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Effect of Gandhamadana Agada in Carrageenan-Induced Inflammation in Albino Wistar Rats: An Animal Study

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Abstract

Background: Gandhamadana Agada is a classical polyherbal formulation described in Ashtanga Hridaya for the management of Keetavisha and Lootavisha. Inflammation is one of the important manifestations associated with such conditions. The present study was undertaken to evaluate the anti-inflammatory activity of Gandhamadana Agada in carrageenan-induced paw edema in albino Wistar rats.

Objective: To evaluate the anti-inflammatory effect of Gandhamadana Agada on carrageenan-induced paw edema and to assess its effect on serum cyclooxygenase-2 (COX-2) and tumor necrosis factor-alpha (TNF-α) levels.

Materials and Methods: Gandhamadana Agada was prepared using eight ingredients, namely Tagara, Lodhra, Vacha, Katvi, Patha, Ela, Tamala Patra, and Kunkuma, in equal proportions. Twenty-four Wistar rats were divided into four groups of six animals each: normal control, carrageenan control, standard treatment with ibuprofen, and test treatment with Gandhamadana Agada. Paw edema was induced by subplantar injection of 1% carrageenan. Paw volume was assessed at predetermined time intervals using a digital plethysmometer. Serum COX-2 and TNF-α levels were estimated at 3 hours after carrageenan administration.

Results: Carrageenan administration produced a significant increase in paw volume compared with the normal control group. Gandhamadana Agada treatment showed an inhibitory effect on paw edema, particularly during the later phase of inflammation. A significant reduction in serum COX-2 and TNF-α levels was also observed in the Gandhamadana Agada-treated group compared with the carrageenan control group. The inhibitory effect was more pronounced for COX-2 than for TNF-α.

Conclusion: Gandhamadana Agada demonstrated anti-inflammatory activity in the carrageenan-induced paw edema model in albino Wistar rats. The observed reduction in paw edema, COX-2, and TNF-α levels suggests its potential role in modulating inflammatory responses. Further studies are required to establish its mechanism of action and therapeutic relevance.

Keywords: Ayurveda, Agada Tantra, Gandhamadana Agada, Inflammation, COX-2, TNF-α

Introduction

Ayurveda is an Indian traditional system of medicine which was practiced since many years. The science

of ayurveda includes eight branches [Ashtang ayurveda] of treatment specialization and Agadatantra is one among the eight branches. Gada literally means disease and Agada means any agent which makes the body free from disease. [1]

In Agadatantra describes various Agada formulations for treating poisoning from sthavara (plants), jangam (animals), dooshivisha (contaminated food), and garavisha (heavy metals). These Agada preparations are highly valued in Ayurvedic medicine for their powerful prabhav, which enables them to effectively counteract and remove the toxic effects of visha (poison).

The Ancient Indian physicians conducted in-depth studies on the effects of poisons from both plantbased (sthavara visha) and animal-based (jangama visha) sources. Specifically, they thoroughly documented the impact of envenomation from various creatures, including keeta, luta and vrishchika detailing the resulting symptoms and developing targeted treatment protocols and medicinal formulations to counteract the venom.

Gandhamadana agada is one of the polyherbal drug combination indicated in keetavisha and lootavisha as per the Aacharya Vagbhata. it is widely used in treatment of insect bites and spider bites. As it minimizes the poisonous effects.[2]

Aacharya Vagbhata stated common symptoms of Keetavisha as follows-

सर्वेषां कर्णिका शोफो ज्वरः कण्डूररोचकः॥
(अष्टाङ्गहृदय, उत्तरस्थान 37/5)

Irrespective of the triggering etiology, inflammation is the initial response of the host defence mechanism towards any infection, irritants or foreign substances. Therefore, control of inflammation becomes essential.[3]

Modern anti-inflammatory drugs are extremely quick acting and efficient. However long-term use of such drugs can induce serious adverse reaction affecting various organs. For e.g. long-term use of Diclofenac causes nephrotoxicity. [4] In this context Ayurveda presents time-tested formulations that are both safe and effective. Herbal medicines offer several advantages, including higher patient compliance, excellent tolerability, and reduced risk of side effects. Additionally, herbal remedies are economically advantageous and often demonstrate greater efficacy than synthetic pharmaceuticals.

