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Studies on the Anti-inflammatory and Analgesic Activities of *Allium stracheyi,* A Medicinal Plant of Alpine Himalayas of Uttarakhand

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Abstract: In the present study, *Allium stracheyi* leaves were explored for their anti-inflammatory and analgesic potential on experimental model and compared to standard drugs. The results showed that methanolic extract show significant reduction in inflammation i.e. 61 % (100 mg/kg) ($p< 0.5$) as compared to the standard drug Diclofenac sodium suspension in 0.1 % Tween 80 (10 mg/kg body weight) as well as the highest analgesic potential i.e. 64.62 % (100 mg/kg) as compared to aspirin 68.62 % at 25 mg/kg body weight. Thus, methanolic extract of the plant can be explored for its anti-inflammatory and analgesic potential which has not been reported so far. The plant extract showed a relative low toxicity hence justifies the folkloric use of plant by the local people in Western Himalayan region for curing inflammation and painful conditions.

Key words: *Allium stracheyi*, anti-inflammatory activity, analgesic activity.

Introduction

Drugs which are presently in use for the management of pain and inflammatory conditions are either narcotics e.g. opioids, or non-narcotics e.g. salicylates, and corticosteroids e.g. hydrocortisone. All these drugs present well known side effects and toxic effects [1,2,3]. Moreover, synthetic drugs are very expensive to develop, since, for successful introduction of a new product approximately 3000-4000 compounds are to be synthesized, screened and tested whose cost of development ranges from 0.5-5.0 million dollars. On the contrary many medicines of plant origin with good absorption, less toxicity and easy availability have been used since long time [4] without any adverse effects. In recent years, nutrient and non-nutrient phyto-compounds are being extensively explored for their potential preventive effects against various disorders. Phyto-compounds with antioxidant, anti-inflammatory, cell cycle modulating apoptotic effects are considered to have therapeutic potential against various diseases. It is therefore essential that efforts should be made to introduce new medicinal plants to develop cheaper and effective drugs [5,6]. Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drug [7].

Allium stracheyi (Jambu) is a perennial herb, flowers are white in color about 35-40 centimeters in height and is traditionally being used by the local people as spice for flavoring. It is mainly found at the height of 2500-3000 meters of Alpine Himalayas of Uttarakhand, India near moist rock, dry rock and steep slope with a strong preference of sunny site. Edible plant part used includes flowers, leaves, root and bulb. The leaf and bulb parts of this plant are used locally in the alleviation of inflammation and painful conditions [8]. Leaves and inflorescences are also used as seasoning agents. Although no specific mention

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of medicinal uses has been for this species, member of this genus are in general very healthy additions to the diet. They contain sulphur rich compounds with antioxidant, antiinflammatory, and antimicrobial properties. Thus, in the present study attempts were made to investigate its anti-inflammatory and analgesic potential with a view of justifying the use of this plant in treatment of inflammatory disease and analgesic locally.

Materials and methods

Material

Whole plant of *Allium stracheyi* was collected from various parts of Uttarakhand, India. The leaves of this plant were dried under shade at 27°C-30°C for 15 to 30 days, after which the leaves of the plant were chopped and grounded into coarse powder.

Preparation of plant extract

The coarse powder of material (200 gm) was successively extracted with solvents in order of increasing polarity like petroleum ether, chloroform, methanol and water using hot soxhlet extractor. The resulting extracts were concentrated by vacuum evaporator keeping the maximum temperature 48°C to 50°C.

Phytochemical screening

The presence of various phytochemical constituents in the extract was determined using standard screening tests [9].

Experimental animals

Wistar rats (150-200g) and Swiss Albino Mice (25-40g) of either sex were used for this study. The animals were obtained from the animal house of the Sardar Bhagwan Singh (P.G) Institute of Biomedical Sciences and Research, Dehradun, Uttarakhand, India. The animals were maintained under standard environmental condition and had free access to food and water before administration of plant extracts.

