

Original Article

ISSN 0975-8216

ANTI-INFLAMMATORY ACTIVITY OF N-BUTANOL FRACTION OF PRUNUS PERSICA L AQUEOUS EXTRACT

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ABSTRACT

Prunus persica L widely distributed in Manipur, India. The leaves were used in folklore/traditional medicine to treat several inflammatory pathologies such as greenish swelling (gland), oedema etc. Reactive oxygen species as well as reactive free radicals such as hydroxyl (OH), nitric oxide (NO) etc. contribute significantly to these pathologies and Which is very popular as a medicinal agent as revealed in a ethnopharmacological survey conducted in different district of Manipur by team of scientists, IBSD, Imphal. Traditional practitioners of the region used leaves of this plant as antihypertensive agent; it's also giving edible fruits. In this study, anti-inflammatory activity of the aqueous extracts (AEPp), of Prunus persica L leaves was evaluated on carrageen in induced oedema. To determine the acute toxicity (LD₅₀) of different products like aqueous extract of Prunus persica L. To evaluate the anti-inflammatory activity of aqueous extract of Prunus persica L leaves on albino rats.

Keywords: Prunus persica, ethnopharmacological.

INTRODUCTION

The use of plants and plant extracts for medicinal purposes has been going on for thousands of years. Herbarium and folk medicine both ancient and modern have been the source of much useful therapy. Some of the plant products currently used either in their natural form or as derivatives, were often used originally for other purposes, such as arrow poisons, as part of religious or other rituals and

even as cosmetics. An examples of such products includes Opium, Belladonna, Cinchona bark, Ergot, Curare, Nutmeg, Colaber beans, Foxglove etc. The earliest mention of medicinal use of plants found in RIG VEDA oldest repository of the human knowledge, having been written about 4000B.C. (Chopra, 1958). Sushrutta samhita written earlier than 1000B.C. contains very comprehensive notes on therapeutics. Charka samhita gives remarkable

materia medica of ancient Hindus. The plant kingdom represents an extraordinary reservoir of novel molecules of estimated 4,00,000-5,00,000 plant species around the globe, only a small percentage of which have been chemically investigated to isolate and identify secondary plant constituent. The vast majority of these secondary compounds are not essential for the normal physiology of plant growth and reproduction. With this background in present study we thought of finding a remedy available at a hand's stretch for the treatment and management of inflammatory response. In addition to this there is a global trend to revive the traditional systems of medicine. In the globalization and post- GATT scenario the cost of allopathic medicines is escalating. The side effects associated with various allopathic drugs are also the cause for renewed interest in traditional systems of medicine. In this background we thought to find out effective safer and cheaper remedy for inflammatory disorders at a hand's stretch. In this connection we had undertaken field surveys and contact programmes with native practitioners of Manipur to explore the possibilities of using locally available herbs for the purpose. In two of our field surveys we found a plant *Prunus persica* L, and native practitioners were claimed that it is highly useful in treating inflammatory disorders. In our present study used animal model to evaluate *Prunus persica* L.

The Carrageenan induced inflammation is a useful model to detect oral action of anti-inflammatory agents. The development of carrageenin induced edema is believed to be biphasic of which the first phase is mediated by release of histamine, serotonin, and kinins in the first hour after injection of carrageenan and the second phase is related to release of prostaglandin like substances in 2-3 hours.

Materials & Methods:

Plant Material

***Prunus persica* L** leaves were collected from the garden of IBSD, Imphal. The plant was identified and authenticated by Dr. Biseswhori Thongam, Scientist – C (Plant Taxonomy), IBSD, Takyelpat, Imphal, Manipur where a voucher specimen were deposited for reference to Plant Taxonomy and conservation Lab, IBSD, Takyelpat, Imphal. The leaves were shade dried at room temperature. The powder obtained was subjected to Soxhlet extraction with the water as solvent. Aqueous extract divided in two equal volumes. One portion concentrated in vacuum evaporator and dried in desiccators and other portion was mixed with equal quantity of petroleum ether and vigorously shaken in separating funnel to separate aqueous and petroleum ether portions. Same aqueous portion was again mixed with Chloroform, n-butanol and ethyl-acetate one after another and separated respective portion in similar manner to get the Chloroform, n-butanol, ethyl-acetate and aqueous fractions respectively.

The aqueous extract and its aqueous, n-butanol, ethyl-acetate and chloroform fractions were used for in vitro antioxidant studies. The products were concentrated under reduced pressure and stored in refrigerator $8 \pm 2^\circ \text{C}$.

Animals:

Albino rats (wister) weighing 150-200g and albino mice weighing 20-25g of either sex were used in this study. They were procured from Regional Institute of Medical Sciences (RIMS), imphal. The animals were acclimatized for one week under laboratory conditions. They were housed in polypropylene cages and maintained at $27^\circ\text{C} \pm 2^\circ\text{C}$ under 12 hours dark / light. They were fed with soya bean chock, Gram and water ad libitum was provided. The litter in the cages was renewed daily to ensure hygienic condition and maximum comfort for animals. Ethical clearance for handling the animals was obtained from the Institutional Animals Ethical Committee (IAEC), IBSD, Imphal prior to the beginning of the project work.

