ANTI-INFLAMMATORY AND ANTI-ARTHRITIS ACTIVITIES OF ROOTS EXTRACT OF *ASTERACANTHA LONGIFOLIA* ROOTS

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Abstract

The present study investigates the anti-arthritis activities of the root extract of *Asteracantha longifolia*. Initial assessments include an acute oral toxicity study to establish the safety profile of the extract. Subsequently, in vivo anti-arthritis activity is evaluated using two rat models: carrageenan-induced paw edema and complete Freund's adjuvant (CFA)-induced arthritis. These models are employed to assess the potential therapeutic effects of the Asteracantha longifolia root extract in inflammatory conditions. The study aims to provide valuable insights into the anti-arthritic properties of the plant extract, shedding light on its potential as a natural remedy for inflammatory joint disorders. The abstract encapsulates the primary objectives and methodologies of the research, offering a succinct overview of the study's focus and design.

Keywords: Anti-inflammatory, Anti-arthritis effect, Medicinal plants, Herbal medicines, Diclofenac, *Asteracantha longifolia*

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Introduction

Inflammation, a type of innate immunity, is a biological reaction of bodily tissues to a variety of potentially hazardous stimuli. It is known to be activated as a normal bodily defence mechanism in response to damage, exposure to pollutants, radioactive substances, toxicants, allergens, and infection by a variety of agents such as bacteria and viruses. The duration and intensity of the inflammatory response are critical factors in its outcome and repercussions. Acute inflammation is characterised by phagocytosis, apoptosis, or activation of pro-inflammatory mediators, which results in the removal of harmful stimuli and the restoration of normal physiology. Chronic inflammation, on the other hand, is a harmful process that causes Alzheimer's disease, cancer, rheumatoid arthritis, type 2 diabetes, obesity, and cardiovascular and pulmonary illness (Chen *et al*., 2017; Janssen & Henson,2012; Abdulkhaleq *et al*., 2018).

Rheumatoid arthritis is an autoimmune inflammatory disease characterised predominantly by synovitis, which is accompanied by extra-articular organ involvement, such as interstitial pneumonia, as well as clinical symptoms such as pain, swelling, joint stiffness, fever, and malaise. Joint destruction occurs quickly after the outset, and once the affected joints are distorted, irreversible physical impairment develops (Anderson *et al*, 1985).

Various nonsteroidal anti-inflammatory & anti-arthritis medications can relieve pain and inflammation by inhibiting the digestion of arachidonic acid by isoforms of the cyclooxygenase enzyme (COX-1 and/or COX-2) and so lowering prostaglandin synthesis. Despite initial effectiveness, severe cardiovascular and renal consequences, as well as gastrointestinal problems in high doses, have been recorded quickly following the debut of selective COX-2 inhibitors. These negative effects are caused by the inhibition of COX-2 constitutive synthesis in some tissues. Thus, the safety of using NSAIDs in clinical practise has been called into question in recent years, as evidence has emerged indicating a substantial risk of acute myocardial infarction, stroke, heart failure, renal failure, and arterial hypertension (Oliveira *et al*., 2019; Harirforoosh, *et al*., 2013).

So, the need for new anti-inflammatory & anti-arthritis medications aids in the advancement of research for better, safer, more effective molecules with fewer side effects and derived from plants. As a result, it is clear that a considerable number of plant-derived compounds are part of modern medicine's therapeutic arsenal. Because of the huge number of species available for investigation, the success of the creation of novel naturally occurring anti-inflammatory medications is essentially dependent on a multidisciplinary effort in the discovery of new leading compounds (Simon & Evan, 2017; Maroon *et al*., 2010).

One such plant is *Asteracantha longifolia* is an essential medicinal herb that is used for a variety of purposes. *Asteracantha longifolia* is an essential medicinal herb that is used in traditional systems of medicine for a variety of ailments such as diuretics, jaundice, dropsy, rheumatism, hepatic, blockages and dissolving of gall stones, kidney stones, liver dysfunction, and urinogenital tract disease. Lupeol, B-sitosterol, stigmasterol, butelin, fatty acids, and alkaloids are found in it (Murthy *et al*., 2017).