Anti-inflammatory activity

Anti-inflammatory activity of *Allium stracheyi* was evaluated by carrageenan-induced rat paw edema method [11]. Twenty five albino rats of either sex were taken and divided into 5 groups, each group contained 5 rats. Group I- received Tween 80 solution (0.1 %) in a volume of 10 ml/kg body weight, Group II- received Diclofenac sodium suspension in 0.1 % Tween 80 (10 mg/kg body weight), Group III- received Petroleum ether extract suspension in 0.1 % Tween 80 (100 mg/kg body weight), Group IV - received aqueous extract suspension in 0.1 % Tween 80 (100 mg/kg body weight) Group V- received methanol extract suspension in 0.1 % Tween 80 (100 mg/kg body weight) in a volume of 10 ml/kg, half hour after carrageenan (0.1 ml of 0.1 %) administration in the sub plantar region of left hind paw of each rat. They were deprived of water during experiment to ensure uniform hydration and to minimize variability in oedematous response [11]. The measurement of the paw volume (cm^3) was done on the principle of volume displacement using digital Plethysmometer. The readings were taken before and at 30 min intervals after the injection of carrageenan for a period of 4 hrs. The edema at each time was calculated in relation to the paw volume before the injection of the carrageenan. The anti- inflammatory activity was determined as the percentage of inhibition of inflammation after it was induced by carrageenan by taking volume of inflammation in control group as 100 %. The percentage inhibition was calculated by using the formula:

% Inhibition =Mean paw inflammation of control –Mean paw inflammation of test / Mean paw inflammation of control x 100.

Acute toxicity study (LD_{50})

The intraperitoneal (i.p) acute toxicity of the Methanolic extract was evaluated in Swiss Albino Mice by method described by Lorke [10]. The method involved the determination of LD_{50} value in biphasic manner. The animals were starved of feed but allowed access to water 24 hours prior to the study. In the initial investigatory step i.e. phase-1 of experiment, a range of doses of the extract producing the toxic effects were established. This was done by intraperitoneal administration of widely differing doses of the extract (50, 100, 500, 1000, 1200 mg/kg i.p) to

four groups of mice (of four each). Based on the result obtained, a phase II investigatory step was done by giving more specific doses (350, 400, 450, 500 mg/kg i.p) to four other groups of mice. The mice were observed for 24 hours for such behavioral signs as nervousness, excitement, dullness, ataxia or death. The LD_{50} was estimated from the geometric mean of the dose that caused 100 % mortality and the dose which caused no lethality. Data were expressed as mean ± S.E. The results were statistically analysed by the Students t-test; $p<0.5$ versus respective control was taken as significant.

Acetic acid-induced writhing in mice

The analgesic investigation was carried out according to the method of Koster [12]. The mice were divided into six different groups (of four mice each). They were differently pretreated with the different extract (100 mg/kg i.p), aspirin (100 mg/kg i.p) and Control normal saline (10 ml/kg i.p) 30, 60, 90 and 120 min after the treatment, 1 % acetic acid solution was administered to the mice (1 ml/100 gm, i.p). They were placed in a transparent observation box. Five minutes after the adminis-tration of acetic acid the number of abdominal constrictions (writhes) made within 20 minutes of every mouse was counted. The results of the treatment groups were compared with those of normal saline pre-treated control. The percentage of the writhes was calculated as:

% of writhes = (Test mean/Control mean) x 100.

Statistical analysis

The calculation of the average edema for the anti-inflammation and percentage writhing reflex for anti-nociception were based on the expression of numerical data as mean ± SEM. The statistical significance between groups were analyzed using two-way analysis of variance (ANOVA), followed by students t-test. p values < 0.5 were taken to be significant.

Results

Phytochemical screening

Various tests were performed for the detection of carbohydrate, result showed the presence only in methanolic and aqueous extract while petroleum ether extract was completely devoid of the carbohydrate. Various tests were also performed for the detection of protein and amino acid. All the test result indicates that all the extract is devoid of protein and amino acids. Salkowski test confirmed the presence of steroids only in petroleum ether while Hensens test confirms the presence of steroids in methanolic extract as well as aqueous extract. For the presence of alkaloids Wagners and Hagers test was performed. Both the test confirmed the presence of alkaloids in methanolic and aqueous extract. Ferric chloride test confirmed the presence of phenolic and flavanoid compounds in methanolic and petroleum ether. Tannins and saponins were also present in methanolic and aqueous extract whereas petroleum ether was devoid of the same. The results of qualitative analysis indicated that petroleum ether and chloroform extract is rich in steroids while methanolic and aqueous extract is rich in alkaloid and saponins (Table 1).

