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Preclinical evaluation of analgesic activities of Barringtonia Racemosa

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Abstract
Background: Pain is the major devastating health problems. Barringtonia racemosa Roxb. (Lecythidaceae) is the one which is frequently used to treat pain by traditional healers in Ethiopian folk medicine. However, the plant has not been scientifically evaluated for its traditionally claimed use. The present study aimed at the investigation of analgesic activities of ethanol root extract of Barringtonia racemosa Roxb in Albino rats (Wistar) model.
Methods: Successive maceration was used as a method of extraction using solvents ethanol. After extraction of the roots, the crude extract was evaluated for its analgesic activities using tail flick and hot plate method. The extract was evaluated at 100, 200 and 400 mg/kg doses. The positive control groups were treated with Aspirin 20 mg/kg i.p, distilled water (10 mL/kg) treated rat were assigned as negative controls.
Results: Barringtonia racemosa Roxb root extract at all test doses showed statistically significant antinociceptive activity in both model in a dose dependent manner ($p < 0.01$ and $p < 0.001$). The greater analgesic activity was observed by the maximum dose of the extract (400 mg/kg) in both tail flick and hot plate method method.
Conclusion: In general, the data obtained from the present study elucidated that the extract possessed a significant analgesic activities and recommended for further studies.

Keywords: Analgesic activity, Barringtonia racemosa Roxb, Tail flick and Hot plate

Introduction
Plants have long provided mankind with medicine, with natural products once serving as the source of all drugs [1]. The rural population of the country is more disposed to traditional ways of treatment because of its easy availability and cheaper cost [2]. Inflammatory and infectious diseases are among those treated using traditional medicine. Inflammation is the response of living tissues to injury; acute and chronic inflammations are a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair [3]. It is known that acute inflammatory response consists of three main vascular effects, namely vasodilation and consequent increased vascular flow; increased vascular permeability and leucocytes migration. Histamine and 5-Hydroxy tryptamine are usually responsible for eliciting the immediate response of inflammation in rats whereas kinins and prostaglandins mediate the more prolonged delayed onset responses [4]. Anti-inflammatory agents exert their effects through a spectrum of different modes of action [5]. Barringtonia racemosa Roxb. (FamilyLecythidaceae) is a moderate evergreen tree with drooping branches found on the west coast of India from Konkan southwards, the Sundarbans, Assam and the Andaman Islands [6] (Plate 1). The plant has a wide range of therapeutic applications. The roots have deobstructant and cooling properties. The fruits are efficacious in cough, asthma and diarrhoea. The seeds are used in colic and opthalmia. The bark and the leaves are used for rat and snake bites, on boils and in gastric ulcer [7, 8]. In certain remote areas of Kerala, these seeds are being used to treat cancer like diseases [9]. Secondary metabolites such as diterpenes, triterpenoids and flavonoids (including polyphenols), steroids and saponins have previously been isolated from B. racemosa [10]. The fruit kernels contain two sapogenins namely, barringtogenol and barringtogenic acid [11].

Materials and Methods

Plant material

The Roots of *Barringtonia racemosa*, was selected for investigation and were procured from Sri Venkateswara University, Tirupathi, Andhra Pradesh, India. The plant material was taxonomically identified and authenticated by Dr. Madhavachetty, Head of Department, Botany, Sri Venkateshwara academia, Tirupathi, Andhra Pradesh. The chit sampling of the hedge plant partakes stood dumped in the Division of Pharmacognosy, of our institution.

Preparation of extract

The Roots of *Barringtonia racemosa* were washed isolated, chopped into small pieces and air dried under shade at room temperature for seven days. The dried parts were powdered by a mechanical grinder and passed through sieve 40 mesh sieve and stored in a closed vessel for future use. This powder was used for the preparation of aqueous and other extracts. The root powder was defatted with petroleum ether. Defatted 500 gm of powder was extracted by 95% ethanol in a soxhlet apparatus for 72hours as by cold maceration followed by concentrated in a rotator evaporator under reduced pressure at temperature 40-50℃ and then lyophilized to get a dry residue. Some part of the total extract was used for qualitative and quantitative phytochemical investigation and rest of the extract was used for preliminary pharmacological screening.

Preliminary Phytochemical Analysis [12]

The extracts were subjected to preliminary phytochemical screening for detection of major chemical groups. In each case test 10% w/v solution of the extract in different solvents were used.

