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EFFECT OF GARAVISHAGHNA LEPA IN COTTON PELLET-INDUCED INFLAMMATION- AN ANIMAL STUDY

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ABSTRACT

Background: Inflammation is a protective biological response of tissues to harmful stimuli such as pathogens, toxins, and injury. However, chronic inflammation can lead to tissue damage and disease. In Ayurveda, inflammation is described as Shopha, which may arise due to factors like Gara Visha (artificial toxins). Classical formulations such as Garavishaghna Lepa are indicated for managing toxic inflammatory conditions and are believed to possess anti-inflammatory and detoxifying properties. **Objectives:** To evaluate the anti-inflammatory effect of Garavishaghna Lepa in cotton pellet-induced inflammation in Wistar rats. To measured serum pro-inflammatory cytokines TNF-α, IL-6. **Materials and Methods:** An in vivo experimental study was conducted on 24 Wistar rats divided into four groups: Normal control, Disease control (cotton pellet-induced inflammation), Standard group (Diclofenac gel), and Test group (Garavishaghna Lepa). Inflammation was induced by subcutaneous implantation of sterile cotton pellets. Treatments were applied locally for 8 days. On day 9, blood samples were collected, and serum levels of inflammatory cytokines, TNF-α and IL-6, were estimated. Data were analyzed using one-way ANOVA followed by Bonferroni post hoc test. **Results:** The disease control group showed a significant increase in TNF-α and IL-6 levels, confirming inflammation. Treatment with Garavishaghna Lepa significantly reduced TNF-α and IL-6 levels compared to the disease control group. The

effect was comparable to the standard drug diclofenac gel, which also showed a marked reduction in inflammatory markers. **Conclusion:** Garavishaghna Lepa demonstrated significant antiinflammatory activity by reducing pro-inflammatory cytokines in cotton pellet-induced inflammation in rats.

KEYWORDS: Shopha, Gara Visha, Garavishaghna Lepa.

INTRODUCTION

Inflammation is a local response of living mammalian tissue to injury due to any agent. Agents causing inflammation are chemical agents (organic and inorganic poisons) physical agents (heat, radiation, mechanical trauma) immunological agents (antigen antibody reaction) infective agents (Bacteria, viruses, fungi, parasites) Inert material such as foreign body. The cardinal Signs of inflammation are ruber (redness), tumor (swelling), color (heat), dolor (pain), functio laesa (loss of function of particular organ).[1]

In ayurveda inflammation is known by the name shopha.[2] The term Shopha originates from the root "Shuth," which translates to swelling or oedema. Classical Ayurvedic literature explains that Shopha arises from the imbalance of the three Doshas; Vata, Pitta, and Kapha, either individually or collectively, leading to localized or systemic swelling.[3] When these Doshas become aggravated, they hinder the normal flow of Rasa and Rakta Dhatus within the body's channels (Strotas), resulting in fluid accumulation and inflammatory changes in the affected tissues.[4]

Classical texts classify shopha into five types: vataj, pittaj, kaphaj, sannipatik and vishaj.[5] Among these, vishaj shopha is associated with toxic etiologies, including influence of gara visha. According to acharya Vagbhatta poison is of two types. One is the akritrim visha (natural poison) which is subdivided into two i.e. sthavaram and jangam visha.

The other one is the gara visha called as kritrim visha (artificial poison). Shopha is one of the signs of gara visha.[6]

Modern pharmacological management of inflammation primarily involves systemic and local drugs. Systemic drugs like non- steroidal anti-Inflammatory drugs and corticosteroids have adverse effects such as epigastric distress, peptic ulceration, osteoporosis, and iatrogenic cushings syndrome which Have limited their use. Similarly local drugs also causes some unwanted effects. Keeping in view all the above facts, there is need of searching out for an

alternative anti-inflammatory medicine having better therapeutic effect with least side effects. Medicinal products derived from plant sources are being explored for their anti-inflammatory potential.[7,8]

In Ayurveda, managing Shopha primarily focuses on pacifying the aggravated Doshas, enhancing circulation, alleviating pain and swelling, and promoting tissue healing. Classical formulations used for Shotha include Kashayam, Lepa, Sneha, and Taila Kalpana.[9]

Lepa is one such treatment which can be used as external application for inflammation. Topical application (Lepa) provides localized action, reduces systemic side effects, enhances drug absorption through the skin, and offers quick relief in swelling, pain, and discoloration compared to oral medications.[10]

According to ashtang sangrah local application in the form of lepa is recommended for shofa, one of the effect of gara visha on skin. Garavishaghna lepa mentioned in ashtang sangrah uttarsthan contains Harenu(Nirgundi), Chandan, Shyama(Priyangu), Nalad(Jatamansi).[11] These ingredients are detoxifying, anti-inflammatory and anti-oxidant.[12,13,14,15]

METHODOLOGY

Study Design

Experimental in vivo study

Study material

Table 1: Contents of Garavishaghna lepa.