The Ayurvedic toxicology text (Agadatantra) describes various medicinal preparations with antiinflammatory potential, extending their utility beyond toxicology to general clinical practice, presenting opportunities for wider therapeutic use.

Gandhamadana Agada, a polyherbal formulation comprising eight ingredients, is a notable example. This preparation is first mentioned in the ancient text Ashtang Hridaya, where it is attributed to the renowned sage Aacharya Vagbhata. According to the Sanskrit verse, Vagbhata created this formulation to counteract the toxic effects of certain poisons, specifically those caused by lootavisha and keetavisha.

Gandhamadana agada is one of the polyherbal drug combinations indicated in Keetavisha and Lootavisha as per the Aacharya Vagbhata. [5]

अगदो मन्दरो नाम तथाऽन्यो गन्धमादनः।
नतालोध्रवचाकट्वीपाठैलापत्रकुङ्कुमैः॥
(अष्टाङ्गहृदय, उत्तरस्थान 37/74)

Since inflammation is the primary clinical manifestation, this study aims to evaluate the scientific basis for Gandhamadana Agada's anti-inflammatory effects. To achieve this, an investigation is conducted, titled "In vivo evaluation of Gandhamadana Agada's anti-inflammatory efficacy in carrageenaninduced inflammation in albino Wistar rats."

Materials and Methods:

The following raw drugs mentioned in Table No. 1 were used for the research work, with the standard references mentioned in *Ashtangahridayam*.

Table No. 1: List of ingredients of Gandhamadana Agada:

Sr. No	Drug Name	Botanical Name	Parts used as per API [6]	Quantity
1.	Tagara	Valeriana wallichii DC.	Roots, Rhizomes	1 Part
2.	Lodhra	Symplocos racemosa Roxb.	Stem bark	1 Part
3.	Vacha	Acorus calamus Linn.	Rhizome	1 Part
4.	Katvi	Picrorhiza kurroa Royle ex.	Rhizome	1 Part
5.	Patha	Cissampelos pareira Linn	Roots	1 Part
6.	Ela	Elettaria cardamomum	Seeds	1 Part
7.	Tamal Patra	Cinnamomum tamala	Dried leaves	1 Part
8.	Kunkum a	Crocus sativus	Dried style and stigma	1 Part

Adjuvent/Vehicle:

1. Ghrit
2. Honey

Table No 2 : Properties [7]: (According to Bhava Prakash Nighantu)

Sr.No.	DRUG	RASA	VIRYA	VIPAKA	GUNA	DOSHAGNATA	KARMA
1.	*Tagara*	Katu, Tikta, Madura	Ushna	Katu	Laghu, Ruksha, Teekshna	Kaphavatahara	Kaphagna, Mukhashodhana, Vedanasthap, Vranaropaka, Swedajanana
2.	*Lodhra*	Kashaya	Sheeta	Katu	Laghu, Ruksha	Pitta Kaphahara	Shothagna, Vranaroapaka, Balya, Chashughna
3.	*Vaca*	Tikta	Ushna	Katu	Laghu, Ruksha	Kap ha-Vatahara	Deepana, Pachana, Kaphanissaraka, Krimighna
4.	*Katvi*	Tikta	Sheeta	Katu	Laghu, Ruksha	KaphaPittahara	Kasahara, Dahahara, Krimighna, Jwaraghna
5.	*Patha*	Tikta	Ushna	Katu	Laghu, Teekshna	VataKaphahara	Vishahara, Vrushya, Shulaghni, Garavishaghna
6.	*Ela*	Katu, Madhura	Sheeta	Madhura	Laghu, Ruksha	Pitta-Vatahara	Deepana, Pachana, Rochana, Vatanulomaka, Sughandhi
7.	*Tamal patra*	Katu, Mdhuara	Ushna	Katu	Laghu, Tikshna, Pischil	Kaph Vatahara	Deepana, Swedajanana, Vatahara, Mutrajanana

8.	*Kunkuma*	Katu, Tikta	Ushna	Katu	Snigdha	Tridoshahara	Varnya, Vishaghna, Vedanahara, Manaprasadakar a, Balya.

