



## ***In-vivo* Anti-inflammatory activity of *Pyrus pashia* Fruit**

Subhash Chandra<sup>\*1</sup>, Sarla Saklani<sup>1</sup>, Sarvesh Kumar<sup>2</sup>

<sup>1</sup>Department of Pharmaceutical Chemistry, H. N. B. Garhwal (A Central University),

<sup>2</sup>Govt. Polytechnic Srinagar Garhwal 246174, Uttarakhand, India.

*Received: 15-03-2016 / Revised: 26-04-2016 / Accepted: 28-04-2016 / Published: 28-04-2016*

### **ABSTRACT**

In the present study, we investigated the in vivo anti-inflammatory activity of the methanolic extract of *Pyrus pashia* fruits. The methanolic extract of *Pyrus pashia* fruits at 100 mg/kg body weight per day given orally as a suspension the paw volume was reduced by 56.61% at after 4 hour, whereas in case of the same extract at 150 mg/kg body weight per day shows 61.12% inhibition at same time. Hence, methanolic extract exhibited considerable anti-inflammatory activity at the dose of 100mg/kg body weight, produced a significant inhibition of inflammation compared with Indomethacin (100 mg/kg) as standard reference and normal saline as control.

**Keywords:** *Pyrus pashia*, Inflammation, Paw edema and Rat.

### **INTRODUCTION**

The term inflammation has been derived from the inflammare a Latin word, which give meaning as burning. Inflammation is considered as a primary physiologic defense mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli, an uncontrolled and persistent inflammation may act as an etiologic factor for many of these chronic illnesses [1]. All types of human body injuries results in chemical changes in the injured area. Generally, the process of inflammation is associated with the activation of IL-1Beta, IL-Gamma and TNF-Alpha, by activated cells which play major role in host defense mechanism. The use of steroidal and non steroidal anti-inflammatory drugs have been used for rapid and effective for only temporary relaxation and are inadequate [2, 3, 4 & 5]. *Pyrus pashia* also known as Mahal belongs to the family of Moraceae, its fruits used for the treatment of different diseases such as astringent, digestive disorder, sore throat, irritability, abdominal pain and anemia. The fruits are also believed to possess activities such as antimicrobial, antioxidant, stomachic and hypoglycemic activities [6 & 7]. Hence in the present study effort has made to establish the scientific validity to the in vivo anti-inflammatory property of methanolic extract of *Pyrus pashia* fruits using Carrageenan induced paw edema in rats as inflammatory models.

### **MATERIALS AND METHODS**

**Chemicals:** All the chemicals and reagents of analytical grade such as Indomethacin (Merck, Bangalore, India) and Carrageenan (Sigma Chemicals, St. Louis, MO, USA) were procured from the respective companies and were used in the study.

**Collection and Identification:** The materials included fresh and dry fruits of *Pyrus pashia* were collected from district Chamoli, Uttarakhand during October-November 2014. These plants were authenticated from Taxonomy Laboratory, Department of Botany, HNB Garhwal University, Srinagar. The voucher specimens GUH 2867 were deposited in the University herbarium for future records.

**Preparation of Plant Extract:** The air dried fruits ground to moderately fine powder and soxhlet extracted with petroleum ether, chloroform, ethyl acetate, acetone, methanolic, ethanolic and water using soxhlet apparatus [8]. Each extract was evaporated to dryness under reduce pressure using rotary evaporator. The extracts thus obtained were stored in air tight container at 4°C until further analysis.

**Experimental animals:** The wistar rats (100-150g) were obtained from the Animal House, National

*\*Corresponding Author Address: Subhash Chandra, Department of Pharmaceutical Chemistry, H. N. B. Garhwal (A Central University), Uttarakhand, India; E-mail: subhashkothiyal@gmail.com*

Centre of Fungal Taxonomy, New Delhi, India. They were housed at a temperature  $24\pm 1^{\circ}\text{C}$ , 12 hour light/dark cycle 35-60% humidity, in polypropylene cages and fed a standard rodent diet with water *ad libitum*. The animals were deprived of food but not water 4 hours before the experiment. The experimental design was approved by the ethical committee for animal experimentation of faculty of Pharmaceutical Science (S. B. S. Ballawala Dehradun Uttarakhand) bearing the number 273/PP/SCEA.

