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Traditional Indian Medicinal Plants as a potential Anti-inflammatory phytomedicine for Psoriasis control

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Abstract
The aim of the present study is to support the traditional claims in the use of medicinal plants Rhinacanthus nasutus leaves and Indigofera *aspalathoides* whole plant assessed for their anti-inflammatory properties, in animal model carrageenan-induced rat paw edema. Both the extracts showed significant (***p<0.0001) decrease in paw volume at 4th hours. *R.nasutus* possessed significant inhibition of edema 61% and *I.aspalathoides* possessed inhibition 65% at the end of 4 hours with carrageenan when compared with standard Indomethacin 70%.

Keywords: Psoriasis, inflammation, anti-inflammatory, carrageenan, plants and phytomedicine.

Abbreviation: *R.nasutus* (*Rhinacanthus nasutus*) and *I.aspalathoides* (*Indigofera aspalathoides*)

1. Introduction

India is a mega rich biodiversity country with large number of species of herbal plants which are still to be undergone through various scientific approach. The traditional knowledge from tribal to various Complementary system of medicine has given root in scientific proving of the drug towards developing the herbal plants in various form for the dreadful diseases. The key effort carried out by the research and development field boosted up the herbal drugs in place of synthetic drug due to its less toxicity as a novel phytobioactive molecules obtained from potentials natural source of crude drugs.

Harmful stimuli to body, such as pathogens, damaged cells or physical injury, are able to trigger an inflammatory response. Upon inflammatory stimulation, macrophages produce nitric oxide (NO), prostaglandin E_2 (PGE_2) and pro-inflammatory, cytokines such as tumor necrosis factor (TNF-α). Overproduction of these mediators is present in macrophage of many inflammatory diseases, including rheumatoid arthritis, atherosclerosis, gout, asthma, diabetes, cancer, cardiovascular disease, psoriasis and neurodegenerative disorders, which represent an important causes of morbidity and mortality worldwide [1].

A significant advance in the study of drugs with anti-inflammatory activity came in the early 1970s. Since then, numerous investigators have addressed their research in developing drugs with the capacity to specifically inhibit inflammatory mediators. Steroidal and non-steroidal anti-inflammatory drugs (SAIDs and NASIDs, respectively) are currently the most widely used drugs in the treatment of acute inflammatory disorders, despite their renal and gastric negative secondary effects [2].

Though these have potent activity, long term administration is required for the treatment of chronic diseases. Furthermore, these drugs have various and severe adverse effects. Therefore, naturally occurring agents, with high effectiveness and very few side effects, are desirable as substitutes for chemical therapeutics. Many new drugs derived from plant secondary metabolites have been applied for the treatment and/or prevention of various diseases. Investigations about natural products have recently regained prominence with the increasing understanding of their biological significance and increasing recognition of their origin and structural diversity [3].

Thus numerous research groups are focusing on the search for new and safe anti-inflammatory agents, and medicinal plants may represent a useful source of molecules for the development of drugs especially designed for the control of chronic inflammatory states. Thus, evaluation of herbal substances that can either reduce levels/activity of pro-inflammatory mediators, or increase levels/ activity of anti-inflammatory mediators, may be a useful strategy for studying their efficacy for the treatment of inflammatory disease [2].

Thus keep in mind, the ethnomedical knowledge of *R.nasutus* [4] (Acanthaceae) and *I.aspalathoides* [5] (Fabaceae) on inflammation process which has be practiced as a folklore

medicine possess anti-inflammatory action is been studied scientifically as a promising source of new molecules entity in the treatment of inflammation disorders since still we are in search of new remedies.

2. Materials and Methods

2.1. Procurement of Plant materials

The whole plant *I.aspalathoides* were collected from grazing field of Tirunelveli districts, Tamilnadu. The leaves of *R.nasutus* were collected from Palakkad district, Kerala. Both the plants were authenticated and voucher specimens (PCOP/ Ph'cog/ 15/06/2014) was deposited at Padmavathi College of Pharmacy, Salem for future reference.

2.2. Extraction of herbs

Herbs for extract were powdered, with tougher herbs (*I.aspalathoides* - whole plant) to moderately fine and softer herbs (*R.nasutus* - leaves**)** powdered to moderately coarse. The leaves of *R.nasutus* were dried at 35-40 ^{0}C for several days and crushed into small pieces and the whole herb *I.aspalathoides* are moderately cut. The 100g of powdered parts of the raw materials were individually taken in a 1 liter round bottom flask and extracted with ethanol by hot continuous extraction method for 6 hours. The extract thus obtained was cooled at room temperature and evaporated to dryness under reduced pressure in a rotatory evaporator [6].

