WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 12, 1280-1288. Research Article ISSN 2277– 7105

LOCAL ANTI-INFLAMMATORY AND ANTINOCICEPTIVE ACTIVITIES OF *PORTULACA QUADRIFIDA* LINN

Manivannan Ekambaram*, Vijayarangan Sivasamy[1], Kothai Ramalingam[2], Arul Balasubramanian[2]

*Department of Pharmacology, Vinayaka Mission's Kirupanandha Variyar Medical College and Hospital, Seeragapadi Salem-636 308, Tamilnadu, India.

[1]Department of Pharmacology, Government Mohan Kumaramangalam Medical College & Hospitals, Salem-636030, Tamilnadu, India.

[2]Vinayaka Mission's College of Pharmacy, Salem-636 008, Tamilnadu, India.

Article Received on
18 Oct. 2016,
Revised on 08 Nov. 2016,
Accepted on 28 Nov. 2016
DOI: 10.20959/wjpr201612-7522

***Corresponding Author**
Dr. Manivannan Ekambaram
Department of Pharmacology, Vinayaka Mission's Kirupanandha Variyar Medical College and Hospital, Seeragapadi Salem-636 308, Tamilnadu, India.

ABSTRACT

The ethanolic extract of *Portulaca quadrifida* was studied in Swiss albino mice against 12-o-tetradecanoyl phorbhol-13-acetate (TPA) and arachidonic acid (AA) induced anti- inflammatory response. While the analgestic effects were studied using the acetic acid induced writhing the mice and formalin induced nociception. The ethanolic extract produced dose dependent anti-inflammatory activity against the pathogens. It also showed an inhibitory effect on pain caused by acetic acid in mice and reduced pain episodes induced by formalin. The result indicated that the ethanolic extract produced significant ($p<0.05$) anti-inflammatory andanti nociceptive activity when compared with the standard and untreated control.

KEYWORDS: *Portulaca quadrifida*; ethanolic extract; anti-inflammatory; anti-nociceptive.

INTRODUCTION

Portulaca quadrifida Linn. a prostrate fleshy annual or stoloniferous perennial herb with somewhat base but sometimes with simple main stems, 5-40 cm tall and generally widespread in warm countries. *Portulaca quadrifida* Linn. belongs to the family portulacaceae. It is a small diffused, succulent, annual herb found throughout the tropical parts of India. It is used as a vegetable and also used for various curative purposes. It is said

to be useful in asthma, cough, urinary discharges, inflammations and ulcers. A poultice of the plant is applied in abdominal complaints, erysipelas and haemorrhoids.[1] *Portulaca quadrifida* Linn. has been reported to possess antifungal activity against *Aspergillus fumigates* and *Candida albicans.*[2] and the neuropharmacological activities were reported by Syed *et al.*[3] A review of literature afforded no information on the anti-inflammatory and anti-nociceptive aspects of this plant.

Prolonged uses of both steroidal and non- steroidal anti- inflammatory drugs are well known to be associated with peptic ulcer formation.[4] Hence search for new anti -inflammatory agents that retain therapeutic efficacy and yet are devoid of these adverse effects are justified. There is much hope of finding active anti- rheumatic compound from indigenous plants as these are still used in therapeutic despite the progress in conventional chemistry and pharmacology in producing effective drugs. Herbal drugs are being proved as effective as synthetic drugs with lesser side effects and they are in line with nature with no hazardous reaction.