Collection of materials:
Collection of raw drugs done from an authenticated source.

Preparation of Gandhamadana agada: -
Gandhamadana Agada was prepared by following the procedure mentioned in *Ashtanga Hridaya*.

1. The raw drugs were identified, collected, and authenticated.
2. The powder was prepared according to the method described in the *Ayurvedic Formulary of India* in a GMP-approved pharmacy.
3. All the ingredients were powdered separately and then mixed together in equal proportions to obtain a uniformly blended powder.

Preclinical study:
Experimental animals:
Wistar rats of either sex, weighing 180-240 grams (6-8 weeks old) were used in the study.

Ethics committee approval: -
Approval for the animal study was obtained from the Institutional Animal Ethics Committee (IAEC) of respected institution constituted under Committee for the control and supervision on animals (CCSEA), Department of Animal Husbandry and Dairying (DAHD), Ministry of Fisheries, Animal Husbandry and Dairying (MoFAH&D), Government of India.

Study Outline:
Requirements-

- Carrageenan
- Distilled water
- Oral gavage needle
- Plethysmometer

Inclusion Criteria:

Animals Strain to be used	Wistar Rats
Animal species	Rattus norvegicus
Number of animals used	24
Number of groups	04
Average weight of animals	180-240 grams
Average age of the animals	6-8 weeks old

Gender of animals	Either sex
Source of animals	Inhouse bred or Registered breeder
Route of test drug administration	Oral

Exclusion Criteria:

- Pregnant and lactating rats were excluded from the study
- Overweight rats weighing more than 350 grams were excluded from the study
- Rats showing no signs of inflammation, mainly edema formation after carrageenan injection were excluded.

Selection of standard ibuprofen dose

The dose of Ibuprofen 100 mg/kg by oral route as standard group has been selected for the present study as per literature and the various documented findings (Thitinarongwate et al., 2022; Kumar et al., 2009)

DOSE CALCULATION OF TEST DRUG: -

The dose of test drug is calculated by converting the therepheutical dose to rat dose.

Dose of churna Kalpana is said to be 1 karsha, 1 karsha is equivalent to 12 gm [8] for rats, conversation factor is 0.018 for 200 gm rat as per Paget and Barnes. [9]

RAT DOSE = HUMAN DOSE X CONVERSION FACTOR OF RATS

= HUMAN DOSE X 0.018

= 12 X 0.018

= 0.216 gm/200 gm rat

=216 mg/ 200 gm rat

Table no 3 : Grouping of Animals

Sr no	Groups	Treatment and dose-Route	No of animals
1	Group I	Normal control	06
2	Group II	Carrageenan control (1 % w/v; 0.1 mL/rats in sub-planter region)	06
3	Group IIII	Carrageenan control (1 % w/v; 0.1 mL/rats in sub-planter region) +Ibuprofen 100 mg/kg, oral	06
4	Group IV	Carrageenan control (1 % w/v; 0.1 mL/rats in sub-planter region + Gandhamadana agada 216 mg/kg, oral	06
Total animals			24

Procedure:

1. **Animals**: Wistar rats of either sex were acclimatized for one week under standard laboratory conditions.
2. **Grouping**: The animals were divided into experimental groups as per Table 3.