3. Anti-inflammatory activity

3.1. Selection of animals

Albino rats of either sex of weight 150-200g were selected and the animals were housed in polypropylene standard cages at 25±2^{0}C in 12/12 h light dark cycle for atleast one week in the laboratory animal room prior to testing. Food and water were given *ad libitum*. All the experimental protocol were performed as per the CPCSEA guidelines and approved by the Institutional Animal ethical committee. (Pharmacology project no. 1143/ac/07/CPCSEA/PCP /IAEC/PhD/140/15).

3.2 Carrageenan induced rat paw edema

Twenty four rats were divided into 4 groups of 6 rats each and the animals are starved overnight. To insure uniform hydration, the rats receive 5ml of water by stomach tube (controls). Thirty minutes later, acute inflammation was produced in all the rats, challenged by a subcutaneous injection of freshly prepared 0.05ml of 1% solution of carrageenan into the plantarside of the left hind paw. Animals were premedicated with extract of plant drugs and the Standard drug Indomethacin, orally 1 h before Carrageenan injection. The paw is marked with the ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume was measured before and after Carrageenan injection and every 30 min, 1h, 2h, 3h and 4th hours by using plethysmometer was recorded. In all the above models, the degree of edema formation was determined as increase in paw volume. Increase in paw volume and percent inhibition was calculated as under [7].

Increase in paw volume = $V_t - V_0$

Where, V_t = Paw volume at t Time, V_0 = Paw volume at 0 time.

% Inhibition in paw volume = $V_C - V_t / V_C \times 100$.

Where, V_C = Increase in Paw volume in control, V_0 = Increase in Paw volume in treatment.

3.2.1. Grouping of animals

Group –I: Control animal treated with 5% saline, orally.
Group –II: Ethanolic extract of *R.nasutus* 1gm/kg orally.
Group –III: Ethanolic extract of *I.aspalathoides* 1gm/kg orally.
Group –IV: Positive control Indomethacin 10mg/kg orally.
Each group having 6 animals.

3.3. Statistical Analysis

The results were expressed as mean ± S.E.M, n=6. Statistical difference between two means are determined by Anova followed by Dunnett's test [8, 9].

4. Result and discussion

4.1. Acute toxicity studies

Toxicity studies conducted as per internationally accepted protocol drawn under OECD guidelines in rats at the dose level of extracts upto 2000 mg/kg body weight. The groups showed no signs of toxic effect and was considered not toxic upto 2000 mg/kg.

4.2. Anti-inflammatory activity – *In-vivo*

The anti-inflammatory activity of plant extract was evaluated using carrageenan induced paw edema models of inflammation. Carrageenan-induced paw oedema as an in vivo model of inflammation was selected to assess the anti-inflammatory activity of natural product and as an acceptable and reliable method for acute anti-inflammatory studies. [10] Both the extracts exhibited significant anti-inflammatory activity against the standard. There was a gradual increase in edema of the paw in control group (carrageenan treated). Statistical analysis of the data by "one way ANOVA" followed by Dunnett's test was performed to find the significance.

Anti-inflammatory effect of the extracts in carrageenan induced paw edema is shown in the tables 1, respectively. Both the extracts showed significant (***$p<0.0001$) decrease in paw volume at 4th hr. Significant anti-inflammatory activity was shown by extracts 100mg/kg of *R.nasutus* at 1st hour (0.62±0.0060) and *I.aspalathoides* at 1st hour (0.62±0.0094) and Standard Indomethacin (0.53±0.0066) at 1st hour. The anti-inflammatory activity gradually increased with time and maximal action was evident at the 1st hour. It has been demonstrated that the suppression of carrageenan-induced hind paw edema after the third hour correlates reasonably with therapeutic doses of most clinically effective anti-inflammatory agents.

Anti-inflammatory activity of Paw volume

Table 1: A dose related paw volume of hind paws edema between 0 and 4 hours was observed.

