

**IN-VIVO ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC
EXTRACT OF LEAVES PART OF *PYRUS PASHIA*****Neha Pandey, Dr. Anita Singh* and Janmejy Pant**

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ABSTRACT

Customary drug experts utilize *Pyrus pashia* (PP) leaves extract for symptomatic alleviation in Cardiovascular, Respiratory and Gastrointestinal disease and furthermore utilized as antineoplastic agent, antimicrobial, cytotoxicity. Previous studies show the anti-inflammatory effect (AIE) of fruit of the *Pyrus pashia* extract. The present examination was aimed to explore the anti-inflammatory effect of leaves of *Pyrus pashia*. The leaf extract was extracted by Soxhlet with 70% methanol at room temperature, separated and the filtrate vanished to dryness. The methanolic extract of *Pyrus pashia* leaves (MEPPL) was suspended in aqueous and utilized per orally in rodents.

MEPPL showed valuable effect in acute anti inflammatory property at 150 mg/kg, 100 mg/kg and 50 mg/kg doses. When compared to the standard drug Indomethacin 10 mg/kg the acute effect of the extract was comparable. These observations established the potential acute AIE of *Pyrus pashia* leaves.

KEYWORDS: *Pyrus pashia*, anti-inflammatory, Carrageenan.**ABBREVIATION:** **AIE:** anti-inflammatory effect**CIPE:** Carrageenan induced paw edema**IND:** Indomethacin**MEPPL:** Methanolic extract of *Pyrus pashia* leaves**PP:** *Pyrus pashia***INTRODUCTION**

Inflammation is the body's reaction against multiple pathogens, which is depicted by heat, swelling, pain, and redness. Many statements have noted that it is besmeared in the

pathogenesis of several ailments like as cancer, neurological disorder, cardiovascular dysfunction, aging, and other attenuating disorders.^[1]

At a basal scale, the acute inflammatory reaction activated through tainting or tissue hurt incorporates the composed movement of blood fragments (plasma and leukocytes) to the area of wound or ailment. This reaction has been depicted excellent for microbial ailments (especially bacterial defilements), in which this is actuated through receptors of the intrinsic immune channel.^[2] This fundamental affirmation of disease is mediated through tissue inhabitant macrophages and mast cells, inciting the era of a diversity of incendiary authorities, like vasoactive amines, chemokines, eicosanoids, cytokines and yields of proteolytic cascades.

A fruitful acute inflammatory reaction outcomes in the expunction of the contagious agents taken after by a determination and improve stage, which is interceded chiefly by tissue-inhabitant and appointed macrophages.^[3]

If response of this acute inflammatory fails to dispense the pathogen, the inflammatory process holds on and gets new attributes. The neutrophil penetrate is replaced with macrophages, and on account of contamination additionally with T cells. On the off chance that the joined impact of these cells is as yet deficient, a chronic inflammatory situation pursues, including the tertiary lymphoid tissues and synthesis of granulomas.^[4] The attributes of this inflammatory situation can contrast contingent upon the effector class of the T cells that are exist.

The family of PP is Rosaceae and their subfamily is Maloideae, PP is a little or average-sized tree. The leaves of PP are protracted 5cm -10 cm, elliptic to extensively javelin like shaped, long-spoted, jagged, hairless and glowing. Flowers of PP are 2cm -2.5 cm over, with white obovate petals with darker veins. Fruits of PP are round, 1.3 cm -2.5 cm, dull dark colored and are palatable when half-spoiled. PP wear is found in the Himalayan region, from Afghanistan to South West China and Burma, at elevations of 750m- 2700 m. Flowering season of PP is from March-April. ⁶ Species of *Pyrus* have various helpful compounds including chlorogenic acids, flavan-3-ols and arbutin and these compounds have various physiological activities. ⁷⁻⁹ *Pyrus pashia* is employed frequently in the traditional medicine as antimicrobial, antioxidant, stomachic and hypoglycemic activities.^[10,11]

MATERIALS AND METHODS

Plant materials

Pyrus pashia leaves were collected in month of August, 2016 from Bhimtal region, Dist. Nainital, North-East India and authenticated by Dr. K. S. Negi, Principal scientist Niglat, District Nainital. The PP leaves were cleaned, then PP leaves spread out in shade dried and then leaves of PP reduces into coarse powder with an electrical blender.

Extraction and sample preparation

For removal of fat materials, prior to extraction the powder of its leaves soaked with the petroleum ether and then Methanolic extraction was done by soxhlet method. By vacuum rotavapor (Perfit) the methanolic extract of MEPPL were concentrated under reduced pressure and then dried in vacuum desiccators. Afterward dried extract of its leaves was kept in refrigerator ($8 \pm 2^\circ\text{C}$) and this *Pyrus pashia* extract was used for CIPE in-vivo study.