3. **Treatment**: One hour prior to carrageenan injection, the rats received the respective treatments according to their group allocation.
4. **Induction of Inflammation**: Carrageenan (1% w/v, 0.1 mL) was injected into the left hind paw of all animals except those in Group I, following the method described by Winter et al. (1962).
5. **Model Rationale**: Carrageenan induced biphasic edema, consisting of an acute phase mediated by histamine and serotonin and a late phase associated with prostaglandins and proteases. Inhibition of edema during the later phase indicated NSAID-like cyclooxygenase activity.
6. **Measurement**: Paw volume was recorded immediately after injection (0 h) and at 30 min, 1 h, 3 h, 6 h, and 12 h using a digital plethysmometer (Orchid Scientific, India).
7. **Biomarker Estimation**: At 3 h, blood samples were collected for estimation of serum TNF-α and COX-2 using ELISA kits.
8. **Histopathology**: After the final paw volume measurement, paw tissue (1 cm) was excised for histopathological examination.
9. **Data Analysis**: Percentage reduction in paw volume was statistically analyzed using one-way ANOVA followed by post-hoc comparison with the carrageenan control group.

OBSERVATIONS: -

During the induction of inflammation, carrageenan (1% w/v) was injected into the subplantar region of the left hind paw of the rats, and the following inflammatory biomarkers were assessed:

- TNF alpha,
- COX II.
- Histopathological studies.

Evaluation criteria:

- Increase in Paw volume using digital plethysmometer
- Paw diameter using vernier caliper
- Inflammation through swelling

Measurement:

- Paw volume was measured by volume displacement method using digital plethysmometer (Orchid Scientific), increase in paw volume indicated as edema formation due to release of prostaglandins (PGE2, COX II) and or histamine at various time points after carrageenan injection.
- Paw volume was measured at 0 minutes, 30 minutes, 1 hours, 2 hours, 3 hours, 6 hours, 8 hours, 12 hours and 24 hours post carrageenan injection.
- Serum TNF alpha and Cyclooxygenase COX II (inflammatory biomarkers) was estimated at 3 hours post carrageenan injections.

Discussion:

Carrageenan-induced edema is biphasic: early phase = histamine/serotonin, late phase = prostaglandins, COX-II, TNF-α. Gandhamadana Agada showed maximum inhibition in late phase, suggesting COX-II and cytokine modulation. This matches phytochemical data: Picrorhiza kutkin inhibits COX-II, Crocus crocin reduces TNF-α, Valeriana has antihistaminic action. Thus, the polyherbal mix acts on multiple inflammatory mediators, validating its classical Shothahara and Vishaghna claim. Unlike NSAIDs that

mainly target COX, this formulation's multi-target action may reduce side effects. Safety in acute toxicity study supports classical claim of Nirvisha.

Table no 4 Showing Statistical analysis :

Treatment and group	Paw volume at various time points (mL, Mean±SD)					
	0 min	30 min	1 hour	3 hours	6 hours	12 hours
Normal control	0.92±0.12	0.92±0.12	0.92±0.12	0.91±0.12	0.92±0.12	0.91±0.13
Carrageenan control	0.84±0.06	1.12±0.06	1.70±0.07$^{###}$	1.84±0.08$^{###}$	1.88±0.08$^{###}$	0.91±0.09
Standard (Ibu+carrageenan)	0.85±0.04	0.97±0.06	1.14±0.07***	1.18±0.12***	0.91±0.07***	0.85±0.04
Gandhamadana agada+Carrageeenan	0.80±0.04	1.33±0.12	1.28±0.11***	1.03±0.12***	1.03±0.11	0.84±0.16
The results are expressed as Mean ± SD (n = 6) and were analyzed by One-Way ANOVA followed by Bonferroni Post Hoc analysis. A statistically significant difference was observed, indicated by $^{###}$P<0.001 when compared to normal control ***P<0.001 when compared to Disease control						

Results were represented and expressed as mean ± SD (n=6). Data was analysed by One-Way ANOVA followed by Bonferroni Post Hoc analysis. *P<0.05 was considered as statistically significant

Result:

Effects of Gandhamadana agada on paw volume in carrageenan induced paw Edema in rats

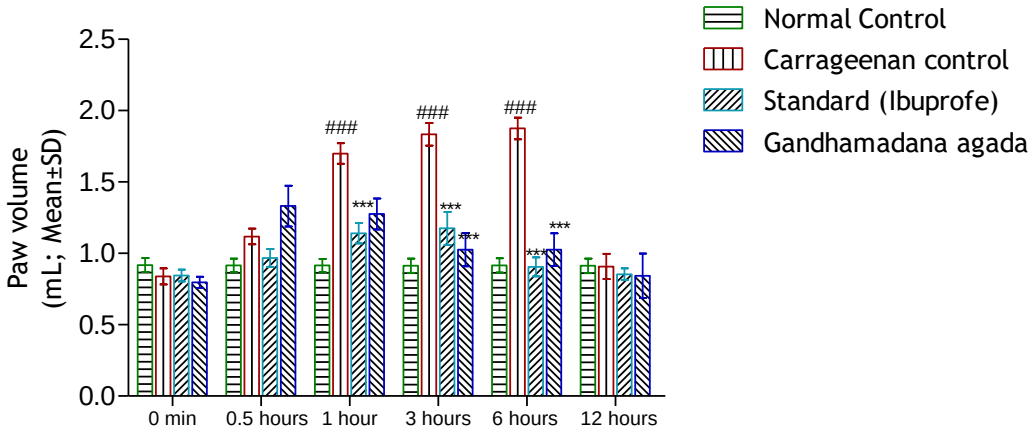

Figure 1: Effect of Gandhamadana Agada on Paw Volume in Carrageenan-Induced Paw Edema in Rats.

The results are expressed as Mean ± SD (n = 6) and were analysed by One-Way ANOVA followed by Bonferroni Post Hoc analysis. A statistically significant difference was observed, indicated by $^{###}$P<0.001 when compared to normal control ***P<0.001 when compared to Disease control

Table No 5 : Effects of Gandhamadana agada on serum COX II and TNF-alpha at 3rd hours in carrageenan-induced paw Edema in rats

Treatment and group	COX II levels	TNF-alpha
	(pg/mL; Mean±SD)	
Normal control	1310.23±264.7	233.69±2.08

Carrageenan control	3609.16±374.9###	407.97±46.28#
Standard (Ibu+carrageenan)	1737.79±226.0***	395.37±16.54
Gandhamadana agada+Carrageeenan	2449.29±119.2***	313.63±135.25*
The results are expressed as Mean ± SD (n = 6) and were analyzed by One-Way ANOVA followed by Bonferroni Post Hoc analysis. A statistically significant difference was observed, indicated by ###P<0.001 when compared to normal control *P<0.05 when compared to Disease control		