Groups	Vehicle	0 mins	30 mins	1 hr	2 hr	3 hr	4 hr
Control	5ml/kg	0.43±0.0079	0.53±0.0066	0.72±0.0076	0.87±0.0076	0.91±0.0066	0.96±0.0079
Standard	10g/kg	0.32±0.0079	0.38±0.0087	0.53±0.0066	0.44±0.0070	0.38±0.0076	0.28±0.0060
R.nasutus	1g/kg	0.34±0.0071	0.47±0.0080	0.62±0.0060	0.57±0.0076	0.49±0.0060	0.38±0.0076
I.asplathoides	1g/kg	0.34±0.0060	0.44±0.0088	0.62±0.0094	0.58±0.0060	0.52±0.0094	0.34±0.0065

Values are mean ± standard deviation, ***p < 0.0001.

The extracts *R.nasutus* showed maximum inhibition of edema 61% and *I.aspalathoides* showed maximum inhibition 65% at the end of 4th hours with carrageenan when compared with standard Indomethacin 70% (Table 2).

Anti-inflammatory activity of % inhibition

Table 2: A dose related % inhibition of hind paws edema between 0 and 4 hours was observed.

Groups	% Inhibition
Control	0
Standard	70
R.nasutus	61
I.asplathoides	65

100mg/kg of *I.aspalathoides* (whole plant) (0.53±0.0066) at 1st hour showed anti-inflammatory activity when compared with ethanolic extract of *I.aspalathoides* leaves at test doses of 100 and 150 mg/kg clearly showed anti–inflammatory effects by reducing paw edema induced by carrageenan in a dose dependent manner in all the treated groups reported by Jothip Priya ganapathy and Dinakaran sundaram 2013 [11].

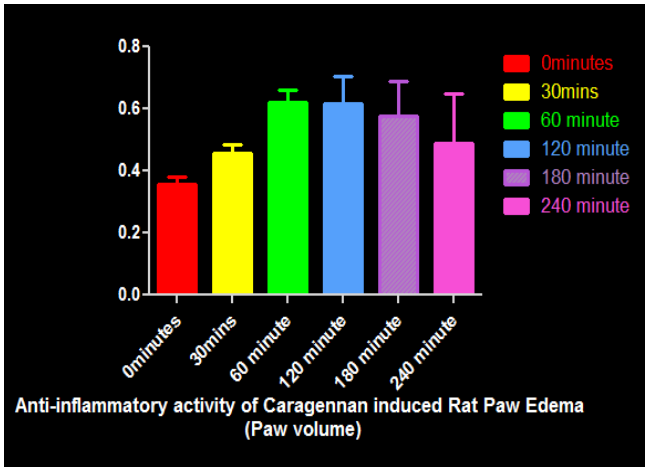
Ethanol extract of *R.nasutus* (leaves) by 100mg/kg showed maximum inhibition of edema 61% at the end of 4th hours with carrageenan and standard Indomethacin 70% which was higher when compared with the report of Standardized *R.nasutus* leaves ethyl acetate extract at the dose of 320 mg/kg produced 54.69% inhibition of edema which was comparable to 59.38% inhibition produced by the standard drug indomethacin (5 mg/kg) at 5 h post carrageenan injection [12].

Oedema formation due to carrageenan in the rat is a biphasic event. The first phase (0-1 hour after Carrageenan) mainly results from concomitant release of inflammatory mediators, namely histamine, serotonin and kinin from surrounding the damaged tissue. The second phase (1-3 hours after Carrageenan) is mediated by bradykinin, leukotrienes and sustained release of prostaglandins produced by the macrophages. The second phase is sensitive to most clinically effective anti-inflammatory drugs [13].

Oral administration of ethanol extract of *R.nasutus* and *I.aspalathoides* suppressed the edematous response after 1st hour and this effect continued upto 4th hour as compared. The observed effect was similar to that of standard Indomethacin. NSAIDS block the synthesis of prostaglandins by inhibiting cyclooxygenase.

Naturally occurring polyphones such as flavonoids, coumarins and tannin might be expected to interfere with the process of synthesis of prostaglandins to produce anti-inflammatory effects [9].