Sr. no.	Drug Name	Latin Name	family	Part Use	Proportion
1	HARENU (NIRGUNDI BEEJ)	Vitex Agnus castus	Verbenaceae	Beej	1 part
2	CHANDAN	Santalum Album	Santalaceae	Kandsar	1 part
3	SHYAMA (PRIYANGU)	Callicarpa Macrophylla	Verbenaceae	Pushpa	1 part
4	NALAD (JATAMANSI)	Nordostachys Jatamansi	Valerianaceae	Mula	1 part

Study population

Table 2: Inclusive criteria.

Animal species used	**Wistar rats**
No. of animals	24
No. of groups	4
Average weight of animals	180-240 gms
Age of animals	6-8 weeks
Gender of animals	Either gender
Route of drug administration	Local application

Table 3: Groups of experimental animals.

Groups	**Treatment**	**No of Animals**	**Administration**
Group I	Normal Group	6	-
Group II	Diseased Control Group	6	Cotton pellet induced inflammation (2 cotton pellets each rats)
Group III	Positive Control	6	Cotton pellet induced inflammation (2 cotton pellets each rats) + Diclofenac gel local application for 8 days
Group IV	Test Drug group	6	Cotton pellet induced inflammation (2 cotton pellets each rats) + Garavishaghna lepa for 8 days

Procedure

A) Pre-procedure stage

24 Wistar rats were selected

All the animals were subjected to 7 days of acclimatization for laboratory Conditions, and then it proceed for the actual experimentation.

Animals were divided into 4 groups as per table no 3.

B) Procedure stage

1) Four sterilized cotton pellets (10 mg) implanted on the either side(2 on each side of the ventral region of the rats)
2) Cotton pellet inserted rats were administered with their respective treatment daily for 8 days.
3) On day 9th, blood was withdrawn and serum was separated and used for estimation of serum inflammatory Cytokines such as TNF alpha and IL-6.

STATISTICAL ANALYSIS

Data was analyzed by One-Way or Two Way ANOVA followed by Bonferroni Post Hoc analysis.

* P<0.5 was considered as statistically significant.

RESULT

Table 4: Effect of *Garavishaghna lepa* on inflammatory cytokines in cotton pellets induced inflammation in rats.

Treatment and dose	inflammatory cytokines (pg/mL; Mean±SD)	
	TNF-alpha	IL-6
Normal control	30.37±14.88	50.77±12.54
Disease control (Cotton pellets)	604.07±34.11$^{###}$	362.22±73.40$^{###}$
Diclofenac gel+ Cotton pellets	63.92±22.17***	67.63±25.03***
Garavishaghna lepa+Cotton pellets	56.62±21.02***	253.25±60.93**
The results are expressed as Mean ± SD and were analyzed by one-Way ANOVA followed by Bonferroni Post Hoc analysis. A statistically significant difference was observed, indicated by $^{###}$P<0.001 compared to normal control group, **P<0.01, ***P<0.001 when compared to the Disease Control		

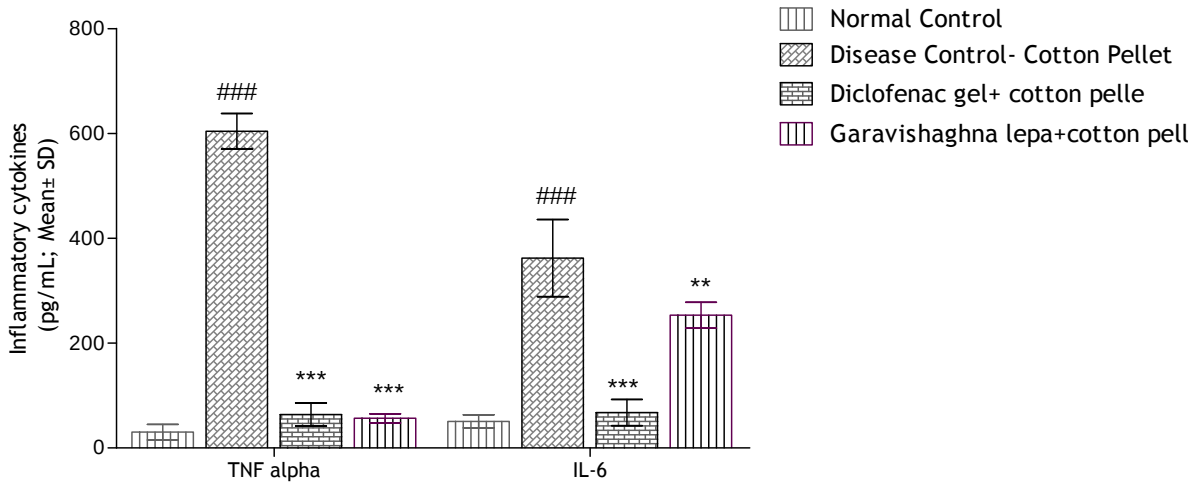

Figure 1: Effect of *Garavishaghna lepa* on inflammatory cytokines in cotton pellets induced inflammation in rats. The results are expressed as Mean ± SD and were analyzed by one-Way ANOVA followed by Bonferroni Post Hoc analysis. A statistically significant difference was observed, indicated by $^{###}$P<0.001 compared to normal control group, **P<0.01, ***P<0.001 when compared to the Disease Control.

