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Anti-Inflammatory activities of metanolic extract of galls of *Pistacia integerrima*

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Abstract
Majority of world population use medicinal plants as they contain compounds which have potential to be used in modern medicine for the treatment of diseases which are not curable. Extracts of *Pistacia integerrima* galls have been dispensed by traditional practitioners of Subcontinent for chest diseases as well as for aches and pains in the body. The galls of the plant are aromatic, astringent, expectorant and also have high value in ayurvedic medicine as a remedy for asthma, phthisis, dysentery, bronchitis, skin diseases, psoriasis, fever, snake bite and as appetizer. They also have been found useful in pelvic inflammatory disorders, diarrhoea and fever in children, excessive menstrual bleeding, pelvic inflammatory conditions and many more. The gall extract has also shown antidepressant and analgesic and has also shown anti-inflammatory activities in several animal models. The present study was planned to evaluate the possible anti-inflammatory effects of galls of Pistacia integerrima extracts on carragenen induced paw edema in rats. In the present investigation methanolic gall extract of *P. integerrima* has shown anti-inflammatory activity in acute and chronic phases of inflammation, strengthening its potential as an anti-inflammatory agent which has enormous potential to be exploited and researched in complicated inflammatory pathology.

Keywords: *Pistacia integerrima*, galls, inflammation, edema

1. Introduction
Inflammation, a response triggered by damage to living tissues is evolved in higher organisms to protect them from infection and injury. Its purpose is to localize and eliminate the injurious agent and to remove damaged tissue components so that the body can begin to heal. The response consists of changes in blood flow, an increase in permeability of blood vessels, and the migration of fluid, proteins, and white blood cells (leukocytes) from the circulation to the site of tissue damage. An inflammatory response that lasts only a few days is called acute inflammation, while a response of longer duration is referred to as chronic inflammation.
Inflammation involves different factors such as infections, chemicals, thermal and mechanical stimuli [1] and reactions ranging from enzyme activation, mediator release to breakdown of tissue and repair [2]. Number of anti-inflammatory drugs is now available which potentially inhibit cyclooxygenase (COX) pathway of arachidonic acid metabolism responsible for production of prostaglandins. Prostaglandins are hyperalgesic, potent vasodilators and also contribute to erythema, edema, and pain [3]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most widely used clinically important medicines for the treatment of inflammation-related diseases like arthritis, asthma and cardiovascular disease owing to their efficacy over wide range of inflammatory conditions [4, 5]. The long-term therapy of NSAID administration may evoke adverse reactions such as gastro-intestinal ulcers, bleeding and renal disorders which are attributed to their non-selective inhibition of both constitutive (COX-1) and inducible (COX-2) isoforms of the cyclooxygenases enzymes [6–8]. Therefore, new anti-inflammatory agents lacking adverse effects of NSAIDS and opiates are being searched across the globe [9, 10].
Nature is a complete storehouse of remedies of all ailments of mankind and plants are important source of new chemical substances which have the potential beneficial therapeutic effects without serious side effects and adverse reactions. In searching anti-inflammatory drugs, the research has also shifted to plants with alleged folklore use as anti-inflammatory agents, therefore viewed as logical research strategy for searching clinically useful new agents [11].
Pistacia *integerrima* (Anacardiaceae) has known as kakra shingi. The plant is known as chakra, chandraspada, shikari in Sanskrit, kakra in Hindi. It's an 18 m moderate sized deciduous tree found in the Himalayas from Indus to Kumaon. *Pistacia integerrima* is

described as a multipurpose tree species. It is found in rocky places of the warm valleys in the Himalayan region. Many species of genus Pistacia are used in traditional medicine against various ailments [3]. In fact their plant extracts have been studied for a variety of biological activities such as bronchodilator, antiemetic, diuretic, analgesic, anti-inflammatory, and antirheumatic effects. The chemical constituents from the oil of *Pistacia integerrima* galls contain 91% monoterpenes which include *α*-pinene, *β*-pinene, limonene, cineol, *α*-terpineol, *β*-farnisene and sabinene [13, 14].
Galls are a kind of swelling growth on the external tissues of plants or animals. Plant galls are abnormal outgrowths of plant tissues, similar to benign tumors or warts in animals. They can be caused by various parasites, from fungiand bacteria, to insects and mites. Typical type of worms make horn shaped galls on the branches and leaves. The galls of pisticia are pale greenish brown or pinkish, elongated, horn-shaped, hollow, twisted, curved or straight. When young they are coriaceous, but later become hard. The dried crushed galls of *Pistacia integerrima* have a very sharp and to some extent bitter in taste and terebinthine odour. This gall is caused by the insect Dasia aedifactor (Homoptera), (plant produce resin against insect. They make these galls by sucking juice from the leaves Then they are called karkatshringi.. Majorly, galls contains resins, pistacienoic acid, tetracyclic triterpenes, camphene, luteolin, pistancin, pistacinin, amino acids, dihydromavalic acids, sterols and tannins (Warrier *et al*., 1995). It has been reported to have depressant, analgesic antiinflammatory activities and hyperuricemic effect.
The galls of plant are aromatic, expectorant and stimulant and have significant value in Indian system of medicine for treatment of asthma, phthisis and other respiratory tract conditions. They are also used in remedy of dysentery, chronic bronchitis, fever, vomiting, skin diseases, psoriasis and as appetizer. Sushruta prescribed galls in combination with other drugs for treatment of snake bite and scorpion sting [12].
Different experimental evidences have suggested that the anti-inflammatory properties of some species of genus Pistacia are due to the enzymatic inhibition of cyclooxygenase and lipoxygenase. These effects seem to be related to the presence of terpenoids and flavonoids.
Based on above findings, *Pistacia integerrima* gall alcohol extract was evaluated for anti-inflammatory effects on experimentally induced inflammation.