Determination of acute toxicity (ALD₅₀)

The acute toxicity for aqueous extracts (AEPp), of *Prunus persica L* leaves was determined in albino mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose (OCED Guideline no. 420) method of CPCSEA was adopted for toxicity studies (Mrs Prema Veeraraghavan, 2003). The tested extracts were

administrated orally. No mortality was observed at 2000mg/kg in the all cases⁵.

Evaluation of Anti-inflammatory Activity

Carrageenan - induced paw edema

The animals were divided into seven groups of 5 animals each. Inflammation was induced by injecting 0.1ml of 1% w/v carrageenan sodium salt subcutaneously in the sub-plantar region of the rat right hind paw in each groups.

Group-I - Animals (Control) were administered 1ml distill water p.o., /animal

Group-II -Animals were administered with diclofenac sodium 8mg/kg bw .

Group-III - Animals were administered with aqueous extracts 200mg/kg/ p.o.,

1 hour after oral administration of reference and test drugs, carrageenan was injected. The hind paw volume was measured plethysmometrically before and after the carrageenan injection, at hourly intervals for 6 hr¹.

$$\% \text{ inhibition of edema} = \left(\frac{V_c - V_t}{V_c} \right) \times 100$$

Where, V_T = mean paw volume of test group.

V_C = mean paw volume of control group.

Results:

1. Determination of acute toxicity (ALD₅₀)

In acute toxicity study of n- butanol fraction and of *Prunus persica L* leaves dose not shown mortality at the dose of 2000mg/kg. Therefore

2000 mg/kg dose was consider as ALD₅₀ cut off the dose (safe dose) so 1/10 of that dose was selected (200 mg/kg) for *in vivo* experiments.

The aqueous extracts (AEPc), of *Prunus persica* L leaves cause significant inhibition of carrageenin, induced edema.

2. Effect of *Prunus persica* L on carrageenin induced paw edema in rats

Table-1 Effect of unknown samples on carrageenin induced paw edema in rats.

Toxin: 0.1ml of 1% w/v carrageenin sodium salt subcutaneously in the sub-plantar region of the rat right hind paw

Treatment	Mean paw volume (ml)					
	0 hrs	1 hrs	2 hrs	3 hrs	4 hrs	6hrs
Normal control (1ml dist. water p.o.)	0.30	0.40	0.60	0.65	0.65	0.55
	0.20	0.45	0.50	0.50	0.50	0.50
	0.20	0.30	0.45	0.55	0.40	0.40
	0.30	0.45	0.60	0.77	0.55	0.55
	0.20	0.40	0.55	0.65	0.60	0.60
Standard Diclofenace Sodium (8 mg/ kg P.O.)	0.20	0.35	0.40	0.40	0.30	0.25
	0.20	0.30	0.45	0.50	0.40	0.30
	0.20	0.40	0.40	0.35	0.30	0.30
	0.30	0.45	0.50	0.50	0.35	0.35
	0.20	0.35	0.35	0.30	0.20	0.20
n- butanol fraction 200 mg/kg	0.30	0.45	0.45	0.40	0.40	0.35
	0.30	0.35	0.40	0.40	0.38	0.30
<i>Prunus persica</i> L	0.45	0.55	0.60	0.55	0.50	0.45
	0.20	0.30	0.35	0.30	0.25	0.25
	0.25	0.35	0.40	0.40	0.35	0.30

Table-2 Effect of unknown samples on carrageenin induced paw edema in rats.

Toxin: 0.1ml of 1% w/v carrageenin sodium salt subcutaneously in the sub-plantar region of the rat right hind paw

Treatment	Mean paw volume (ml)					
	0 hrs	1 hrs	2 hrs	3 hrs	4 hrs	6hrs
Normal control (1ml dist.water p.o.)	0.24	0.4	0.54	0.62	0.54	0.52
	± 0.02449	± 0.02739	± 0.02915	± 0.04665	± 0.04301	± 0.03391
Standard Diclofenace Sodium (8 mg/ kg P.O.)	0.22	0.37	0.42	0.41	0.31	0.28
	± 0.02000	± 0.02550	± 0.02550	± 0.04000	± 0.03317	± 0.02550
n- butanol fraction 200 mg/kg P.O.	0.3	0.4	0.45	0.41	0.376	0.33
	± 0.04183	± 0.04472	± 0.04183	± 0.0400	± 0.04032	± 0.03391
<i>Prunus persica</i> L						

Conclusion:

At sites of inflammation, increased free radical activity is associated with the activation of the neutrophil NADPH oxidase and/or the uncoupling of a variety of redox systems, including endothelial cell xanthine dehydrogenase. Our present study revealed that *Prunus persica* L able to cause protection against above mentioned pathologies of inflammatory disorder in experimental animals that also claimed by traditional practitioners. Where aqueous extract of *Prunus persica* L leaves caused significant acute anti-inflammatory effect, but that was less than respective reference standard. *Prunus persica* L leaves may safe anti-inflammatory for next generation. However, further work on phytochemicals present in the plant and its details pharmacological properties is carrying out in the pharmacology laboratory, IBSD, Imphal.

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