Knowledge of plant chemical constituents is desirable not only for the discovery of therapeutic agents, but also for revealing new sources of economic phytocompounds for the synthesis of complex chemical substances and for determining the true significance of folkloric remedies. So, this study aims at elucidating in vivo anti-inflammatory & anti arthritic activity of *Asteracantha longifolia*.

Materials & Methods

Materials

In August 2021, roots of Asteracantha longifolia were procured from the local market in Bhopal. Following collection, the roots underwent a processing phase to ensure thorough cleaning, aimed at preventing the deterioration of phytochemicals present in the plant.

Extraction procedure

Defatting of plant material

The shade dried plant material was subjected to extraction with petroleum ether by soxhlation extraction method. The extraction was continued till the defatting of the material had taken place (Khandelwal, 2005; Kokate, 1994).

Successive extraction with different solvents by soxhlet method

160 gm of powdered plant materials were extracted successively with chloroform ethyl acetate, ethanol and water using Soxhlet apparatus at 55-85°C for 8-10 hrs. in order to extract the polar and non-polar compounds (Elgorashi and Staden, 2004). For each solvent extraction, the powdered pack material was air dried and then used. The solvents of the respective extracts were reduced under room temperature and stored at 4°C for further use.

***In vivo* anti-arthritis activity**

Animals:-

Wistar rats (180±20 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Mice received standard rodent chow and water *ad libitum*. Animas were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of mice was used for each set of experiments. The Institutional Animal Ethics Committee (IAEC), established by the Ministry of Environment and Forests, Government of India, New Delhi, India, to oversee and supervise the use of experimental animals, gave its approval to the animal experiments.

Acute oral toxicity study

Acute oral toxicity study was performed as per OECD-423 guidelines ((https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl423.pdf)). *Ethyl acetate Extract of Astracantha longifolia* (5, 50, 300, and 2000 mg/kg) was administered orally for 4 days of six groups of mice (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-arthritic effect.

Experimental designs (Carrageenan induced paw edema)

Group I: Control rats were served as a normal group

Group –II: Carrageenan control (0.1 ml of 1% w/v)

Group –III: Carrageenan + ethyl acetate extract of *Astracantha longifolia -100*

Group–IV: Carrageenan + ethyl acetate extract of *Astracantha longifolia -200*

Group –V: Carrageenan + Diclofenac Sodium (5 mg/kg per day).

Anti-arthritis activity

Freund's adjuvant-induced arthritis in rats: Animals were divided into six groups containing six animals each. Arthritic syndrome was induced by subcutaneous injection of 0.1ml of complete Freund's adjuvant (10mg of heat-killed mycobacterium tuberculosis per ml of paraffin oil) into the planter surface of the left hind paw.

Experimental design (CFA-induced arthritis in rats)

It consisted of four groups (n=6) of six animals each.

Group I: Control rats were served as a normal group

Group II: Rats were administered Complete Freund's Adjuvant (CFA) to induce arthritis

Group III: CFA-induced arthritic rats treated with ethyl *acetate extract of Astracantha longifolia* -100 mg/kg/p.o. per day

Group IV: CFA-induced arthritic rats treated with *ethyl acetate extract of Astracantha longifolia* - 200 mg/kg/p.o. per day

Group V: Arthritic rats induced with CFA were treated with Indomethacin (10 mg/kg per day)

On 1st day, excluding the control group, all the other groups of rats were given a single dose of 0.1 mL of collagen into the right hind footpad intradermally. The rats of III-VI groups were given *ethyl acetate extract of Astracantha longifolia* (100 and 200 mg/kg/p.o.) and rats of diclofenac sodium were administered with diclofenac sodium (5 mg/kg per day) up to 28th days. The changes in paw volume were measured weekly by using a Plethysmograph.

Results & Discussion

In the assessment of the anti-inflammatory potential of the ethyl acetate extract from Astracantha longifolia, a dose of 100 mg/kg exhibited a reduction in paw edema thickness, measuring 1.14 ± 0.07mm, 1.62 ± 0.13mm, 2.1 ± 0.12mm, and 1.68 ± 0.17mm at 1, 2, 3, and 4 hours, respectively. Increasing the dosage to 200 mg/kg orally resulted in a more pronounced effect, preventing carrageenan-induced paw edema with thickness reductions of 0.94 ± 0.12mm, 1.44± 0.13mm, 1.9 ± 0.08 mm, and 1.56 ± 0.17mm at 1st, 2nd, 3rd, and 4th hour, respectively.