Anti-inflammatory activity

In the study the pro-inflammatory effect was observed for methanol, petroleum ether and aqueous decoction against the standard Diclofenac sodium (Table 2). Out of the three extracts methanolic extract showed significant percent reduction (61%) ($p<0.5$) as compared to the diclofenac sodium that showed 72 % inhibition of paw volume (Figure 1). Non-significant inhibition was observed for petroleum ether extract and aqueous extract with 46 % and 20 % inhibition when the dose administered was 100 mg/kg. Statistical analysis showed that the extract of methanol showed highest anti-inflammatory potential than petroleum ether extract while the least activity was found with the aqueous plant extract. Maximium activity was found at three hour interval with each dose. *Allium* species, are characterized by their rich content of thiosulfinates and other organosulfur compounds, such as the well known lachrymatory factor. The thiosulfinates or alkane(ene) thial-*S*-oxide are formed by the action of the enzyme alliinase (E.C. 4.4.1.4) from their respective S-alkenyl cysteine sulfoxides which are the main responsible of

Table 1. Qualitative chemical analysis of different extracts of *A. stracheyi*

Test performed	Petroleum ether extract	Methanol extract	Chloroform extract	Aqueous extract
Test for carbohydrates				
Fehling's test	(-)	(+)	(-)	(+)
Molish's test	(-)	(-)	(-)	(-)
Barfoed's test	(-)	(-)	(-)	(+)
Benedict's test	(-)	(+)	(-)	(-)
Selivinoff's test	(-)	(+)	(-)	(+)
Test for proteins and amino acid				
Millon's test	(-)	(-)	(-)	(-)
Biuret test	(-)	(-)	(-)	(-)
Ninhydrin test	(-)	(-)	(-)	(-)
Test for steroids				
Salkowski test	(+)	(-)	(+)	(-)
Gilberman-Buchard's test	(+)	(-)	(+)	(-)
Hensen's test	(-)	(+)		(+)
Test for alkaloids				
Wagner's test	(-)	(+)	(-)	(+)
Hager's test	(-)	(+)	(-)	(+)
Mayer's test	(-)	(-)	(-)	(-)
Test for phenolic and flavonoid compounds				
Vanilin-HCL test	(-)	(+)	(-)	(+)
Ferric chloride test	(+)	(+)	(+)	(-)
Zinc hydrochloric acid-reduction test	(-)	(-)	(-)	(-)
Test for tannins	(-)	(+)	(-)	(+)
Test for saponins	(-)	(+)	(-)	(+)

Table 2. Inflammation measured after oral administration of different extract (100 mg/kg) of *Allium stracheyi* in rat hind paw

Extracts	Paw Volume (ml)	Inhibition with reference to control (%)
Control (Normal saline)	0.29 ± 0.015	-
Diclofenac sodium	0.081 ± 0.005	72.49
Aqueous extract	0.23 ± 0.012	20.69
Methanolic extract	0.112 ± 0.07	61.38
Petroleum ether extract	0.156 ± 0.07	46.21

onion flavor and produce the eye-irritating compounds that induce lachrimation. However, depending on the *Allium* species, and under differing conditions, thiosulfinates can decompose to form additional sulfur constituents, including diallyl, methyl allyl, and diethyl mono-, di, tri-, tetra-, penta-, and hexasulfides, vinyldithiins, and (*E*)- and (*Z*)-ajoene. It also contain sulfur compound (allicin, allin and agoene), volatile oils, enzymes (allinase, peroxidase and miracynase),

