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Research Article

PRECLINICAL EVALUATION OF THE ANALGESIC ACTIVITY OF *MAHA AGAD* USING THE HAFFNER'S TAIL CLIP MODEL IN SWISS ALBINO MICE

Ashish Vaishnava[1], Ramesh Chandra Tiwari[2]*, Bhawana Mittal[3], Vipin Kumar[4]

[1]Post Graduate Scholar, *[2]Professor & HOD, [3]Assistant Professor, P.G. Department of Agad Tantra evam Vidhi Vaidyaka, Uttarakhand Ayurved University, Rishikul Campus, Haridwar, Uttarakhand, India.

[4]Assistant Professor, Department of Pharmaceutical Sciences, Gurukul Kangri Deemed to be University, Haridwar, Uttarakhand, India.

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ABSTRACT

Pain is one of the earliest and most frequent manifestations observed in animal bites *(Jangam Visha).* It acts as a vital protective response indicating tissue injury or pathological conditions; however, persistent pain can markedly impair quality of life. Conventional analgesic therapies such as opioids and non-steroidal anti-inflammatory drugs (NSAIDs) are commonly employed for pain management, but their long-term use is associated with adverse effects and risk of dependency. This has encouraged increasing interest in herbal and natural alternatives that may offer safer and more holistic therapeutic benefits. The present study was undertaken to evaluate the analgesic activity of the Ayurvedic formulation *Maha Agad* using the Haffner's Tail Clip model in mice. **Material and Methods:** Healthy Swiss albino mice were divided into three groups. Group I (control) received Carboxyl Methyl Cellulose (CMC), Group II (test group) received *Maha Agad* at a dose of 200mg/kg, and Group III (standard group) received diclofenac sodium at a dose of 10mg/kg. Analgesic activity was assessed by measuring the response time following tail clip stimulation. Statistical evaluation was performed using One-Way ANOVA followed by Dunnett's post hoc test. **Results:** Both *Maha Agad* and Diclofenac sodium produced a significant increase in response time when compared with the control group ($p=0.017$ and $p=0.032$, respectively), indicating significant analgesic activity. These findings support the validity of the experimental model and demonstrate the pain-relieving potential of *Maha Agad*. **Discussion:** The observed analgesic activity of *Maha Agad* substantiates its classical *Ayurvedic* indication in the management of pain-related conditions. The study also contributes toward integrating traditional Ayurvedic knowledge with modern evidence-based research, thereby supporting the development of safer, plant-based analgesic therapies for pain management.

INTRODUCTION

Animal bites or stings can produce various toxic manifestations in the body, including pain, swelling, fever, redness, itching, numbness, and tingling sensations either locally or systemically.[1] Pain serves as a crucial protective mechanism that alerts the body to injury or disease; however, when persistent or uncontrolled, it can greatly diminish an individual's quality of life.[2]

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In Ayurveda, the term *Gada* denotes disease, discomfort, pain, or poison, whereas *Agad* refers to therapeutic formulations used as antidotes against such harmful conditions.[3] Although modern analgesic therapies such as opioids and non-steroidal anti-inflammatory drugs (NSAIDs) are commonly employed for pain management, their use is often associated with adverse effects and the risk of dependency. Consequently, there has been growing interest in herbal and natural remedies that offer safer and more holistic therapeutic approaches. Ayurveda describes several antitoxic (*Agad*) formulations traditionally used for the management of pain, fever, inflammation, and toxic conditions[4].

One such formulation is *Maha Agad*, mentioned by *Acharya Vagbhata*[5] for the treatment of disorders caused by animal bites (*Jangam Visha*) and associated symptoms such as pain, fever, inflammation, burning sensation, and related manifestations.[6] *Maha Agad* is composed of *Trivrit, Vishalya (Langli), Madhuka, Nishadwaya (Haridra* and *Daruharidra), Panchalavana (Saindhava Lavana, Sauvarchala Lavana, Vida Lavana, Samudra Lavana,* and *Romaka Lavana), Manjistha,* and *Trikatu (Pippali, Maricha,* and *Shunthi)*. The ingredients are illustrated in Figure 1.

The present study was undertaken to evaluate the analgesic activity of *Maha Agad* in mice using the Haffner's Tail Clip model and to compare its efficacy with Diclofenac, a widely prescribed NSAID. The study further aims to integrate traditional Ayurvedic knowledge with modern evidence-based scientific research, thereby supporting the development of safer, plant-derived analgesic therapies for pain management.

MATERIAL AND METHODS

Animals

Healthy Swiss albino mice weighing between 20–50gm were used for the study. Prior to experimentation, the animals were acclimatized under standard laboratory conditions for seven days with a 12:12 hour light-dark cycle. They were provided with a conventional laboratory diet and water ad libitum, as illustrated in Figure 2.