In comparison, Diclofenac sodium at a dose of 5mg/kg orally exhibited significant anti-inflammatory activity, reducing paw edema thickness to 0.94 ± 0.13mm, 1.21 ± 0.11mm, 1.57 ± 0.09mm, and 1.24 ± 0.14mm at 1, 2, 3, and 4 hours, respectively.

The anti-arthritic activity was further investigated using complete Freund's adjuvant (CFA). On day 28, the paw volume for groups administered Astracantha longifolia at doses of 100 mg/kg and 200 mg/kg was observed to be 0.63±0.031ml and 0.61±0.028ml, respectively. In comparison, the *Indomethacin* -treated group exhibited a paw volume of 0.56±0.036ml.

These results suggest that the ethyl acetate extract from Astracantha longifolia possesses notable anti-inflammatory and anti-arthritic effects,

showcasing its potential as a natural remedy in the management of inflammatory conditions.

Results of anti-inflammatory activity

Carrageenan induced paw edema

Table 1: Effect of extracts of ethyl acetate extract of *Astracantha longifolia* at doses of 100 and 200 mg/kg, and diclofenac sodium as compared to carrageenan control group at different hours in carrageenan-induced paw edema model

Group	Drug and Dose	Change in paw thickness (mm) ± SD			
		1st h	2nd h	3rd h	4th h
Group I	Normal Control	-	-	-	-
Group II	Carrageenan	1.4 ± 0.13	2.57 ±0.14	3.82± 0.12	3.92 ± 0.17
Group III	Carrageenan + ethyl acetate extract of *Astracantha longifolia -100*	1.14 ± 0.07	1.62 ± 0.13[a]	2.1 ± 0.12	1.68 ± 0.17*
Group IV	Carrageenan + ethyl acetate extract of *Astracantha longifolia -200*	0.94 ± 0.12	1.44± 0.13[a]	1.9 ± 0.08*	1.56 ± 0.17*
Group V	Carrageenan + Diclofenac Sodium (5 mg/kg per day)	0.94 ± 0.13	1.21 ± 0.11*	1.57 ± 0.09*	1.24 ± 0.14*

All values are expressed as mean ± SEM;

*$P < 0.05$ v/s carrageenan control

Results of anti -arthritic activity

Table 7: Effect of ethyl acetate extract of *Astracantha longifolia* against CFA-induced arthritis in rats

Paw volume (mL)					
Group	**Drug and Dose**	**Day 7**	**Day 14**	**Day 21**	**Day 28**
Group I	Normal Control	0.28±0.047	0.24±0.06	0.32±0.41	0.23±0.04
Group II	Complete Freund's Adjuvant (CFA)	0.73±0.031	0.83±0.041	0.85±0.032	0.86±0.041
Group III	CFA + ethyl acetate extract of *Astracantha longifolia -100*	0.72±0.031	0.72±0.024*	0.66±0.040*	0.63±0.031**
Group IV	CFA + ethyl acetate extract of *Astracantha longifolia -200*	0.74±0.032	0.66±0.023*	0.63±0.042*	0.61±0.028**
Group V	CFA + *Indomethacin (10 mg/kg per day)*	0.74±0.041**	0.66±0.030**	0.64±0.032***	0.56±0.036***

Values are expressed as mean ± S.E.M. (n = 6). Values are statistically significant at *** p<0.001, ** p<0.01, and *p<0.05 (One-way ANOVA followed by Tukey's test).

Conclusion

To provide a scientific explanation for the folk use of *Astracantha longifolia*, we researched the biological effects of its extracts, focusing on the inflammatory & anti rheumatic process. The present research clearly demonstrated that extracts of *Astracantha longifolia* roots had anti-inflammatory activity & anti rheumatic activity, as evidenced by the extremely significant responses of extracts preventing inflammation. Because of its strong anti-inflammatory activity, these findings suggest that *Astracantha longifolia* extract can be used as a functional material and traditional treatment. However, in the near future, appropriate mouse models should be used to isolate bioactive components from the examined plant extracts and analyse the molecular pathways responsible for the putative anti-inflammatory activity.

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