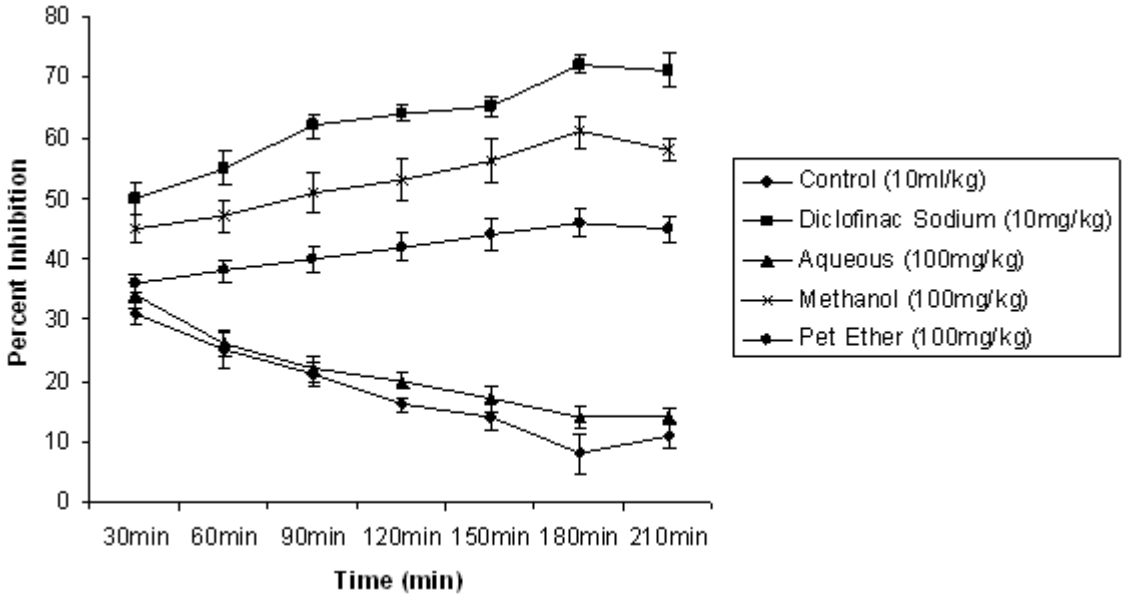

Figure 1. Comparative Anti-inflammatory activity of different extract of *Allium stracheyi* in Carrageenan induced paw oedema in rat compared to the same point in control group (Normal saline)

carbohydrates (sucrose and glucose), minerals (selenium), amino acid such as cysteine, glutamine, isoleucine and methionine which help to protect cell from the harms of free radicals, bioflavoids such as quercetin and cyaniding, allistatin 1 and allistatin 2 and vitamins C, E and a which help to protect us from oxidation agents and free radicals, and other vitamins such as niacin, B_1 and B_2 β-carotene. The presence of alkaloids and organosulphur compounds, which has been indicated in analgesic and anti-inflammatory activities, supports the traditional and local use of *Allium stracheyi*. More recently, organosulfur compounds have been found to decrease the production of inflammatory signaling molecules in cultured macrophages and human whole blood [13]. Further, it has been demonstrated that a garlic extract and its sulfur-containing compounds inhibited nuclear factor kappa B (NF-κB) activation induced by various receptor agonists including lipopolysaccharide (LPS). They have stated that ethyl acetate fraction of garlic inhibited the LPS-induced dimerization of TLR4, resulting in the inhibition of NF-κB activation and the expression of cyclooxygenase 2 and inducible nitric oxide synthase [14].

Acute Toxicity Study (LD_{50})

Out of the three solvent extracts used only methanolic extract showed a significant anti-inflammatory activity therefore LD_{50} value were only estimated for the methanolic extract. Mice treated with doses 500 mg/kg i.p. were dull, panted, showed occasional abdominal stretching and died within 24h of treatment. The adverse signs and deaths were however, not seen at doses below 500 mg/kg i.p. The LD_{50} of Methanolic extract of *Allium stracheyi* was estimated to be 445.5 mg/kg i.p. The 50 revelation of the median lethal dose LD of the Methanolic extract of A. stracheyi to 445.5 mg/kg i.p is probably an indicator that the extract may be 50 relatively safe where as estimated LD value > 1 g is considered safe. This is of importance [10] because a cumulative toxic effect could not occur if the extract is taken over time.

Acetic acid-induced writhing

The result represented in (Table 3), shows that extracts of *Allium stracheyi* at the doses of 100 mg/kg and aspirin 25 mg/kg exhibited significant ($p<0.5$) inhibition of the control writhes at the rate of 68.6 %, 55 %, 64.6 % and 38.4 % for aspirin, petroleum ether, methanol and aqueous extract respectively when compared to that of

Table 3. Comparative study of analgesic activity of *Allium stracheyi* extracts vs aspirin

Treatment	Dose	No. of writhings (Mean ±Std. Deviation)	Percent inhibition
Saline (control)	10 ml/kg	23.0±5.9	-
Aspirin	25 mg/kg	7.2±0.75	68.6
Ether extract	100 mg/kg	10.33±1.86	55.00
Methanol extract	100 mg/kg	8.1±1.16	64.6*
Aqueous extract	100 mg/kg	14.16±0.75	38.47

*P< 0.5

control. The result shows that all the extracts of *Allium stracheyi* at the doses of 100 mg/kg and aspirin 25 mg/kg exhibited significant (p<0.5) inhibition of the control writhes at the rate of 68.62 %, 55 %, 64.62 % and 38.47 % for aspirin, petroleum ether extract, methanolic extract and aqueous extract respectively when compared to that of control. In addition, extracts of *Allium stracheyi* at the above mentioned doses, potentiated analgesic activity of aspirin shown by further decreasing the writhing response when given in combination. Statistical analysis showed that Methanolic extract has highest analgesic potential than petroleum ether extract of 64.62 % of inhibition as compared to the standard aspirin that gave 68.62 % inhibition.

