: Analgesic activity of MERO by Hot Plate Method

DISCUSSION

In modern medication, the use of chemicals as medicine to treat various diseases leads to different adverse effects. Medicines from nature bounty have wide therapeutic range with minimal side effects. The drugs which are used as analgesics and antipyretics are Non steroidal anti-inflammatory drugs (NSAIDs). NSAIDs commonly produce gastro intestinal tract (GIT) damage (ulcerogenic). As per World Health Organisation (WHO) about 80% of world population rely on the traditional medicine to treat various diseases.^[13] Identification of newer drug from phytochemical constituents has wider safety margin with greater therapeutic index.

The antipyretic and analgesic activity of methanolic extract of *Riveaornata* was studied by employing Brewer's yeast induced hyperpyrexia method, tail immersion and hot plate method respectively. The result showed that methanolic extract of *Riveaornata* exhibits significant antipyretic and analgesic activity.

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