Study tools

The chemical Carrageenan was procured from HiMedia Laboratories Pvt. Ltd and Indomethacin was procured from Yarrow Chem products, Mumbai. Plethysmograph instrument required for fundamental surgical method were utilized for the study.

Animals

Rats of either gender of weighing 150gm-200gm were utilized for evaluate AIE. Animals (rats) were procured from Departmental animal house of Kumaun University, Campus Bhimtal. The rats were housed in polypropylene cages and kept up at $27^\circ\text{C} \pm 2^\circ\text{C}$ and standard 12 hr dark / light cycle and acclimatized for one week. They were fed with standard food and water ad libitum. The waste in the cages was renewed day by day to ensure sterile condition and most extreme comfort for animals. The perusal protocol was approved by institutional Animal ethics committee KUDOPS/67

Acute toxicity study

The methodology was followed to OECD 423 rules. The dose 2000 mg/kg of MEPPL was administered orally according body weight to different groups of rats. Neurological toxicity, behavioral and mortality signs observed for 14 days.¹²

Determination of AIE (Anti-inflammatory Effect)**CIPE**

In this method, rats were randomly partitioned into five gatherings, each containing six rats and all the rats received the drugs as shown

Group I: Control (Distilled water)

Group II: Test drug (MEPPL 50mg/kg)

Group III: Test drug (MEPPL 100mg/kg)

Group IV: Test drug (MEPPL 150mg/kg)

Group V: Standard drug (Indomethacin 10mg/kg)

The animals were pre-treated with oral medications 1 hour before administration of carrageenan injection. 0.1ml of 1% carrageenan was injected sub planter surface of right hind paw of each rodent. Paw edema was measured by plethysmograph at various time interims i.e. at - 60 minutes (1 hour preceding carrageenan injection at time of drug administration), 0 hour (at the time of carrageenan injection) and at 1, 2 and 3 hours after carrageenan injection. Percentage inhibition (protection) edema development was carried as a record of acute anti-inflammatory activity, calculated by

$$\text{Percentage inhibition} = \frac{\{(V2-V1) \text{ control} - (V2-V1) \text{ treated}\}}{(Vt-V0) \text{ control}} * 100$$

Where V1- Mean paw volume in ml prior to injection of carrageenan at 60 minutes

V2- Mean paw volume in ml at different time periods after carrageenan administration

Statistical Analysis

Information was expressed as mean \pm Standard Error Mean (SEM). Disparity were assayed as significant at *** $P < 0.001$, or ** $P < 0.01$ or * $P < 0.05$ when compared test (different concentration of MEPPL) groups v/s control (distilled water) group. For numerical outcomes, one-way analysis of variance (ANOVA) with Tukey-Kramer Multiple Comparisons post tests were performed using GraphPad InStat Version 3 (GraphPad Software) and all graphs were made by utilizing Microsoft office 2007 software.

RESULTS

Acute toxicity study

The MEPPL was studied for acute toxicity at doses of 2000 mg/kg, p.o. The extract was found devoid of mortality of all animals. Hence, the doses selected for AIE were 50, 100, and 150 mg/kg, p.o.

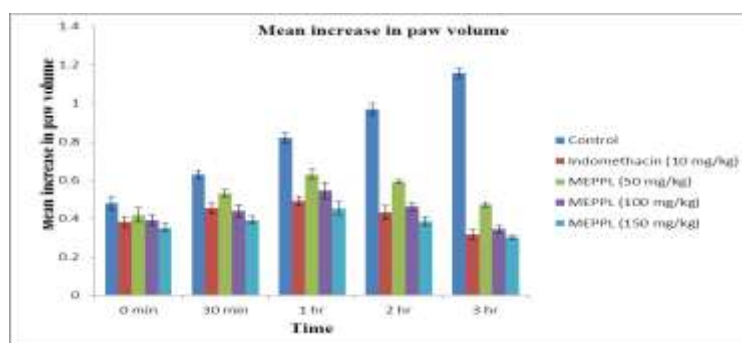
S/No.	Group (n=3)	Weight (g) Rats	Dose (mg/kg)	Toxicity symptoms	Mortality
1.	Group I	150-200	5	Nil	No
2.	Group II	150-200	50	Nil	No
3.	Group III	150-200	300	Nil	No
4.	Group IV	150-200	2000	Nil	No

Anti-inflammatory activity

The AI effect of the *Pyrus pashia* leaves extract against acute paw edema has been denoted in Table 1. Which showed significant AIE and the outcomes were comparable with (distilled water) control. It was found that the (MEPPL) (150 mg/kg, p.o.) exhibits maximum (AI) activity against (CIPE). The inhibition obtained with *Pyrus pashia* (150 mg/kg) at 30 mins, 1 hr, 2 hr and 3hr was 26.6, 37.7, 44.8 and 60.1% respectively (Table 1).

