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EVALUATION OF ANALGESIC ACTIVITY OF EXTRACT OF *POLYALTHIA LONGIFOLIA* SEEDS ON EXPERIMENTAL ANIMALS

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ABSTRACT

Aim and Objective: The aim of this study was to evaluate analgesic activity of ethyl acetate extract of *Polyhalthia longifolia* seeds on experimental animals. **Method:** The analgesic activity of *Polyalthia longifolia* seed extract in mice was investigated by using Eddy's hot plate method. The mice were categorized into four groups: the control group received a vehicle solution, the standard group was administered Diclofenac sodium, and the test groups were given oral doses of *Polyalthia longifolia* seed extract at 200mg/kg and 400mg/kg, respectively. Analgesic activity was measured by assessing the mice basal reaction times on a hot plate maintained at 55±0.5°C. Observations were recorded at intervals of 0, 30,60, and 90 minutes after administration, with a 15-second cut off to avoid paw injury. The parameters like licking their fore or hind paws and jumping response were measured. **Results:** The administration of the dose (200mg/kg and 400mg/kg) of ethyl acetate extract of *Polyalthia longifolia* seed produces significant increase in the basal hot plate latency period ($P<0.01$, $P<0.001$). This increase in basal hot plate latency period of extract when compared to control, indicates the significant analgesic activity of the *Polyalthia longifolia* seed extract. **Conclusion:** The outcome of the present provides the evidence that the ethyl acetate extract of *Polyalthia longifolia* seeds exhibits a dose-dependent analgesic effect.

KEYWORDS: *Pain, Analgesic, Polyalthia longifolia, Eddy's hot plate.*

INTRODUCTION

Pain is a complex unpleasant physiological and psychological phenomenon composed of sensory experiences originating from damaged tissues or abnormal physical condition.[1] It is a designation for a spectrum of sensations of highly divergent character and intensity ranging from unpleasant to intolerable.[2] Pain is classified broadly into three main categories e.g., nociceptive, inflammatory and pathological pain.[3]

Every individual experiences pain in one or various forms in their life time.[4] Unrelieved acute pain can cause chronic pain and untreated chronic pain can cause anatomical and even genetic changes in the nervous system.[5] It is a major health concern that has a significant influence on life quality and economic status.[3] The perception of pain arises through a complex cascade of peripheral signaling, central processing, cortical activation and finally behavioral response.[6]

The pain reaction is transmitted over the reflex arc by sensory fibers in the dorsal horn of the spinal cord by synapsing motor neurons in the anterior horn. Due to harmful stimulus anatomic pattern of sensory and motor neurons move quickly, nerve impulses altering the individual to move away from such stimuli are simultaneous sent along efferent nerve fibers from the brain. Bradykinin, histamine, prostaglandins are major mediators of pain.[7]

Analgesic drugs are a group of drugs that have the activity of reducing pain without removing consciousness. Analgesics are used to relieve symptoms such as headache, toothache, menstrual pain, muscle pain, abdominal pain, fatigue and so on.[8] They are divided into opioids and non-steroidal anti- inflammatory Drugs (NSAID). Opioids are indicated in deep-seated visceral pain, while NSAIDs are in pain associated with inflammation and tissue injury.[9] However, prolonged use of these drugs causes severe adverse effects. Opioid analgesics like morphine, have a high potential for addiction and a number of negative side effects, such as drowsiness, nausea, respiratory depression and decreased gastrointestinal motility. While NSAIDs, pose a risk of toxicity to liver cells, renal glomeruli, the brain's cortex and cardiac muscles.[10] Analgesics decrease PGE2 production by acting at the COX enzyme and inhibits the bradykinin and lipoxygenase pathway of nociception.[11] NSAID's inhibit cyclo-oxygenase enzyme, which results in inhibition of prostaglandin synthesis.[7] Diclofenac sodium is a commonly used non-selective NSAID that is widely used to alleviate pain and comes in a variety of formulations.[12]