The enzyme phospholipase A2 is known to be responsible for the formation of mediators of inflammation such as prostaglandins and leukotrienes, which by attracting polymorphonuclear leucocytes to the site of inflammation would lead to tissue damage probably by the release of free radicals. Phospholipase A2 converts phospholipids in the cell membrane into arachidonic acid, which is highly reactive and is rapidly metabolized by cyclooxygenase (prostaglandin synthase) to prostaglandins, which are major components that induce pain and inflammation.[5,6] So the present study is therefore an attempt to assess the efficacy of this indigenous herb for its anti-inflammatory and anti-nociceptive activities and to propose a possible mechanism of action

MATERIALAND METHODS

Plant material

The aerial parts of the plant were collected from the foothill of Yercaud, Salem, in the month of June 2015 and cleaned to remove the debris. The collected plant was identified and authenticated by a botanist Dr. A. Marimuthu, Department of Botany, Government Arts College, Attur. A voucher specimen (PQM-1) has been kept in our museum for future reference. The plant parts were dried at room temperature for 10 d and coarsely powdered with the help of a hand-grinding mill and the powder was passed through sieve No. 60.

Preparation of the extract

The powder of aerial parts of *P. quadrifida* was extracted separately by continuous hot extraction process using soxhlet apparatus with different solvents in increasing order of polarity from petroleum ether, chloroform, acetone, alcohol, to finally chloroform:water.[7] After extraction, the extracts were concentrated under reduced pressure in tared vessel. The marc of crude drug powder was then once again subjected to successive extraction with other solvents and the extractive values were calculated with reference to the air-dried drug. The dry extracts were subjected to various chemical tests to detect the presence of different phytoconstituents.

Animals

Swiss albino mice of either sex and of approximately the same age weighing about 20- 30g were used for the study. The were housed in polypropylene cages and fed with standard chow diet and water ad *libitum*. The animal were exposed to alternative cycle of 12 h of darkness and light each .Before each test, the animals were fasted for atleast 12h. The experimental protocols were subjected to the scrutinization of the institutional animal ethics committee and were cleared by the same.

Acute toxicity studies

The acute toxicity studies were conducted as per the guidelines of OECD (guideline 423). and were observed for mortality till 48 h and the LD50 was calculated.

TPA/ AA induced ear inflammation

Anti- inflammatory activity was assessed by the method described by young *et al.*[8] The rats were divided into three groups of six animals each. An edema was induced on the right ear by topical application of 2.5 µg of TPA (sigma chemical co, St Louis MO,USA) or 2mg of AA (Sigma chemical co, St. Louis MO,USA) in 20 µl of acetone. The left ear acts as a control and received the vehicle acetone. First group (positive control) received 0.5 mg/ear indomethacin, second and third groups received 0.5 and 1.0 mg/ear of ethanolic extract of *P.quadrifida* respectively and applied simultaneously with TPA or AA. The edema was measured initially and after 4h after challenge to phlogestic agent to assess an increase in ear thickness due to treatment.[9-12] Ear edema was calculated by subtracting the thickness of the left ear (vehicle control) from the right ear (treated ear).

Percent inhibition of inflammation was calculated using the formula % inhibition =100 [1-(Vt/Vc)], where "Vc" represents edema in control and Vt edema in group treated with test extracts.

Acid induced writhing in mice

The method described by Koester *et al.*[13] was used in this experiment. Swiss albino mice were divided in to four groups of six each. The first group served as control and the second group received acetyl salicylic acid at a dose of 200 mg/kg p.o. third and fourth groups received the ethanolic extract of *P.quadrifida* (100 and 200 mg/kg p.o) The number of abdominal construction was observed 5 min after stimulation during a period of 30 min.

Formalin induced nociception in mice

The method of Hunskar *et al.*[14] was used. Each group of six mice each, was treated with the test extract (100 and 200 mg/kg p.o.,) normal saline and indomethacin 10 mg/kg p.o. One hour after treatment all animals were injected with 20 µl of 1% formalin in saline solution in to the planer surface of the left hind paw. Licking time was measured in the test chamber over 30 min, in two phases. The first phase was from time zero to 5 min. after formalin injected and the second phase was from 20 to 30 min after formalin injection.

STATISTICAL ANALYSIS

All values were expressed as mean ±SEM. The data were statistically analyzed using one way ANOVA followed by Newman Keut's multiple range test and difference below $P< 0.05$ are considered as significant.