Experimental animals

Albino rats (Wistar) weighing 150-200g and Swiss albino mice weighing 20-25g of either sex were used in this study. They were procured from Sri Venkateshwara Enterprises, Hyderabad. The animals were housed, stabilized for 1 week; they were maintained under standard condition at 25℃ ± 2℃ temperature; 60 ± 5% relative humidity and 12-hour light dark cycle. They had been given standard pellet diet obtained from Gold Mohur Lipton India Ltd., and water ad libitum throughout the course of the study. The litter in the cages is renewed thrice a week to ensure hygeinity and maximum comfort for animals. Ethical clearance was obtained from Institutional Animal Ethical committee (IAEC) _____

Acute Toxicity Studies [13]

The acute toxicity test aims at establishing the therapeutic index. The acute toxicity study was done according to OECD (Organization of Economic Co-operationand Development) guidelines 420- Fixed Dose Procedure (FDP). The suspension of ethanolic roots extract of *Barringtonia racemosa* (EREBR) was administrated orally to overnight fasted Albino Wistar Rats (number of animals = 6) in dose of 1000, 2000 and 4000 mg/kg body weight respectively. The animals were observed continuously for the initial 4hrs for behavioral changes and mortality and intermittently for the next 6 h and then again at 24 hrs and 48 h after dosing.

Selection of Doses: In this study dose of 1000 to 4000 mg / kg was found to be safe and no mortality was observed, so dose 1/10th i.e. 100, 200 and 400 mg / kg of the extract was chosen for the experimentation.

For screening of Analgesic activity, mice and rats were divided into five different groups. The first group served as a control group. The second group was used as reference standard. Three groups received extracts of plants at three different doses (100, 200 and 400 mg/kg).

Evaluation of Analgesic Activity

Hot plate method [14]

Principle: The method originally described by Woolfe and Mac Donald .The paws of rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The time until these responses occur is prolonged after administration of analgesics is recorded.

Procedure: Following administration according to respective grouping rats were placed on a hot plate maintained at 55 ± 1 ℃. Latency of nociceptive response such aslicking, flicking of a hind limb or jumping was measured. Measurements were performed at time 0 before and 30, 60 and 120 min after drug administration, with a cut-off time of 15 seconds to avoid lesions to the animal paws

Group I: Served as control (vehicle treated) 10 ml/kg orally for seven days,

Group II: Served as positive control (Aspirin treated) 20 mg/kg intraperitoneally as a standard drug for seven days,

Group III: Received ethanolic roots extract of Barringtonia racemosa (EREBR) 100 mg/kg orally for seven days

Group IV: Received ethanolic roots extract of Barringtonia racemosa (EREBR) 200 mg/kg orally for seven days

Group V: Received ethanolic roots extract of Barringtonia racemosa (EREBR) 400 mg/kg orally for seven days

The basal reaction was time taken by observing hind paw licking or jump response (whichever appear first) in animals while placed on hot plate, which was maintained at constant temperature 55^0C. A cut off period of 10 seconds was observed to avoid damage to the paws. The percentage increase or decrease in reaction time (as index of analgesia) at each time interval was calculated.

Tail-flick method [15]

Principle: The application of thermal radiation to the tail of rats provokes the withdrawal of the tail by a brief vigorous movement. It is the reaction time of this movement that was recorded.

Procedure: Tail-flick latency was assessed by the Analgesiometer. The strength of the current passing through the naked nichrome wire was kept constant at 5 ampere. The distance between heat source and the tail was 1.5 cm and the application site of the heat on the tail was maintained within 2 cm, measured from the root of the tail. To avoid any tissue injury during the process the cutoff reaction time was taken as 10 sec. The time taken by Rat to withdraw (flick) the tail was taken as the reaction time. The animals were subjected to the same test procedure at 0 before and 30, 60, and 120 min after administration of treatment as described in the grouping and dosing section.

Group I: served as control (vehicle treated) 10 ml/kg orally for seven days,

Group II: served as positive control (Diclofenac sodium 10 mg/kg orally) as a standard drug for seven days,

Group III: received ethanolic roots extract of *Barringtonia racemosa* (EREBR) 100 mg/kg orally for seven days

Group IV: received ethanolic roots extract of *Barringtonia racemosa* (EREBR) 200 mg/kg orally for seven days

Group V: received ethanolic roots extract of *Barringtonia*

racemosa (EREBR) 400 mg/kg orally for seven days

Statistical Analysis

Results were expressed as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test. $P < 0.05$ was considered statistically significant.

Results

Herbal drugs play a restorative role in many diseases; most of them speed up the natural healing process. Previous literature indicates that a number of plant extracts and compounds isolated from various plant sources and minerals have shown activity against the various disease conditions. In the present investigation we have studied the Analgesic Activity of ethanolic roots extract of *Barringtonia racemosa* in Albino Wistar Rats.