The experimental procedures were approved by the Institutional Ethical Committee of Uttarakhand Ayurved University (UAU/RC/IEC/2025/PG/266) and the Institutional Animal Ethical Committee of Gurukul Kangri Deemed to be University (GKV/AHF/67/2025), in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA), India.

Test Drug

After identification and authentication of all the ingredients of *Maha Agad* by experts from the P.G. Department of *Dravyaguna* (DG/RC/UAU-279), 100 g of *Langali Moola* was initially purified by soaking it in *Gomutra* for one day, followed by shade drying. Thereafter, according to the guidelines mentioned in *Sharangdhara Samhita*, all ingredients of *Maha Agad* were taken in dried form and individually converted into coarse powder using an iron mortar and pestle. The materials were then finely powdered, sieved separately through sieve no. 80, and thoroughly mixed.

Subsequently, *Bhavana* was administered using *Basta Mutra* (goat urine), which was freshly collected in sterile containers during the morning hours. A total of 1356gm of the formulation was subjected to the *Bhavana* process. Each *Bhavana* required approximately 2–3 days for completion, with 5–6 hours of processing daily. Altogether, five *Bhavana* cycles were given to *Maha Agad*. After completion of the process, the final weight of the formulation increased to 1410g. The prepared *Churna* (powder) was dried and stored in an airtight container for further experimental use. Images of the final *churna* are shown in Figure 3.

Standard Drug

Diclofenac sodium was used as the standard reference drug for comparison of analgesic activity.

Experimental Design

The mice were randomly allocated into three groups, with six animals in each group (n = 6):

- **Group I (Control):** Received 0.25% CMC at a dose of 10 ml/kg orally once daily. The body weight and dose details are presented in Table 1.
- **Group II (Test Drug):** Received *Maha Agad* at a dose of 200mg/kg orally once daily. The body weight and dose details are provided in Table 2.
- **Group III (Standard Drug):** Received Diclofenac sodium at a dose of 10mg/kg orally once daily. The body weight and dose details are shown in Table 3.

In-vivo Experiment (Haffner's Tail Clip Method)

For identification purposes, each animal was marked individually as Head (H), Back (B), Tail (T), Head-Back (HB), Back-Tail (BT), and Head-Tail (HT). Following six days of once-daily administration, the analgesic activity was assessed on the seventh day. Sixty minutes after drug administration, a metal artery clip was applied approximately 1cm away from the base of the tail, as depicted in Figure 4.

The response latency, indicated by tail flicking or biting behavior, was recorded in seconds with a maximum cut-off time of 10 seconds to prevent tissue injury, as shown in Figure 5. An increase in response latency was considered indicative of enhanced analgesic activity. The observations recorded for each animal in all groups are presented in Table 4.

Table 1: Body weight and Dosing chart of each mouse in group 1 (Control group)

Group 1- Control (0.25 %, CMC, 10 mL/kg, P.O., OD)		
Marking	**Weight (gm)**	**Dose in ml**
H	35	0.35
B	37	0.37
T	42	0.42
HB	40	0.40
BT	36	0.36
HT	40	0.40

Table 2: Body weight and Dosing chart of each mouse in group 2 (Test group)

Group 2- Test Drug (*Maha Agad*- 200mg/kg, P.O., OD suspension concentration: 2mg/mL in 0.25% CMC)			
Marking	**Weight (gm)**	**Dose in mg**	**Dose in ml**
H	36	7.2	0.36
B	43	8.6	0.43
T	45	9	0.45
HB	40	8	0.4
BT	39	7.8	0.39
HT	37	7.4	0.37

Table 3: Body weight and Dosing chart of each mouse in group 3 (Standard group)

Group 3 - Standard Drug (Diclofenac Sodium-10 mg/kg, P.O., OD suspension concentration: 2mg/mL in 0.25 % CMC)			
Marking	**Weight (gm)**	**Dose in mg**	**Dose in ml**
H	38	0.38	0.19
B	42	0.42	0.21
T	46	0.46	0.23
HB	37	0.37	0.18
BT	40	0.40	0.2
HT	42	0.42	0.21

Table 4: Individual animal response time (in seconds) in all groups

Markings	Group 1- Control (CMC)	Group 2- Test drug (*Maha Agad)*	Group 3- Standard (Diclofenac sodium)
H	0.45	7.76	38.00
B	0.80	19.54	9.34
T	0.32	37.22	5.22
HB	1.22	4.50	24.46
BT	0.56	23.74	3.24
HT	1.84	9.44	17.70