Sekhar Shailasree *et al.*, (2012) proposed that the plant-derived bioactives such as Polyphenols, flavonoids, terpenes, quinines, catechins and alkaloids are compounds targeted for anti-inflammatory activity. Potent anti-inflammatory plant compounds include guggulsterone [4,17(20)-pregnadiene-3,16-dione], a plant sterol from *Commiphora mukul*, boswellic acid, a pentacyclic triterpenic acid and its derivatives viz., acetyl-b-boswellic acid, 11-ketob- boswellic acid and acetyl-11-keto-b-boswellic acid, curcumin from turmeric, resveratrol from red grape seeds, genistein from Soy, quercetin (onions), silymarin (artichoke), withanolides (Ashwagandha), tea polyphenols, cranberries and peanuts. Medicinal plants viz., *Andrographis paniculata, Biophytum sensitivum, Boswellia serrata, Butea monosperma, Embelia ribes, Terminalia chebula* and *Tribulus terrestries* have the reported ability to down regulate NFkB activation. *Acacia catechu, Alstonia scholaris, Artocarpus hirsutus, Bacopa monnieri* and *Myristica fragrans* have reported COX-2 inhibitory activity. Further *Acacia catechu, Alstonia scholaris, Bacopa monnieri* and *Garcinia indica* have LOX inhibitory activity [14].



Graphical data 1: Anti-inflammatory of Carrageenan induced Rat Paw edema (Paw volume)

S. Kumar *et al.*, (2013) and Joao B Calixto *et al.*, (2004) discussed the core chemical classes of anti-inflammatory agents from natural sources to engage a vast range of compounds such as polyphenols, flavonoids, terpenoids, alkaloids, anthraquinones, lignans, polysaccharides, saponins and peptides.

It has been reviewed that anti-inflammatory activity is due to the presence of chemical class of compounds in ethanolic extract of root of *A. heterophyllym* which contains alkaloids, flavonoids, sterols, *Bacopa monnieri* contains triterpenoids,

bacosides, alkaloids, flavonoids, terpenes, sugar, glycosides. Crude saponins extract revealed the presence of various constituents like terpenoid and steroidal saponins, tannins and flavonoids which had been reported to have anti-inflammatory activity. Terpenoids may affect different mechanism relevant to inflammations arising in response to varied etiological factors.

From the study done so far, it has been elucidated that flavonoids are major anti-inflammatory agents. Some of them act as phospholipase inhibitors and some have been reported as TNF-α inhibitors in different inflammatory conditions. Biochemical investigations have also shown that flavonoids can inhibit both cyclooxygenase and lioxygenase pathways of arachidonic metabolism depending upon their chemical structures [15, 16].

Arya Vikrant *et al.,* (2011) and Enkelejda Goci *et al.,* (2013) reported that various secondary metabolites from barks like Coumarins, terpenes, flavonoids, sterols, saponins, glycosides etc. have been exerting wide range of anti-inflammatory activity [17, 18]

The phytochemical analysis of *I.aspalathoides* showed steroids, triterpenes, alkaloids, phenolic groups, flavones, saponin, tannin, sugar, catachine, amino acid and reducing sugar [19]. Pavala rani *et al.,* (2013) study revealed the identification of flavonoids in ethanolic extract of *I. asphalathoides* leaves and its aerial parts was confirmed by HPTLC and FT-IR spectroscopy [20]. Preliminary phytochemical testing of methanol extract shows the presence of various phytoconstituents such as glycosides, alkaloids, carbohydrates, steroids, glycosides, proteins, and phenolic compound [21]. The results of HPLC finger print analysis of flavonoids contains gallic acid, caffeic acid, rutin, quercetin and ferulic acid were performed in leaf and stem of *I. aspalathoides* DC [22]. Preliminary phytochemical screening of the methanolic root extract of *I. aspalathoides* revealed that the presence of flavonoid, phenols, tannins, saponins, steroidal terpenes and quinines [23].

Preliminary phytochemical screening of the hexane, ethyl acetate, methanol and water leaf extracts of *R.nasutus* showed positive results for the presence of secondary metabolites like steroids, saponins, triterpinoids, alkaloids, carbohydrates, flavonoids, polyphenols and glycosides. Tannins are absent in all extracts prepared [24].

5. Conclusion

The results of the present study indicated that ethanolic extract of *Rhinacanthus nasutus* and *Indigofera aspalathoides* significantly inhibited the formation of rat hind paw oedema. This effect may be due to influence on inflammatory mediators and also on pathway of prostaglandins synthesis which may be due to the presence of flavonoids, coumarins, polyphenolic compounds and other secondary metabolites present in plant extracts. However the chemical constituents and mechanism responsible for the pharmacological activities remain investigated.

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