**Acute Toxicity Study:** To determine the minimum lethal dose, acute oral toxicity studies were performed as per OECD guidelines [9]. Adult albino rats of either sex weighing 100-150gm were used. The animals were divided into five groups of six each. Group I was given 2 ml of 1% saline and group II received 2 ml of 1% vanillin both acted as control. The other three groups were administered 50, 100 and 150 mg/kg between of the methanolic extract with 2 ml of 1% vanillin orally using intra Gastric Catheter respectively. All the experimental rats were fasted overnight. They were observed continuously for any gross behavioral changes and toxic manifestations like hyperactivity, grooming, convulsions, sedation, hypothermia and mortality during the first three hours. Thereafter the animals were continuously monitored at regular intervals for 7 days. No adverse effect or mortality was detected in this study up to 500 mg/kg between doses. Hence sub-lethal doses of 50, 100 and 150 mg/kg between doses of the extract were selected for the following experiments.

## RESULTS AND DISCUSSION

Table 1. In vivo anti-inflammatory effect of methanolic extract of *Pyrus pashia* fruit on Carrageenan Induced Paw Edema in rats.

Group	Dose mg/kg	Paw volume in ml $\pm$ SEM and percentage inhibition			
		+1 Hour	+2 Hour	+3 Hour	+4 Hour
I	Control	0.638 $\pm$ 0.01	0.687 $\pm$ 0.03	0.698 $\pm$ 0.02	0.756 $\pm$ 0.04
II	Indomethacin	0.462 $\pm$ 0.02	0.405 $\pm$ 0.03	0.363 $\pm$ 0.02	0.312 $\pm$ 0.01
III	50	0.568 $\pm$ 0.04	0.579 $\pm$ 0.03	0.551 $\pm$ 0.01	0.532 $\pm$ 0.02
IV	100	0.501 $\pm$ 0.02	0.458 $\pm$ 0.03	0.371 $\pm$ 0.03	0.328 $\pm$ 0.02
V	150	0.454 $\pm$ 0.03	0.397 $\pm$ 0.04	0.326 $\pm$ 0.04	0.294 $\pm$ 0.03

Table 2. In vivo anti-inflammatory effect percent of inhibition of methanolic extract of *Pyrus pashia* fruit on Carrageenan paw edema in rats.

Dose	1 Hour	2 Hour	3 Hour	4 Hour
Indomethacin (100 mg/kg)	27.58%	41.04%	47.99%	58.73%
50 mg/kg	10.97%	15.72%	21.06%	29.62%
100 mg/kg	21.47%	33.34%	46.84%	56.61%
150 mg/kg	28.84%	42.21%	53.29%	61.12%

## METHODOLOGY

**Carrageenan induced rat paw edema assay:** Acute anti-inflammatory activity studies were performed following the Carrageenan induced hind paw edema suggested [10]. The rats were divided into five groups of six rats each. Group 1 acted as control and was given 1% saline, Group 2 received 100 mg/kg between of standard reference drug – Indomethacin, Group 3, 4 and 5 were administered 50, 100 and 150 mg/kg between of the methanol extract with 2 ml of 1% vanillin respectively. 0.1 ml of 1% solution of Carrageenan was injected intradermally to the rats into the plantar surface of the right hind limb to induce paw edema. The paw volume was measured plethysmographically before induction (0 H) and after at one hour intervals for four hours. The paw volume in group II, III and IV were compared with that of the control. Percentage inhibition was calculated using the formula,

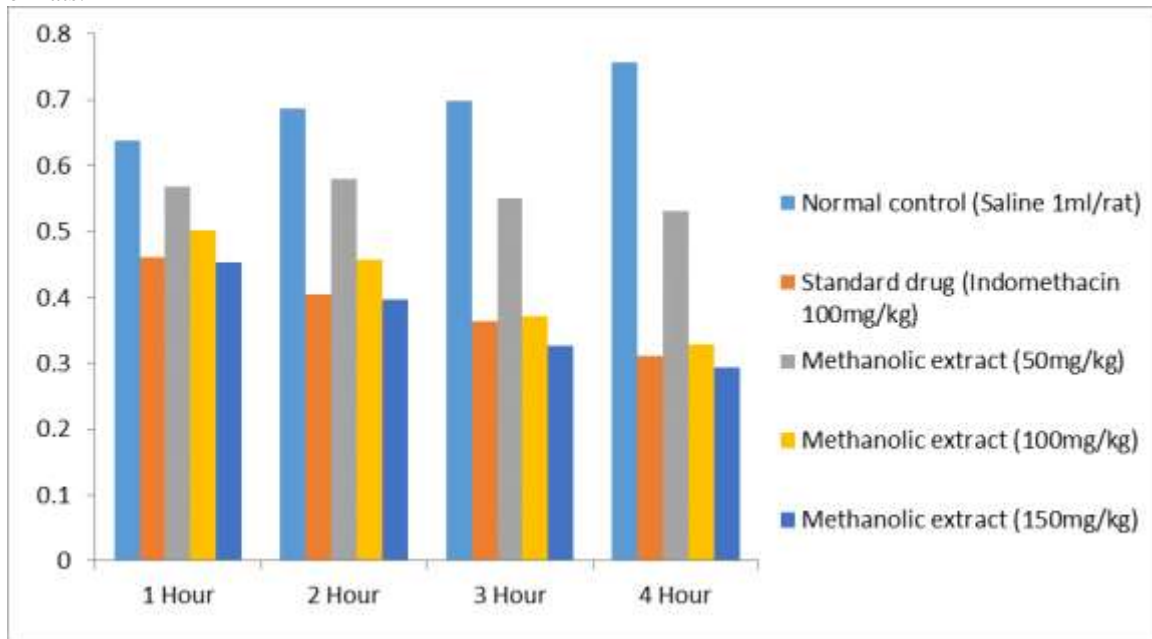
$$\% \text{ Inhibition} = (V_C - V_T / V_C) \times 100$$