The analgesic profiles of many plant species widely used in folk medicine, such as *Hyptis pectinate* Poit. (Lamiaceae), *Hyptis fruticose* Salzm. ex Benth. (Lamiaceae), and *Erythrina velutina* Willd. (Fabaceae) have been studied.[13] Some of these plants include: *Allium sativum, Zingiber officinale, Nigella sativum, Albuca abyssinica, Ruta chalepensis,* and *Moringa stenopetala*.[5]

MATERIALS AND METHODS

Collection and authentication of *Polyalthia longifolia* seeds

Polyalthia longifolia seeds used for the present studies were collected from local areas of Puttur on August 2024. It was authenticated by Dr. Siddaraju M. N, Assistant Professor and Research guide, Dept of Botany, University College Mangalore.

Preparation of Ethyl Acetate Extract of *Polyalthia longifolia* Seeds[14]

The fresh seeds of *Polyalthia longifolia* were collected and air dried under normal environmental condition and homogenized to coarse powder. 200 g of grinded seeds were soaked and macerated in 1000 ml of ethyl acetate for 7 days at room temperature. Extracts were then filtered by using Whatman filter paper No.1. The filtrate was concentrated in vacuum using rotary evaporator (at optimum temperature between 40-45℃ to avoid denaturation of active ingredients). The extract was stored in refrigerator for further use.

Preliminary Qualitative Phytochemical Analysis[15]

Preliminary phytochemical screening was conducted to detect the presence of chemical constituents like alkaloids, glycosides, flavonoids, terpenoids, carbohydrates, steroids, amino acids, saponins.

Experimental animals

Healthy Swiss albino mice (20 to 25gms) of either sex used for the experiment were procured from the animal house of Srinivas College of Pharmacy, Mangalore. They were maintained under standard conditions (temperature 22 ± 2°C, relative humidity 60±5% and 12hr light/dark cycle). The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard palette diet and water ad libitum. The institutional animal ethics committee approved the experimental protocol (approval no. SCP/IAEC/JUL/2024-233). All the animals received human care according to the criteria outlined in the “Guide for the Care and Use of Laboratory Animals” prepared by the “National Academy of Science” and published by the “National Institute of Health”. The

animals were acclimatized for at least one week before use. All the procedures were performed in accordance with Institutional Animal ethics committee constituted as per the direction of CPCSEA, under the ministry of animal welfare division, Government of India, New Delhi, India.

Ethical considerations

All efforts were made to minimize animal suffering and to reduce the number of animals used in experiment. The animals received human care and all the experiments were conducted strictly in accordance with approved guidelines by the IAEC regulated by the CPCSEA. According to Government of India accepted principles for laboratory animals use and care. The study protocol was approved by IAEC, Srinivas College of Pharmacy, Valachil, Mangalore **(Ref no: SCP/IAEC/JUL/2024-233).**

Preparation of stock solution of the extract for dosing:

The ethyl acetate extract of *Polyalthia longifolia* was weighed and suspended in 1% tween 80.A fresh preparation of the extract was prepared before administration each time. The extract was administered post orally at the constant volume of 200mg/kg and 400mg/kg for each animal.

Dose Fixation[14]

Dose of 200mg/kg and 400mg/kg body weight was chosen as per previous works.

ANALGESIC ACTIVITY

Eddy's Hot Plate Method[16]

Purpose and rationale

Eddy's hot plate is commonly used in pharmacological research to measure the analgesic (pain- relieving) effects of drugs. This method is used to screen analgesic drugs by comparing an animal's reaction time before and after administering a test compound. If the reaction time increases after administration, it suggests that the compound has analgesic effects.

Apparatus

Eddy's Hot Plate consisting of 25x25cm heating surface with perspex enclosure and solid state temperature controller with Micro-controller based Digital Temperature Indicator controller to set surface temperature between 30° to 80°C.