Material and Methods: The galls of *Pistacia integerrima* were collected from the hilly region of Darlaghat, District Solan, Himachal Pradesh and was authenticated by Sr. Scientist, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. **Preparation of Extract**: Shade dried coarsely powdered plant material was packed into thimble as per the quantity sufficient according to volume of extractor. The packed thimbles were placed into the soxhlet extractor and repeatedly extracted using soxhlet extraction method in a 2000ml round bottom flask with solvents starting from petroleum ether, chloroform and methanol. The reflux time for each solvent was about 72 hours or until the solvent coming down the siphon tube became colourless. The excess of solvent in the extract was distilled off and the extracts were cooled at room temperature and evaporated to dryness.

Acute Toxicity Study: The dose progression slope 2 (OECD 425) was followed throughout the study with the starting dose of 175mg/kg body weight of methanolic extract of *Pistacia integerrima*. Rat number 1 was treated at the dose level of 175mg/kg body weight. No mortality was observed at this dose level hence, rat number 2 was treated at the higher dose level of 550mg/kg body weight. As rat number 2 survived, rat number 3 was treated at higher dose level of 1750mg/kg body weight. The rat number 3 also survived therefore, rat number 4 was treated at limit dose level of 5000mg /kg body weight. As rat number 4 survived, two rats (number 5 and 6) were sequentially treated at same dose level of 5000mg/kg body weight. All the animals were found safe at dose of 5000mg/kg and observed for mortality, clinical signs, body weight and pathology. No mortality was found but toxic symptoms were observed at 5000mg/kg body weight dose levels. So on the basis of finding of dose range finding study dose level of 100mg (low dose) and 200mg (high dose) were selected for efficacy studies.

Anti-inflammatory Activity: Wistar albino rats (180 - 250 g) of either sex were acclimatized for 7 days under standard husbandry conditions, i.e. room temperature 25±1^0 C, relative humidity 45-55% and light/dark cycle 12/12 h. The experimental protocols were approved by the Institutional Animal Ethical Committee.

Carrageenan induced rat pedal inflammation: The rats were divided into four groups of six animals each. Group A: saline control; Group B: 10mg/kg Indomethacin; Group C: Methanolic Extract of *P. integrimma* 100mg/kg; Group D: Methanolic Extract of *P. integrimma* 200mg/kg. Acute pedal inflammation was induced one hour after oral administration of alcoholic extracts of crude drug, saline control and indomethacin by injecting 0.1ml of 1.0% Carrageenan in 0.9% NaCl. Paw volume was measured with the help of plethysmometer by mercury displacement method from 0 to 3 hours [15].
The percentage inhibition of pedal oedema was calculated using the formula:
Percentage inhibition= $(1- V_t /V_c) \times 100$
Where, V_t = paw oedema in the drug treated group.
V_c= paw oedema in the control group.

Results: The mean paw volume and the percentage of inhibition of the carrageenan induced paw oedema by alcoholic extracts of *P. integrimma* (100, 200 mg/kg) and indomethacin are depicted in table no.1. Carrageenan was injected into the paw one hour after orally administered extract. Measurement of paw was taken at 0, ½, 1, 2 and 3, hours after injection. Significant reduction in paw volume was observed (68.49%, 71.23%) at both dose levels as compared with standard drug Indomethacin (69.86%) at the end of 3 hours.

Treatment	Dose mg/kg	Time, mean paw volume ± SEM (% inhibition)				
		0 hr	1/2 hr	1 hr	2 hr	3 hr
Control	0	0.25 ± 0.02	0.42 ± 0.01	0.56 ± 0.02	0.64 ± 0.04	0.73 ± 0.02
Indomethacin	10	0.24 ± 0.03	0.34 ± 0.03 (19.05)	0.30 ± 0.01* (46.43)	0.27 ± 0.02* (57.81)	0.22 ± 0.02* (69.86)
Methanolic extract of	100	0.23 ± 0.01	0.33 ± 0.02 (21.43)	0.35 ± 0.03*(37.50)	0.29 ±	0.23 ± 0.02*

P. integrimma					0.01*(54.69)	(68.49)
	200	0.21 ± 0.02	0.32 ± 0.01 (23.81)	0.33 ± 0.02*(41.07)	0.24 ± 0.02* (62.50)	0.21 ± 0.02* (71.23)

Discussion

The current investigation on methanolic extract of *P. integrimma* galls establishes the anti-inflammatory activity in the *in-vivo* animal model. Carrageenan induced paw oedema persist as frequently used *in-vivo* inflammation animal model used to assess the anti-inflammatory activity of natural products. It is a useful model to assess the contribution of mediators involved in the vascular changes associated with acute inflammation. The oedema formation is believed to be biphasic. The early phase (0-2hr) is mediated by release of histamine, serotonin and synthesis of Prostaglandins in the damaged surroundings of tissue. The late phase is mediated by bradykinin, leukotrienes, and release of prostaglandins [16].

In the present study the orally administered extract showed anti-oedematous activity at both doses from first hour and maximum activity at end of third hour, suggesting activity may be due to inhibition of mediators of inflammation such as histamine, serotonin released during the first phase and bradykinin and prostaglandins during second phase.

Based on the above findings it can be concluded that methanolic extract of gall of *P. integrimma* possesses an anti-inflammatory activity in acute and chronic phases of inflammation.

The multipurpose medicinal plant (P. integerrima), is the unique source of various types of compounds having diverse chemical structures. Very little work has been done on the biological activity and plausible medicinal applications of these compounds and hence extensive investigation is needed to exploit their therapeutic utilityto combat diseases. The present results therefore, offer a scientific basis for the traditional use of the various extracts of P. Integerrima (17)

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