Phytochemical studies

Table 1: Preliminary Phytochemical studies of Ethanolic roots extract of *Barringtonia racemosa*

Sl. No.	Phytoconstituents	Results
1	Reducing sugars	+
2	Alkaloids	+
3	Glycosides	+
4	Steroids	-
5	Gums	+
6	Saponins	-
7	Flavonoids	+
8	Tannins	-
9	Terpenoids	+

Acute toxicity studies

No mortality and signs of any toxicity were evidenced after the administration of a limit dose of 1000 mg/kg, 2000 mg/kg and 4000 mg/kg ethanolic roots extract of *Barringtonia racemosa* in acute oral toxicity test hence, for oral administration the doses selected were 100 mg/kg, 200 mg/kg and 400 mg/kg.

Analgesic activity

Tail flick method evaluation

For evaluation of analgesic activity by tail flick method in Group 1 *(*Control) Normal saline was administered. In Group 2 (Standard) Aspirin 20 mg/kg orally was administered. In Group 3 ethanolic roots extract of *Barringtonia racemosa* in dose of 100 mg/kg was administered orally. In group 4 ethanolic roots extract of *Barringtonia racemosa* in dose of 200 mg/kg was administered orally. In group 5 ethanolic roots extract of *Barringtonia racemosa* in dose of 400 mg/kg was administered orally.

In Group 1 there was no Analgesic Effect seen at 0, 30, 60 and 120 minutes. In group 2 the response to heat application was not seen at 0 minute interval but the Tail flick latency period was slightly increased after 30 minutes which gradually increased after 60 and 120 minutes. In Group 3 at 0 minute there was no analgesic effect seen, but Tail flick latency period increased at 60 and 120 min. In Group 4 the analgesic effect was Tail flick latency period increased between 60 and 120 minutes. In Group 5 the analgesic effect was not seen at 0 minutes but Tail flick latency period increased progressively from 30 minute to 60 min and up to 120 min. The highest Tail flick latency period was observed in Group 2 and 5 at 120 min. At all time of point, the tail-flick latency period differed significantly between the extract and Aspirin treated Groups being greater in the Group 2. Comparing different doses of the extract revealed that there is positive relationship between reaction time and increase dose of the extract in which, protection against heat application with 400 mg /kg was significant compared to all doses of the extract.

Table 2: Analgesic Activity by Tail Flick Method

Groups	0 min	30 min	60 min	120 min
Group I (Control)	5.33± 0.51^{ns}	5.00 ±0.00 ^{ns}	5.83 ±0.75^{ns}	6.66 ± 0.51^{ns}
Group II (Standard)	5.33 ± 0.51 ^{ns}	7.66 ± 1.36 **	10.50 ± 1.04**	13.00 ± 0.89**
Group III (EEBR 100 mg/kg)	5.50 ± 0.54^{ns}	6.00 ± 0.63*	7.66 ± 0.51**	8.66 ± 0.51**
Group IV (EEBR 200 mg/kg)	5.50 ± 0.54 ^{ns}	8.50 ± 0.54**	9.00 ± 0.63**	11.33 ± 0.81**
Group V (EEBR 400 mg/kg)	5.5 ± 0.54^{ns}	7.5 ± 0.54 **	9.33 ± 1.03**	12.00 ± 0.89**

*P < 0.01-significant, **P<0.001- highly significant, values are tail Flick Latency time in seconds