RESULTS

The plant *P. quadrifida* was collected from the foothill of Yercaud, Salem, air-dried and extracted by continuous hot extraction process using soxhlet apparatus. The average percentage yield of ethanolic extract of *P. quadrifida* was found to be 3.8% w/w. The LD50 was found to be 2000 mg/kg for ethanolic extract of *P. quadrifida*.

The ethanolic extract did not exhibit and toxic effects up to 1000 mg/kg when administered to mice as a single i.p. dose. The effect of ethanolic extract of *P. quadrifida* on TPA induced ear inflammation in mice is shown in Table 1. The results obtained indicate that the ethanolic extract was found to have significant anti- inflammatory activity in mice. The ethanolic

extract of *P. quadrifida* reduced the inflammation induced by TPA by 61.78 and 71.22% on administration of 0.5 and 1mg/ear inhibited the edema volume by 78.90%.

Inflammation in mice is shown in table 2.The percentage inhibition of animals treated with the ethanolic extract of *P. quadrifida* (0.5 and 1.0mg/ear) was found to be 45.30 and 65.97%. Treatment with Indomethacin (0.5mg/ear) produced an inhibition of 80.63%. The ethanolic extract of *P. quadrifida* and Indomethacin, both inhibited the ear inflammation in mice. In order to determine the peripheral analgestic action of *P. quadrifida* acetic acid induced writhing in mice was used. The ethanolic extract of *P. quadrifida* at 100 and 200 mg/kg, p.o, showed an inhibition of 45.79 and 64.97%. The standard drug acetyl salicylic acid inhibit 76.74% of writhing induced by acetic acid. It was found that both doses (100 and 200 mg/kg p.o) of the ethanolic extract of *P. quadrifida* exhibited a significant dose dependent inhibition of nociception induced by acetic acid in mice. Animals treated with saline showed response times of 54.50 ± 1.22s and 76.67 ±0.65s in the first and second phases (Fig1), respectively. Animals treated with indomethacin (10 mg/kg p.o.,) showed only the first phase (40.83± 0.90s) the second phase was totally inhibited animals those treated with the ethanolic extract of *P. quadrifida* showed both phases (52.67±1.14s and 21.50±1.55s, for 100mg/kg p.o and 47.83 ± 0.57s and 12.33± 0.82s, for 200 mg/kg p.o).

Table 1: Effect of ethanolic extract of *P.quadrifida* on TPA induced inflammation in mice

Treatment	Dose mg/ ear	Difference in ear thickness (mm-3)	% of inhibition
Control	20µl	67. 17 ± 1.88	-
Indomethacin	0.5	14.17±0.90	78.90
Ethanolic extract of *P.quadrifida*	0.5	25.67±1.63	61.78
Ethanolic extract of *P.quadrifida*	1	19.33±0.33	71.22

P<0.05 when compared with control. Values are expressed as mean ±SEM (n=6).

Table: 2. Effect of ethanolic extract of *P.quadrifida* on AA induced inflammation in mice

Treatment	Dose mg/ear	Different in ear thickness(mm-3)	% of inhibition
Control	20µl	63.67±0.49	-
Indomethacin	0.5	12.33±0.82	80.63
Ethanolic extract of *P.quadrifida*	0.5	34.83± 0.90	45.30
Ethanolic extract of *P.quadrifida*	1	21.67±1.14	65.97

Table: 3 Effect of ethanolic extract of *P.quadrifida* on acetic acid induced writhing in mice

Treatment	Dose mg/ear	Difference in earb thickness (mm-3)	% of inhibition
Control	1ml	65.17±3.67	-
Indomethacin	10	15.16±1.39	76.74
Ethanolic extract of *P.quadrifida*	100	35.33±1.31	45.79
Ethanolic extract of *P.quadrifida*	200	22.83±2.20	64.97

P<0.05 when compared with control. Values are expressed as means ±SEM (n=6).