Procedure

For this experiment, animals of each group were tested for paw lick or jump response after placing them on eddy's hot plate, and only those that reacted in the range of 3 to 5 sec were used for the experiment. Time interval from placing animals on the surface of the hot plate to licking of hind paws or jumping is termed as hot plate latency period. This is called a basal hot plate latency period. After recording the basal hot plate latency period of each animal in all four groups, selected animals of each group received their respective drug as per body weight. The animals of each group were placed on Eddy's hot plate analgesiometer one by one, which was maintained at 55 ± 1°C temperature 30 min after dosing. Hot plate latency period for each animal was noted by using stopwatch at 0, 30, 60 & 90min after dosing. The mean hot plate latency period for each group was calculated. Cut-off time was 15 sec. As after dosing response time increases, those who did not react till 15 sec were removed from hot plate to reduce chances of burn.

Experimental design[17]

The Swiss albino mice (20-25gms) of either sex were selected. The mice were randomly divided into following groups (n=6) as follows

Group I: Vehicle control (1% Tween-80 solution in water, 10ml/kg, p.o)

Group II: Standard group (Diclofenac sodium 10mg/kg, p.o)

Group III: Test sample (Ethyl acetate extract of *Polyalthia longifolia* 200mg/kg, p.o)

Group IV: Test sample (Ethyl acetate extract of *Polyalthia longifolia* 400mg/kg, p.o)

Treatment

The treatment was given through oral route. All animals were pre-treated with respective drugs 30 minutes before the evaluation.

Evaluation

The above mentioned parameter of standard and test compounds was carefully evaluated and compared to find the analgesic activity of the test compounds.

Statistical analysis

Results were prepared as Mean ± SEM. One way ANOVA was used followed by multiple comparison tests. For all tests, 'p' value of 0.05 or less was considered for statistical significance.

RESULTS

Extraction of plant material

The percentage yield of *Polyalthia longifolia* seed extract was calculated, and it was found to be 9.66%.

Table 1: Percentage yield of crude extract of *Polyalthia longifolia* seeds.

Solvent	Color	Method	Percentage yield
Ethyl acetate	Dark brown	Maceration	9.66%

Table 2: Preliminary phytochemical screenings of Ethyl acetate extract of *Polyalthia longifolia* seeds.

Sl. No.	Test	Result
1.	Carbohydrates	+ve
2.	Alkaloids	+ve
3.	Steroids	+ve
4.	Glycosides	+ve
5.	Saponins	+ve
6.	Flavonoids	+ve
7.	Terpenoids	+ve
8.	Tannins	-ve
9.	Phenolic compounds	-ve

Table 3: Effect of Analgesic activity of Ethyl acetate extract of *Polyalthia longifolia* seeds (EAEPLS) in mice using Eddy's Hot plate method.

Group No.	Treatment	Mean latency (sec) before and after drug administration				% inhibition		
		0 min	30 min	60 min	90 min	30 Min	60 min	90 min
I	Control	2.56± 0.17	3.01±0. 02	3.70±0. 19	3.77±0.3 4	-	-	-
II	Diclofenac Sodium 10mg/kg	2.49± 0.03	4.30±0. 21***	6.20±0. 39***	6.64±0.3 7***	30	40.32	43.22
III	200mg/kg EAEPLS	2.59± 0.05	3.63±0. 19**	4.70±0. 49**	5.10±0.5 5**	17.07	21.27	26.07
IV	400mg/kg EAEPLS	2.54± 0.24	4.19±0. 24***	5.93±0. 24***	6.22±0.1 6***	28.16	37.60	39.38
Values are expressed as Mean ± SEM; (n=6), ** P<0.01, *** P<0.001 compared to controlanimals. Data analysis was performed using Tukey's test								