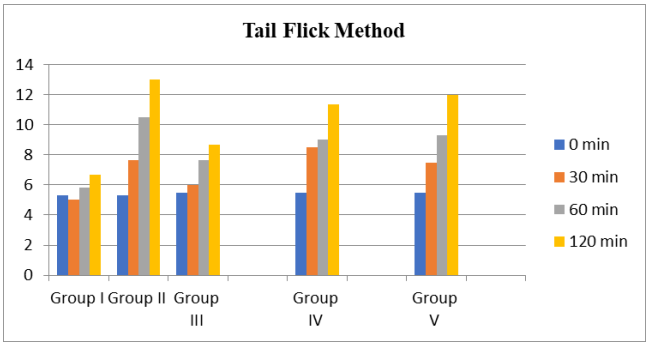

Fig 1

Hot Plate Method

For evaluation of analgesic activity by Hot Plate Method in group 1(control) Normal saline was administered. In group 2 (standard) Diclofenac sodium 10 mg/kg was administered orally. In group 3 ethanolic roots extract of *Barringtonia racemosa* in dose of 100 mg/kg was administered orally. In group 4 ethanolic roots extract of *Barringtonia racemosa* in dose of 200 mg/kg was administered orally. In group ethanolic roots extract of *Barringtonia racemosa* in dose of 400 mg/kg was administered orally. In Group 1 there was no analgesic effect seen at 0, 30, 60 and 120 minutes. In group 2 the response to heat application was not seen at 0 minute interval but the prolongation of reaction time was slightly increased after 30 minutes which gradually increased after 60 and 120 minutes. In Group 3 at 0 minute there was no analgesic effect seen, but prolongation of reaction time increased at 60 and 120 min. In Group 4 the prolongation of reaction time effect was increased between 60 and 120 minutes. In Group 5 the analgesic prolongation of reaction time was not seen at 0 minutes but increased progressively

from 30 minute to 60 min and up to 120 min. The highest prolongation of reaction time was observed in Group 2 and Group 5 at 120 min. At all time of point the prolongation of reaction time differed significantly between the extract and Diclofenac treated Standard groups being greater in the Group 2. Comparing different doses of the extract revealed that there is positive relationship between reaction time and increase dose of the extract in which, protection against thermal stimuli with 400 mg /kg was significant compared to all doses of the extract.

Table 3: Analgesic Activity by Hot Plate Method

Groups	0 min	30 min	60 min	120 min
Group I (Control)	1.61 ± 0.12^{ns}	2.11 ±0.14^{ns}	2.16 ±0.12^{ns}	2.26 ± 0.10 ^{ns}
Group II (Standard)	1.56 ± 0.12 ^{ns}	3.3 ± 0.26 *	4.23 ± 0.15**	8.98 ± 0.26**
Group III (EEBR 100 mg/kg)	1.7 ± 0.08 ^{ns}	2.58 ± 020^{ns}	2.93 ± 0.018*	3.23 ± 0.16*
Group IV (EEBR 200 mg/kg)	1.71 ± 0.11 ^{ns}	2.8 ± 0.18*	4.06 ± 0.16**	7.16 ± 0.17**
Group V (EEBR 400 mg/kg)	1.8 ± 0.14 ^{ns}	4.1 ± 0.43**	6.3 ± 0.26**	7.88 ± 0.45**

*P < 0.01-significant, **P<0.001- highly significant, values are tail Flick Latency time in seconds

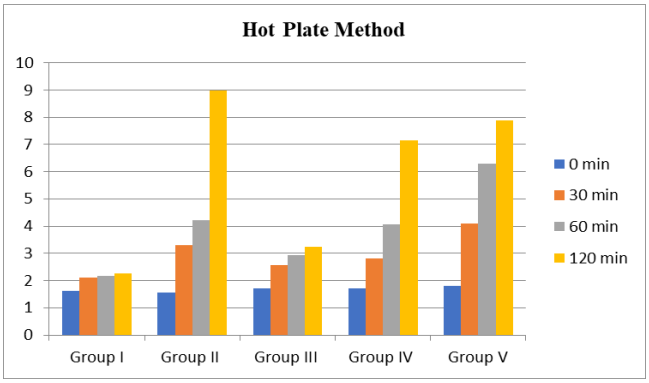

Fig 2

Discussion

Acute toxicity study revealed the non-toxic nature of the extract at dose of 4000 mg/kg. Experiment was carried out on normal healthy rats. No deaths were observed in rats the extract treated group and the behavior of the treated groups also appeared normal. There was no toxic reaction found at any dose selected until the end of the study.

In our study we found the presence of reducing sugar, glycosides, gum, flavonoids, terpenoids and alkaloids. Alkaloids group are mostly basic nitrogen atoms chemical compounds which are naturally occurring and possess a variety of pharmacological actions. We also found presence of flavonoids which are are a sub-group of polyphenolic compounds having a benzo-γ -pyrone structure and are ubiquitous in plants .Studies have consistently shown that high intake of flavonoids has protective effects against many infectious (bacterial and viral diseases) and also has a protective action against degenerative diseases such as cardiovascular diseases, cancers, and other agerelated diseases [16-18].

Based on the costs imposed to the society for pain and inflammation drug therapy and having the current knowledge about the numerous adverse effects of the available analgesics, the need for new analgesic drugs with higher efficacy and fewer side effects is need of the time. As a consequence searching for medicinal plants which have been widely used in traditional medicine to treat different pain conditions, and also yield novel substances, with anti-inflammatory activity are of especial importance in this regard. Therefore, *Barringtonia racemosa* which is commonly used in indian folklore medicine to treat different ailments and pain conditions was chosen for this study.