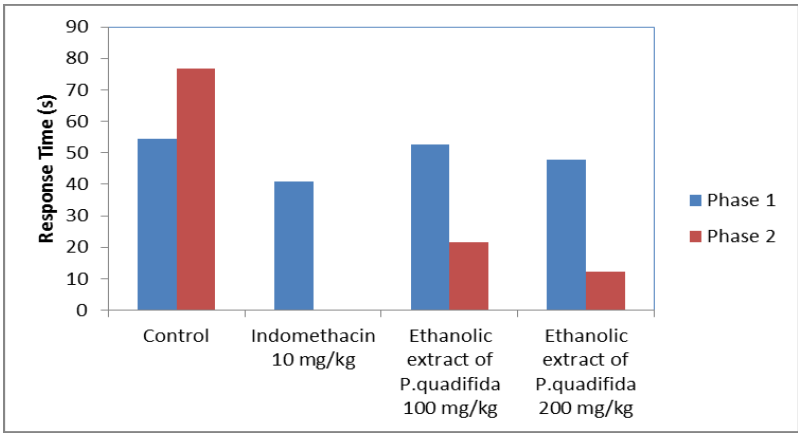

Fig: 1 Effect of ethanolic extract of *P.quadrifida* on formalin induced nociception in mice

DISCUSSIONS

Due to the increasing frequency of intakes of NSAID'S and their reported common side effects, there is need to focus on the scientific exploration of herbal drugs having fewer side effects, So, there is a continuous search for indigenous drugs, which can provide relief to inflammation. The traditional medical practitioners of kollinhills, Tamilnadu are using this plant to cure inflammation. To give a scientific validation to this plant, an attempt was made to study the anti- inflammatory and anti- nociceptive activities

TPA a phorboleaster provides a skin inflammation model suitable for evaluation of topical and systemic anti inflammatory agents. The majority of its activities appear to involve or depend on arachidonic acid release and metabolism and interaction with protein kinase C.[9] Topical application of TPA induces long lasting inflammation response, associated with increase in prostanoid production. Moreover, topical administration of cyclooxygenases appears more effective at inhibition the edema response than lipooxygenase response.[15] While AA induce ear inflammatory response is paralleled to the generation of prostaglandin andleukotrienes.[9,15] Thus, local anti inflammatory activity of the extract appears to be

complex in nature involving mainly an action on pathways associated with biosynthesis of prostaglandin and leukotrienes or involving arachidonic acid release and metabolism or interaction with protein kinase C.

The abdominal constriction response induced by acetic acid in the nociceptive activity test in mice was a very sensitive procedure that enabled the detection of anti-nociceptive activity of ethanolic extract of *P.quadrifida* dose levels undetectable by the tail –flickassay.[16,17] The abdominal constriction response is thought to be involve local peritoneal receptors, thus suggesting that the observed analgesic effect.

In contrast formalin produces a distinct biphasic (early and late) phases of pain and can be used to elucidate the mechanisms of pain and analgesia.[18] Centrally acting drugs such as narcotics, inhibit both phases of pain equally.[19] While peripheral acting drugs, such as aspirin, oxyphenbutazone, hydrocortisone and dexamethasone inhibit only the late pain phases.[20-22] The extract of *P.quadrifida* also produced dose dependent anti- inflammatory effects in mice. Tjolsen *et al.*[18] have demonstrates that the second pain phases of formalin test depends on an inflammatory reaction in the peripheral tissues, suggesting that the inhibition of the second anti- nociceptive phases in the formalin test is partially produced by the peripheral action of the extract of inflammation. *P.quadrifida* is endowed with peripheral analgesic activity, possibly related to an inhibition of cyclooxygenase. According to selbert *et al.*[23] it may be inferred that the extract inhibits cyclooxygenase-2, while the available data do not allow to assume that *P.quadrifida* also inhibits cyclooxygenase-1.Thus, it can be concluded that the plant *P.quadrifida* possess significant anti inflammatory and anti nociceptive activity in mice. Further studies involving the purification of the chemical constituents of the plant and the investigation in the biochemical pathways may result in the development of a potent anti- inflammatory and-nociceptive agents with a low toxicity and better therapeutic index.

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