Where,  $V_C$ = Paw volume in control group,

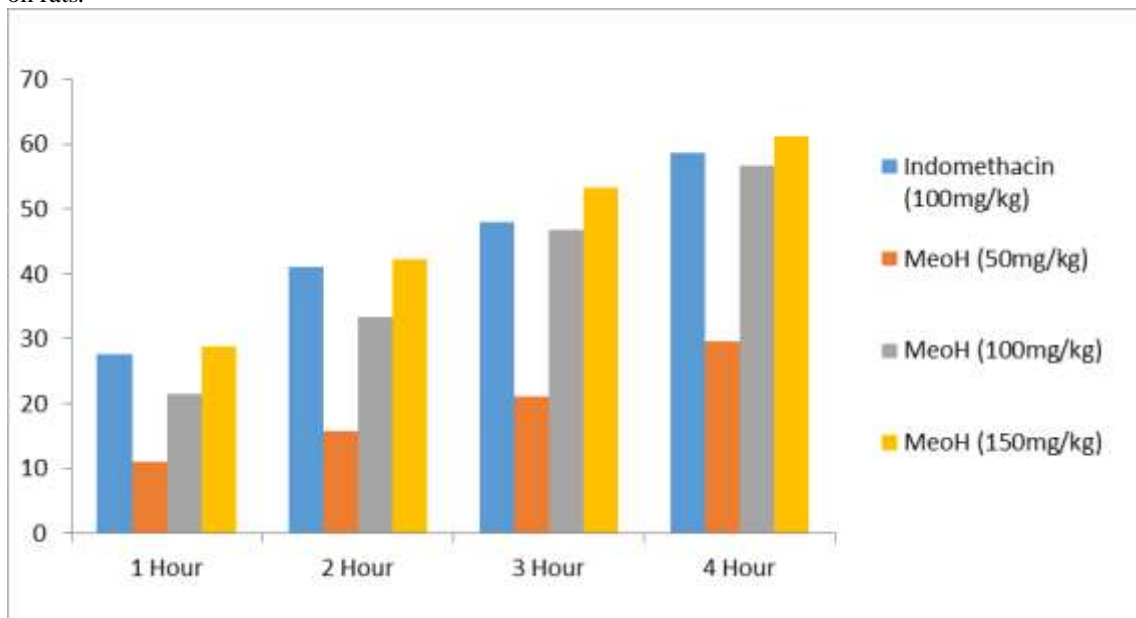
$V_T$ = Paw volume in drug treated group.

**Statistical analysis:** Results were expressed as mean  $\pm$  standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by multiple Tukey's comparison tests. A students 't'-test value with  $p < 0.05$  was considered statistically significant [11].

**Figure 1.1** Comparison of standard drug (Indomethacin) with different methanolic extract of *Pyrus pashia* fruit on rats.



**Figure 1.2** % Inhibition of standard drug (Indomethacin) with different methanolic extract of *Pyrus pashia* fruit on rats.



## DISCUSSIONS

Carrageenan-induced rat paw edema model is a suitable test for evaluating anti-inflammatory drugs, which has frequently been used to assess the antiedematous effect of the drug. Carrageenan is a strong chemical used for the release of inflammatory and proinflammatory mediators (prostaglandins, leukotrienes, histamine, bradykinin, TNF- $\alpha$ , etc.). The course of acute inflammation is biphasic. First

phase starts with the release of histamine, serotonin, and kinins after the injection of phlogistic agent in the first few hours. While the second phase is related to the release of prostaglandins like substances in 2, 3 & 4 hours. Second phase is sensitive to both the clinically useful steroidal and nonsteroidal anti-inflammatory agent. Prostaglandins are the main culprit responsible for acute inflammation. We observed

that methanolic extract of all doses (50, 100 and 150mg/kg body weight) showed significant inhibition against carrageenan-induced paw edema in the dose dependent manner.