In the present study, the abdominal constriction (Writhing) model adopted is thought to partly involve local peritoneal receptors [15,16,17]. The ability of the methanolic extract to cause a significant reduction in the number of acetic-acid induced writhes in mice probably suggests an anti-nociceptive property. The use of abdominal constriction (writhing) model is known to be very sensitive when compared with other models such as tail flick model [16,18]. This study also showed that anti-nociceptive effect of the aqueous and chloroform extract showed very less effect in same duration of time as compared to methanolic extract.

The result obtained complimented the earlier investigation that methanolic extract of Allium species contains copious amount of flavonoids, organosulphur compounds [19]. Previous study on different Allium species also shows that these flavonoids and organosulphur compounds are potent analgesic and anti-inflammatory agents. These compounds prevent the formation of pro-inflammatory messengers. This is in line with some other reports that some metabolites are as potent or even more potent in some activities as the parent compound. The carragenan induced edema model showed the anti-inflammatory property of the extract. Although the mechanisms of action for the anti-nociception and anti-inflammation are not yet elucidated, the combination of both effects can be taken advantage of, therapeutically. Significantly (p<0.5) high anti-inflammatory activity of methanolic extract (100 mg/kg body weight) of *A. stracheyi* may be due to inhibition of the mediators of inflammation such as histamine, serotonin and prostaglandin. Thus, the research work justified the traditional use of the plant as a spice in food supplements and in the treatment of pro-inflammatory disease.

Further studies will be carried out on pharmacodynamics pattern to establish the mechanism of the action of the plant extracts.

Conclusion

The use of active phyto-constituents containing pharmacological activity as anti-inflammatory and analgesic activity has been reported for many plant species. The present study focused on the assessment of pharmacological properties of *Allium strachey.* That is being widely used in the traditional health care system and as condiment in food preparation by the local communities of Uttarakhand. The results obtained complimented the earlier investigation that the *Allium* sp. contains copious amount of flavanoids and organosulphur compounds which, are potent anti-

inflammatory agents [19]. These compounds prevent the formation of pro-inflammatory messengers and also inhibit COX-2 enzyme and LOX (lipoxygenase). Development of oedema induced by carrageenan is commonly correlated with early exudative stage of inflammation [20]. This study has shown that the methanolic extract of the leaves of *Allium stracheyi* possessed a significant anti-oedematogenic effect on paw oedema induced by carrageenan. Since carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation [21]. This inhibition action is similar to the non-steroidal anti-inflammatory drugs. From above study it is clear that methanolic extract of *A. stracheyi* have compounds which are potent anti-inflammatory agent. Phytochemical screenings showed that glycosides were isolated from the leaves of *Allium stracheyi*. Anti-inflammatory activities of many plants were attributed to their high sterol/ triterpene or flavonoids contents. The mechanisms of anti-inflammatory activity may be related to the anti-phlogistic action of tannins that were also observed in the methanolic leaf extract of *Allium stracheyi*. In conclusion, the results of the present investigation indicate that the methanolic extract of *A. stracheyi* shows profound anti-inflammatory activity and has provided some justification for the folkloric use of the plant in several communities for conditions such as stomach ache, pain and inflammations, but caution should be exercised in its use for medical purpose. The analgesic effect of the foliage leaf samples that is been prominently used in the traditional system has not been previously reported and seems to possess the properties which are mediated via peripheral inhibitory mechanisms. In conclusion, the findings of this experimental animal study indicate that the leaf methanolic extract of *Allium stracheyi* possesses analgesic properties, and thus lend pharmacological credence to the suggested ethnomedical, folkloric uses of the plant in the management and/ or control of painful, arthritic and other inflammatory conditions and can be used as the basis for the development of the drug in near future.

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