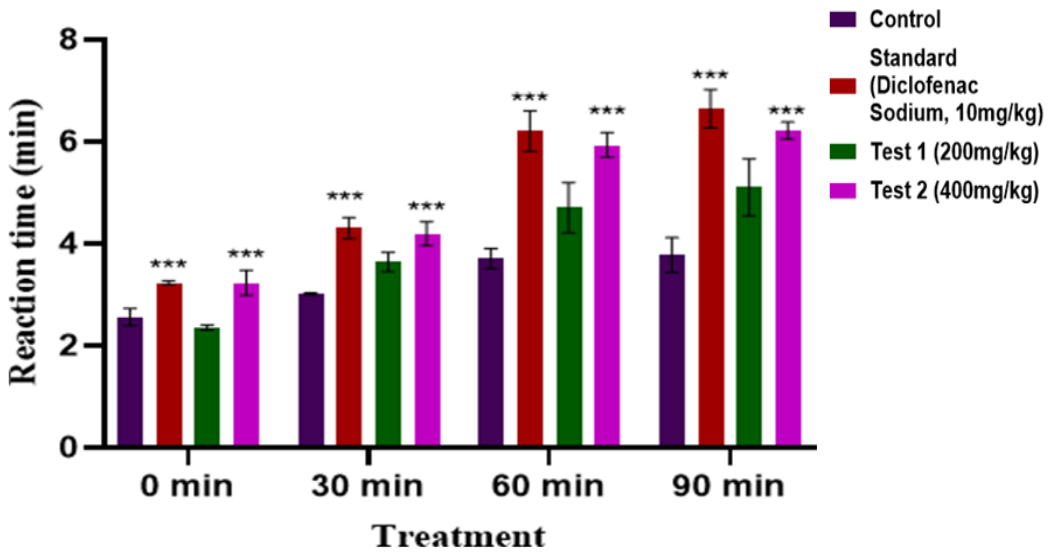

Fig. 1: Effect of Analgesic activity of EAEPLS in mice using Eddy's Hot Plate Method.

DISCUSSION

The purpose of this study was to evaluate analgesic activity of *Polyalthia longifolia* by using analgesic models. Stimulation of nociceptors or their afferent nerve endings causes pain. Therefore, people are always looking for solutions to eliminate or reduce pain.

Eddy's hot plate is commonly used in pharmacological research to measure the analgesic effects of drugs. This method is used to screen analgesic drugs by comparing an animals basal reaction time before and after administering a test compound. If the reaction time increases after administration, it suggests that the compound has analgesic effects.

Diclofenac sodium is an FDA-approved drug used in the treatment and management of acute and chronic pain. It shares similar adverse effects as other NSAIDs due to its inhibition of COX enzymes. However, since diclofenac seems to target COX-2 more selectively, it carries a higher risk of cardiovascular and gastrointestinal issues, so there is a shift of choice from allopathic to ayurvedic and naturopathy, where herbs and spices are very common ingredients of medicine.

The phytochemical analysis of ethyl acetate extract of *Polyalthia longifolia* seeds was conducted, establishing the presence of multiple phytoconstituents, including alkaloid, flavonoids, terpenoids, saponins, carbohydrates, glycosides and steroids possess analgesic activity. These phytochemicals inhibits prostaglandin synthesis by blocking cyclooxygenase(COX), thereby reducing inflammation and pain perception.

This study investigates the analgesic activity of the Ethyl acetate extract of *Polyalthia longifolia* seeds using animal models, specifically by Eddy's Hot Plate method. The study demonstrated that the effect of analgesic activity of ethyl acetate extract of *Polyalthia longifolia* seeds was comparable to that of standard drug, diclofenac sodium at a dose of 10 mg/kg. The extracts at the doses of 200 mg/kg and 400 mg/kg showed dose-dependent increase in time, suggesting effective pain relief.

The results obtained in this study suggest that the ethyl acetate seed extract of *Polyalthia longifolia* possesses analgesic properties.

CONCLUSION

The present study can be concluded with the fact that the ethyl acetate extract of *Polyalthia longifolia* seeds unveils a significant analgesic effect in Swiss albino mice using analgesic models namely Eddy's hot plate.

The precise mechanism underlying the analgesic effect of *Polyalthia longifolia* remains unclear, but it appears to be associated with specific compounds present in *Polyalthia longifolia.* Further research is needed to identify, isolate and assess the active chemical constituents responsible for this analgesic activity. Thus, ethyl acetate extract of *Polyalthia longifolia* has potential clinical applications in the management of algesia.

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