Pain induced by thermal stimulus by the hot plate model and thermal radiation by tail flick model is specific for centrally mediated activity. They were selected for this study because of several advantages including sensitivity to strong analgesics, limited tissue damage, accuracy of results and they are also less time consuming [19]. In the hot plate method of evaluation, a plate was heated at a constant temperature which produces two behavioral components which were measured in terms of their reaction times, namely paw licking and jumping. Both are considered to be supra-spinally integrated responses. As far as analgesic substances are concerned, the paw-licking behaviour is affected by opioids. Similarly the duration of jumping reaction is increased equally by less powerful analgesics such as acetylsalicylic acid or paracetamol [20].

In this method, duration of time for attaining peak activity was longer in the extract treated group at (60 min) than for the standard drug treated group at (30 min), this time gap may be due to the time delay between drug absorption and distribution into the target site or formation of an active metabolites that are capable of analgesic activity. This might also be the reason for the longer duration of analgesic activity of the extract at doses of 200mg/kg and 400mg/kg throughout the study period as compared to the standard. Better activity of 400 mg/kg of the extract compared to Diclofenac and Aspirin, suggests that there may be other constituents that contribute for the analgesic activity of the extract in addition to opioid like constituents.

Opioid-like central analgesic activity of the extract was assessed by using Tail Flick Method. The application of thermal radiation to the tail of rat provokes the withdrawal of the tail by a brief vigorous movement which is integrated spinally. Usually withdrawal time is within 2 &10sec. The lengthening of this reaction time by animal seen after administration of a drug is interpreted as analgesic action [21]. Unlike the hot plate test, in the tail flick test peak activity for the extract and aspirin was achieved at 30 min, and there was a constant decline in activity with time. The possible mechanism may be due to the adaptability to this method by animal leading to habituation and learning phenomena which leads to progressive shortening of response reaction time. There was also significant difference in the analgesic activity between the standard and extract treated groups at dose of (200mg/kg and 400mg/kg) throughout the study period. The results could be due to the presence of constituents which possess opioid-like activity and could have possibly interacted with opioid receptors even at low doses.

The effect of the extract on tail flick response and hot plate method are indicative of analgesic activity of ethanolic roots extract of *Barringtonia racemosa*. *Barringtonia racemosa* showed a central anti-nociceptive activity by increasing the

latency to discomfort and may have acted in a manner of the centrally active drugs, which by activating the periaqueductal grey matter (PAG) cause the release of endogenous peptides (i.e., endorphin or enkephalin). These endogenous peptides act via spinal cord and function as inhibitors of the pain impulse transmission at the synapse in the dorsal horn. The phytochemical ingredients found in the Leaf Extract of this plant like Flavonoids which potently inhibit prostaglandins, especially the endoperoxidase, tannins, alkaloids could also contribute to the anti-nociceptive action of this plant. Flavonoids, such as quercetins, are known to be effective in reducing inflammatory symptoms. These flavonoids possess potent inhibitory effects on various enzymes, such as protein kinase C, phospholipase A2 and phosphodiesterase. The mechanism of action postulated in these studies could be due to prostaglandin inhibition and the action on the central pain receptors [22].

A similar study was done by B. Medhi *et al*. where they found alcoholic extract of the root bark in mice using acetic acid-induced writhing method for analgesic activity .they found that root bark possess marked analgesic activity as evidenced through acetic acid induced writhing method [23].

Conclusion

The study shows that plant contains phytochemicals like reducing sugar, alkaloids, flavonoids, glycosides, terpenoidss could be responsible for the potential activity of the plant.

The ability of the extract to increase tail flick latency period and hot plate latency confirms the analgesic activities of the extract. The data collectively indicates the ethanolic roots extract of *Barringtonia racemosa* possesses analgesic properties, which are probably mediated by both central and peripheral inhibitory mechanisms as well as via inhibition of prostaglandin synthesis. The *Barringtonia racemosa* plant can therefore be proposed to have a potential benefit in the management of pain disorders.

In conclusion animal models have demonstrated the analgesic effects of *Barringtonia racemosa* root. Many phytochemicals may be involved in the analgesic process. Bioactive compounds such as flavonoids naturally present in *Barringtonia racemosa* root, may be involved in to analgesic process. Further studies should be devoted to investigate the potential analgesic action and the mechanism of action of other bioactive compound naturally present in *Barringtonia racemosa* root. Finally, human studies are needed to evaluate the analgesic properties of *Barringtonia racemosa* root also in human beings.

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