Table 1:

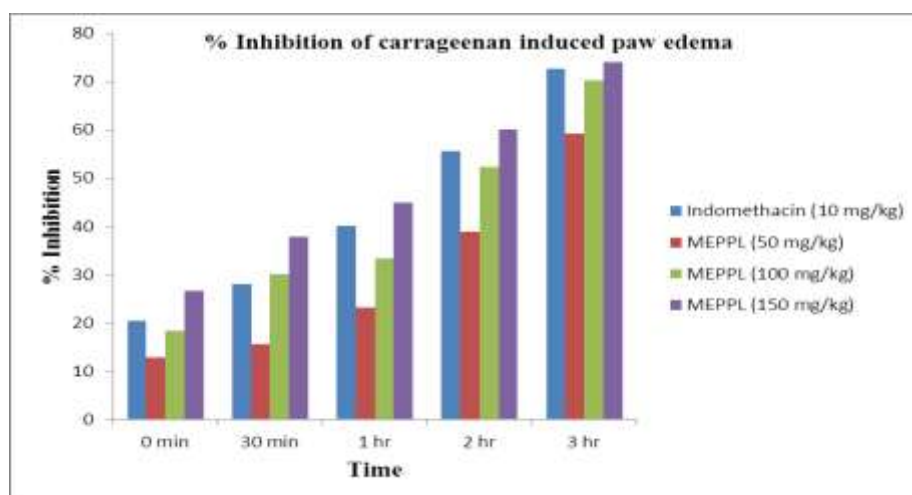
Treatment (mg/kg)	Mean of paw volume increase			
	30 minutes	1 hour	2 hours	3 hours
control	0.63 ± 0.02121	0.82 ± 0.02864	0.968 ± 0.032	1.158 ± 0.02437
Indomethacin (10 mg/kg)	0.454±0.02839	0.491±0.0239	0.43±0.03728	0.318±0.02728
MEPPL (50 mg/kg)	0.532±0.01908	0.63±0.02608	0.592±0.009695	0.472±0.01281
MEPPL (100 mg/kg)	0.44±0.03302	0.546±0.04142	0.462±0.02154	0.346±0.01806
MEPPL (150 mg/kg)	0.392±0.01985	0.452±0.03484	0.386±0.02379	0.302±0.01158



Graph 1: Mean paw volume against carrageenan induced acute inflammation.

Table 2- % inhibition by *Pyrus pashia* (PP) against carrageenan induced acute inflammation.

Group	30 min	1 hr	2 hr	3 hr
Indomethacin	20.41666667	27.93650794	40.12195122	55.57851
T1	12.91666667	15.55555556	23.17073171	38.84298
t2	18.33333333	30.15873016	33.41463415	52.27273
t3	26.66666667	37.77777778	44.87804878	60.12397



Graph 2- % inhibition by *Pyrus pashia* (PP) against carrageenan induced acute inflammation.

DISCUSSION

Through the chemical constituents present in the *Pyrus pashia* leaves which are liable for treatment in inflammation, we researched in-vivo anti-inflammatory property of MEPPL. By this research we can say that the MEPPL is effective biologically with lower side effect as compare to marketed drugs. Our study demonstrated that MEPPL have significant in-vivo anti-inflammatory property at doses 50 mg/kg, 100 mg/kg and 150 mg/kg, by inhibits the edema formation which is formed by inject of carrageenan on the subplantar region.

The MEPPL which demonstrated the highest AIE, displayed likewise very highly significant statistic values ($P < 0.001$) at doses 150 mg/kg, $P < 0.01$ at 100 mg/kg and $P < 0.05$ at 50 mg/kg for CIPE after the treatment with the phlogistic agent. It is clear that carrageenan is a sulphated polysaccharide obtained from ocean weed (Rhodophyceae) and is normally used to induce acute inflammation.

Inflammation becomes in 2 conditions, first condition happens because of arrival of 5HT and histamine (0-120 min) and the second condition occurs because of swelling is ascribed to PG

(Prostaglandin) discharge (> 240 mins). By these views we can say that MEPPL might suppress either the release of 1st stage mediators like 5HT and histamine or 2nd stage mediators like COX and hence reduce pain.

CONCLUSION

From above discussion it can be concluded that the MEPPL promising anti inflammatory effect AIE. And from the above data we can also say that the MEPPL is more effective at dose 150 mg/kg as compare to standard drug (Indomethacin). So we can say that herbal extract may be remunerative effect with lower side effect as compare to standard drug (Indomethacin) for the management of inflammation.

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