Table 5: Effect of *Garavishaghna lepa* on Serum Liver enzymes in cotton pellets induced inflammation in rats-individual data.

Treatment and dose	Animal no	Inflammatory cytokines (ng/mL)	
		TNF-alpha	IL-6
Normal Control	Animal No 1	44.8	46.3
	Animal No 2	14.1	63.8
	Animal No 3	16.1	45.3
	Animal No 4	48.0	69.1
	Animal No 5	21.9	39.5
	Animal No 6	37.3	40.6
Disease Control cotton pellets	Animal No 1	579.0	460.3
	Animal No 2	603.4	290.1
	Animal No 3	590.6	357.3
	Animal No 4	611.5	409.4
	Animal No 5	572.8	266.7
	Animal No 6	667.1	389.5
Standard (diclofenac gel) + cotton pellets	Animal No 1	37.6	69.3
	Animal No 2	83.2	40.2
	Animal No 3	94.4	89.0
	Animal No 4	43.4	104.4
	Animal No 5	58.0	46.7
	Animal No 6	66.9	56.2
Garavishaghna lepa+cotton pellets	Animal No 1	28.7	326.6
	Animal No 2	40.8	277.9
	Animal No 3	78.3	179.4
	Animal No 4	80.3	273.9
	Animal No 5	64.4	177.5
	Animal No 6	47.2	284.2

DISCUSSION

The present study investigates the anti-inflammatory effect of Garavishaghna lepa in cotton pellets induced inflammation in rats. In the present study, the disease control group showed a significant elevation in TNF-α and IL-6 levels, indicating an active and sustained inflammatory response. The marked increase in these cytokines confirms successful induction of inflammation.

Administration of Garavishaghna Lepa resulted in a statistically significant reduction in both TNF-α and IL-6 levels compared to the disease control group. This indicates that the formulation effectively modulates the inflammatory response, possibly by inhibiting cytokine production and reducing cellular infiltration at the site of inflammation. The reduction in TNF-α was more pronounced, while IL-6 levels, although significantly reduced, remained comparatively higher, suggesting a differential modulatory effect on inflammatory mediators.

The anti-inflammatory activity observed in this study can be attributed to the synergistic action of the individual components of Garavishaghna Lepa. Nardostachys jatamansi (Jatamansi) possesses antioxidant and anti-inflammatory properties, which help in reducing oxidative stress and cytokine expression. Vitex negundo (Nirgundi) is known to inhibit inflammatory mediators and reduce edema. Santalum album (Chandan) exhibits antioxidant activity and stabilizes inflammatory processes. Callicarpa macrophylla (Priyangu) has demonstrated anti-inflammatory and free radical scavenging activity in experimental studies. The combined effect of these drugs contributes to the overall therapeutic efficacy of the formulation.

From an Ayurvedic perspective, inflammation (Shopha) is primarily caused by the vitiation of Doshas and may also occur due to the action of Gara Visha (artificial toxins). Garavishaghna Lepa, being Vishaghna (detoxifying) and Shophaghna (anti-inflammatory), acts by neutralizing toxic factors and restoring Dosha balance. The local application of Lepa (Bahya Chikitsa) allows direct drug action at the affected site, facilitating better absorption and targeted therapeutic effect, thereby reducing swelling and associated symptoms.

The findings of the present study suggest that Garavishaghna Lepa possesses significant anti-inflammatory activity, as evidenced by the reduction in key pro-inflammatory cytokines.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

CONCLUSION

We measured serum pro-inflammatory cytokines mainly TNF-α, IL-6, since both cytokines initiating and amplifying the inflammatory response by promoting leukocyte recruitment and stimulating fibroblast proliferation, which contributes to granuloma formation and proliferation of inflammatory and connective tissue cells. Our findings revealed that, rats from cotton pellets inserted disease control group showed significant increased levels of TNF-α and IL-6 indicating active chronic inflammation. Treatment with Garavishaghna lepa significantly attenuated both TNF-α and IL-6 compared to disease control group, Similarly, the diclofenac gel also showed reduction in TNF-α and IL-6 levels, indicting both Garavishaghna lepa or diclofenac treatment correct the chronic inflammatory response. Garavishaghna lepa was able to demonstrate its inhibitory effects on serum TNF-α and IL-6

levels, thereby showing its anti-inflammatory effects in cotton pellets induced inflammation in rats.

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