H-head, B-back, T-tail, HB-head back, BT- back tail, HT- head tail

Table 5: Parameters showing reaction times with error representing SEM

Parameter	Group 1 Mean ± SEM	Group 2 Mean ± SEM	Group 3 Mean ± SEM
Haffner's Tail Clip Model (in seconds)	0.865 ± 0.234	17.03 ± 5.03	16.33 ± 5.42

Table 6: One-way ANOVA followed by Dunnett's multiple comparisons test

Haffner's Tail Clip Model					
Dunnett's multiple comparisons test	**Mean Difference**	**95.00% CI of difference**	**Significant**	**Summary**	**Adjusted P Value**
Group 1 vs. Group 2	-16.17	3.15 to 29.19	Yes	*	**0.017**
Group 1 vs. Group 3	-15.47	1.42 to 29.52	Yes	*	**0.032**

*- Significant

Fig 1. Ingredients of *Maha Agad*

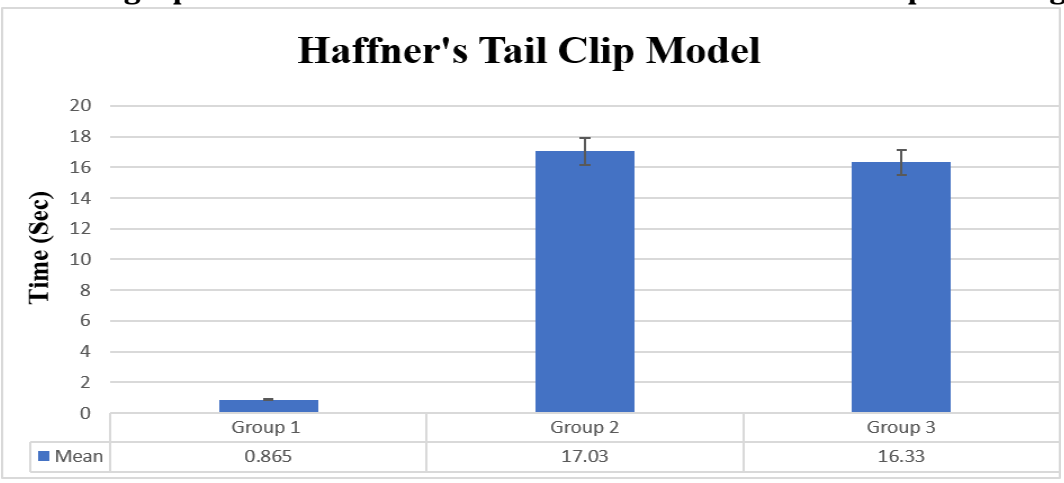
Fig 2. Mice were housed under standard laboratory conditions with free access to food and water

Fig 3. Prepared *Maha Agad churna*

Figure 4: Applying metal artery clip to the mice tail 1cm away from the body

Figure 5: Mice responding to pain by flick and bite

Graph 1: The graph visualizes the reaction times with error bars representing SEM



Statistical Analysis

The obtained data were represented as Mean ± SEM, as presented in table 5. Statistical evaluation was carried out using One-way ANOVA followed by Dunnett's post hoc test. A p-value of less than 0.05 was considered statistically significant. The comparative analysis is summarized in Table 6 and graphically illustrated in Graph 1.

RESULT

The results demonstrated a significant increase in reaction time in Group 2 (SEM 17.03±5.03) compared to Group 1 (SEM 0.865±0.234), with a mean difference of -16.17 seconds (p=0.017), indicating marked analgesic activity of the test drug, *Maha Agad*. Animals in the control group (Group 1) responded rapidly to the tail clip stimulus, reflecting the absence of analgesic action.

Similarly, Group 3 (SEM 16.33 ± 5.42) also exhibited a significant rise in reaction time when compared with Group 1, with a mean difference of -15.47 seconds (p=0.032), thereby confirming the analgesic efficacy of the standard drug, Diclofenac sodium. Mice treated with *Maha Agad* in Group 2 showed prolonged reaction times, suggesting a potent analgesic effect, which in certain individual cases appeared greater than that produced by the standard drug.

DISCUSSION

The present study was conducted to evaluate the analgesic potential of the Ayurvedic formulation *Maha Agad* using the Haffner's Tail Clip Model, a well-established experimental method commonly employed in preclinical research for assessing analgesic activity in mice.[7] In the control group (Group 1), animals administered with CMC exhibited a mean response time of (0.865±0.234 seconds), indicating an immediate reaction to the nociceptive stimulus. This finding was consistent with the expected outcome for the negative control group and validated the reliability of the experimental model.