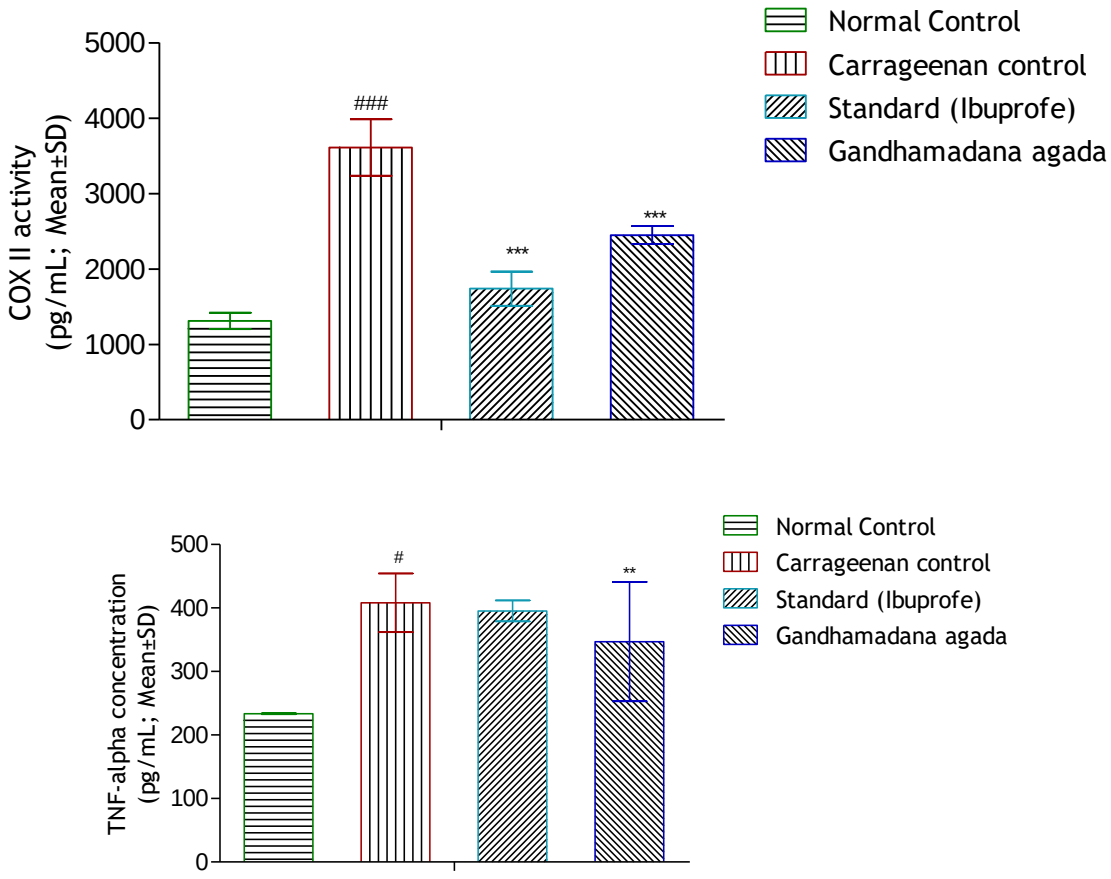

Figure 2: Effect of Gandhamadana Agada on Serum COX-II and TNF-Alpha Levels at 3 Hours in Carrageenan-Induced Paw Edema in Rats.

The results are expressed as Mean ± SD (n = 6) and were analysed by One-Way ANOVA followed by Bonferroni Post Hoc analysis. A statistically significant difference was observed, indicated by ###P<0.001 when compared to normal control *P<0.05 when compared to Disease control

Conclusion:

Our experimental findings revealed that, the carrageenan injected animals showed significant (p<0.001) increase in paw volume due to inflammatory action compared to normal control group in time dependent manner, paw volume were found to higher at 6th hours compared to 0 hour and remain higher until 6th hour post carrageenan injection. Treatment with gandhamadana agada showed inhibitory action on paw volume at 3 hours onward, the inhibitory action on paw volume were found to significant at 6th hour compared to

carrageenan control. Standard Ibuprofen exerted inhibitory effects from 1st hour and remain significant throughout the study duration of 12 hours (Table 4, Figure 1). Furthermore, our findings also demonstrated that carrageenan injection resulted into significant ($p<0.001$) elevation of COX II enzyme and TNF alpha an inflammatory cytokine (Table 5, Figure 2). The COX II enzymes involved in the production of various inflammatory prostaglandins such as PGD2, PGE2. Treatment with Gandhamadana agada able to showed inhibitory effects on COX II enzyme and TNF alpha. Moreover, the inhibitory was found to elicited better for COX II enzyme than that of TNF-alpha indicating Gandhamadana agada as anti-inflammatory agent. Standard ibuprofen also elicited better anti-inflammatory activity by virtue of its effects on reduction in paw volume and COX II enzyme and TNF alpha.

Thus, in conclusion the Gandhamadana agada treatment elicited anti-inflammatory activity by its effects on paw volume and COX II enzyme and TNF alpha.

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