The result of anti-inflammatory activity of fruits extract of *Pyrus pashia* against carrageenan induced paw edema is shown in Table 01. Paw volume was significantly reduced ( $P < 0.01$ ) in all treated groups as compared to control group [Fig 1.1]. Methanolic extract at dose 150mg/kg showed more significant inhibition of edema ( $0.294 \pm 0.03$ ) than 100mg/kg dose ( $0.328 \pm 0.02$ ). However the anti inflammatory activity showed by two different doses of methanolic extract were found to be significant but less effective than reference standard compound i.e. Indomethacin ( $0.312 \pm 0.01$ ) [Fig 1.2]. When methanolic fruits extract of *Pyrus pashia* at 100 mg/kg body weight per day given orally as a suspension the paw volume was reduced by 56.61%, whereas in case of the same extract of *Pyrus pashia* at 150 mg/kg body weight per day shows a 61.12% inhibition, which indicate that the effect of methanolic extract of *Pyrus pashia* reflected in a dose-dependent manner. Hence, both doses of methanolic extract showed an inhibitory effect on carrageenan-induced paw edema thus

exhibiting anti-inflammatory effect against acute inflammation.

## CONCLUSION

Thus it can be concluded that the methanolic extract of *Pyrus pashia* fruits showed potent anti-inflammatory activity. A preliminary phytochemical screening of the methanolic extract of *Pyrus pashia* fruits showed the presence of flavonoids, terpenoids, phenolic compounds and tannins, which is may be responsible for the anti-inflammatory activity. Further studies on the isolation and structure determination of the active principle and its mode of action are suggested for the development of a new drug candidate in the treatment of inflammatory diseases.

## ACKNOWLEDGEMENT

This work is financially supported by UCOST, Dehradun [UCS&T/R&D/CHEM-16/09/10/ 6539/1]. The authors would like to thank Dr. Abhishek Mathur Sr. Scientist, National Center of Fungal Taxonomy, New Delhi, for animal's activity.

## REFERENCES

1. Kumar V, Abbas AK, Fausto N (eds.) In: Robbins and Cotran pathologic basis of disease. 7<sup>th</sup> ed. Philadelphia, Elsevier Saunders, 2004, PP 47-86.
2. Gautam R, Jachak SM, Recent developments in anti-inflammatory natural products, *Medicinal Research Reviews*, 2009, 29, 767-820.
3. Cho, Shin, Noh, Cho, Hong, Park, Lee, Anti-inflammatory effects of methanol extract of *Patrinia scabiosaefolia* in mice with ulcerative colitis, *Journal of Ethnopharmacology*, 2001, 136, 428-435.
4. Jani N, Regueiro MD, Medical therapy for ulcerative colitis *Gastroenterol. Clin. N. Am.*, 2002, 31, 147-166.
5. Lakatos PL, Lakatos L, Ulcerative proctitis: A review of pharmacotherapy and management, *Expert Opin. Pharmacother.*, 2008, 9, 741-749.
6. Shui, G., & Leong, L. P, Separation and determination of organic acids and phenolic compounds in fruit juices and drinks by HPLC. *Journal of Chromatography A*, 2002, 977(1), 89-96.
7. Maga J. A, Simple phenol and phenolic compounds in food flavour, *Critical Review in Food Science and Nutrition*, 1978, 10, 323-372.
8. Lin J, Opak War, and Geheeb-Keller M, Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and antimicrobial activities. *Journal of Ethnopharmacology*, 1999, 68, 267-274.
9. Organization for Economic Cooperation and Development (OECD), Acute Oral Toxicity, Up and Down Procedure. Available from, <http://www.oecdbookshop.org/oecd/index.asp?lang=en>, 2008, pp. 1-27. July 11. Section 4, Health Effects: Test No. 425.
10. Winter CA, Risley EA and Nuss GW, Carrageenan induced edema in hind paw of the rat and an assay for anti-inflammatory drugs, *Proceeding of the Society of Experimental Biology and Medicine*, 1962, 111, 544-547.
11. Snedecor GW and Cochran WG, Statistical Methods, Iowa state University Press, Iowa, USA, 1980, 75.