Mice treated with *Maha Agad* (Group 2) at a dose of 200mg/kg showed a marked prolongation in response time (17.03±5.03 seconds) with statistical significance (p=0.017). The increased latency period suggests a considerable reduction in pain perception, thereby indicating significant analgesic activity of the test formulation, possibly through centrally mediated antinociceptive mechanisms. Furthermore, the absence of sedation or altered behavioral activity in the treated animals suggests that the analgesic effect was not due to generalized central nervous system depression, but rather a specific pain-relieving action.

Similarly, Group 3 animals treated with Diclofenac sodium (10mg/kg), a standard non-steroidal anti-inflammatory drug (NSAID), demonstrated a mean reaction time of (16.33±5.42 seconds) with p=0.032, which was significantly higher than the control group, though slightly lower than the response observed with *Maha Agad* treatment.

Pain is a multifactorial phenomenon involving complex processes such as transduction, transmission, perception, and modulation within the nervous system.[8] According to Ayurvedic principles, pain *(Shoola)* is mainly caused by the aggravation of *Vata Dosha,* often precipitated by *Visha* (toxins), injury, or *Aam* (metabolic toxins), which disrupt the normal physiological balance of the body.[9]

Interestingly, *Maha Agad* exhibited comparatively greater analgesic activity than Diclofenac sodium, as reflected by the higher mean response time observed in the experimental model. This finding suggests that the formulation may exert its analgesic effect through modulation of both peripheral and central pain pathways. The therapeutic efficacy of *Maha Agad* may be attributed to the synergistic action of its various herbal and mineral constituents. *Trivrit*, described in Ayurvedic literature for its potent *Vishaghna* and *Sukhvirechana* (purgative) property, may facilitate elimination of toxic substances from the body.[10] *Vishalya, Madhuka, Haridra,* and *Daruharidra possess Krimighna* and *Shoolaghna* properties, which may contribute to anti-inflammatory, and analgesic effects.[11] *Panchalavana* is traditionally recognized for its *Tridoshaghna, Deepana, Rochana,* and *Shoolaghna* actions,[12] thereby supporting digestion, metabolism, and pain alleviation. *Manjistha*, known for its *Vishaghna Raktashodhaka* (blood purifying) property, may aid in detoxification and reduction of inflammatory processes.[11]

The inclusion of *Trikatu (Shunthi, Maricha, and Pippali)* further enhances the *Deepana-Pachana* effect of the formulation, facilitating digestion and elimination of *Aam,* which is considered responsible for obstruction of body channels and aggravation of pain. Additionally, the *Yogavahi* property of *Trikatu* may improve the bioavailability and absorption of active constituents present in the formulation.[13]

Overall, *Maha Agad* appears to be a promising herbal alternative for pain management that not only alleviates symptoms but also addresses the underlying pathophysiological factors involved in pain generation. The findings of the present study support the integration of classical Ayurvedic concepts with modern experimental pharmacology and encourage further scientific exploration of this formulation.

CONCLUSION

The findings of the present study demonstrate the significant analgesic potential of *Maha Agad*. The formulation showed a greater mean response time than the standard drug diclofenac sodium and produced a specific antinociceptive effect without causing sedation in experimental animals. Furthermore, the statistically significant outcomes obtained through Dunnett's multiple comparison test validate the reliability of the experimental data and strongly support the analgesic efficacy of the test formulation.

The results substantiate the hypothesis that this classical Ayurvedic formulation exerts pain-relieving activity through both central and peripheral mechanisms. The synergistic action of its constituents, including *Trivrit, Vishalya, Madhuka, Nishadwaya, Panchalavana, Manjistha, and Trikatu,* may contribute to its therapeutic efficacy by addressing fundamental pathological factors such as *Visha, Aam,* and *Vata* vitiation, in accordance with Ayurvedic principles.

The study successfully bridges traditional Ayurvedic knowledge with modern scientific evaluation and provides encouraging evidence for the development of safe, plant-based alternatives in pain management. However, due to limitations of time and resources, the present experiment was confined to the induction of physical pain using the tail clip model. Therefore, further investigations employing venom-induced experimental models, larger sample sizes, multicentric approaches, and double/triple-blind clinical study designs are recommended to establish the efficacy and mechanism of action of *Maha Agad* more comprehensively.

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***Address for correspondence**
Dr. Ramesh Chandra Tiwari
Professor & HOD,
P.G. Department of Agad Tantra evam Vidhi Vaidyaka, Uttarakhand Ayurved University, Rishikul Campus,
Haridwar, Uttarakhand, India.
Email: agadtantra786@gmail.com