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Evaluation of hydro-alcoholic extract of *Aristolochia bracteolata* leaves and *Peganum harmala* seeds in carrageenan-induced inflammation in ratssharma Rakesh^{1*}, Dr. Gilhotra Umesh Kumar²¹PhD Research scholar, Rajasthan University of health sciences, Jaipur, Rajasthan 302033²Professor, Department of Pharmacology, G.D. Memorial College of Pharmacy, Jodhpur, Rajasthan 342005**Article Information**

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Keywords*Aristolochia bracteolata*,
Peganum harmala, anti-
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sedimentation rate, C-reactive
protein.**ABSTRACT**

Background: Inflammation is a key pathological process in various diseases, and its modulation is crucial for therapeutic intervention. *Aristolochia bracteolata* and *Peganum harmala* are traditionally used medicinal plants with reported anti-inflammatory properties. This study aimed to evaluate their anti-inflammatory effects in carrageenan-induced paw edema in rats and assess their impact on inflammatory biomarkers. **Methods:** Paw edema was induced in Wistar rats by subplantar injection of carrageenan. The anti-inflammatory effects of *Aristolochia bracteolata* (200 mg/kg and 400 mg/kg) and *Peganum harmala* (200 mg/kg and 400 mg/kg) extracts were assessed by measuring paw volume at different time intervals. Indomethacin (10 mg/kg) served as the standard drug. The percentage inhibition of edema was calculated, and systemic inflammation was evaluated by measuring erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels. **Results:** Carrageenan injection induced significant paw edema, with peak inflammation observed at later time points. *Aristolochia bracteolata* exhibited dose-dependent anti-inflammatory activity, with the 400 mg/kg dose showing significant inhibition from 1 hour onwards. *Peganum harmala* (400 mg/kg) demonstrated anti-inflammatory effects only at later time points, with maximum inhibition (75.26%) at 5 hours. Indomethacin significantly reduced inflammation from 1 hour onwards. Both plant extracts significantly reduced ESR levels ($p < 0.05$ and $p < 0.01$), while CRP reduction was observed but not statistically significant. **Conclusion:** *Aristolochia bracteolata* and *Peganum harmala* exhibit promising anti-inflammatory activity, with *Aristolochia bracteolata* providing early and sustained effects, whereas *Peganum harmala* shows delayed yet potent inhibition. These findings support their traditional use and highlight their potential as anti-inflammatory agents for further pharmacological exploration..

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INTRODUCTION:

Inflammation is a complex vascular tissue reaction to noxious stimuli, pathogens, damaged cells, or irritants. It is a process of elimination of the offending agent and triggers healing^{12, 18}. Inflammation is caused by infectious, chemical, or physical stimuli like microorganisms, toxins, and trauma. The process manifests itself clinically in the form of redness, heat, swelling, and pain, which serves to isolate and eliminate tissue damage¹⁷. While acute inflammation plays a vital role in healing, chronic inflammation goes on to produce serious health ailments like ischemic heart disease,

chronic kidney disease, cancer, diabetes, neurodegenerative, and autoimmune diseases.

Inflammation-related diseases afflict millions of individuals across the globe, greatly reducing quality of life through restricted mobility and other health complications². Non-steroidal anti-inflammatory agents (NSAIDs) are one of the most commonly utilized medications for treating inflammation owing to their well-established efficacy^{1, 8}. These medications work by suppressing prostaglandin production through cyclooxygenase (COX) enzyme inhibition, thus diminishing inflammation and accompanying symptoms. Although NSAIDs are useful in the management of arthritis, muscle pain, menstrual pain, and acute trauma, they have substantial side effects such as gastrointestinal disturbances, cardiovascular disease, and impairment of renal function^{7, 25}. Doctors usually prescribe the minimal dose necessary to treat for as brief a time as possible to avoid these side effects^{8,24}.

Due to the drawback of NSAIDs, other therapy, such as medicinal plants, has become of growing interest. Medicinal plants have been traditionally used for decades in Ayurveda, Traditional Chinese Medicine (TCM), and African traditional herbal medicine for the management of inflammatory diseases¹⁶. Most herbal remedies are economical, easily accessible, and pose fewer side effects than synthetic agents⁵. Affordability and accessibility render medicinal plants as an asset especially in developing countries where availability of orthodox medicines is compromised^{8,20}.

Of these medicinal herbs, *Aristolochia bracteolata* and *Peganum harmala* have long been used medicinally for their therapeutic effects. These herbs are likely to display anti-inflammatory action because of the presence of certain bioactive phytoconstituents. *Aristolochia bracteolata* or Bracted Dutchman's Pipe or Worm Killer contains bioactive compounds like aristolochic acids and flavonoids, which may be responsible for its possible anti-inflammatory activity⁴. *Peganum harmala*, known by the alias Syrian Rue, contains a plethora of alkaloids that include harmine and harmaline and might modulate pro-inflammatory pathways¹⁴.

To establish the anti-inflammatory activity of these plants, the current study assesses their activity in the carrageenan-induced paw edema model. The study may give useful information on the potential of plant therapies for inflammatory diseases, opening the way to safer and less expensive therapeutic alternatives.

MATERIAL AND METHODS:

Plant Collection and Extraction:

Leaves of *Aristolochia Bracteolata* and Seeds of *Peganum harmala* were procured from local market and were authenticated at Janani Organics, Hubali. The dried plant material was coarsely powdered and subjected to Soxhlet extraction using a hydroalcoholic solvent (70% ethanol and 30% water). The extraction process was carried out for 24 hours, ensuring efficient recovery of bioactive compounds. The extract was filtered, allowed to dry completely. The dried extract was weighed and preserved in an airtight container at 5°C until further analysis. Preliminary phytochemical screening of the hydroalcoholic extracts was performed to identify the presence of alkaloids, flavonoids, saponins, tannins, steroids, and other secondary metabolites using standard qualitative tests.

Animals and Ethical Approval:

Swiss albino mice (20-40gm) and female Wistar albino rats (180-220 gm) was procured from Local vendor and were maintained at $25 \pm 2^\circ \text{C}$ and relative humidity of 45 to 55% and under standard environmental conditions (12 h light: 12 h dark cycle) at animal house. The animals had free access to food and water throughout study. The study was conducted in accordance with CPCSEA guidelines, and ethical approval was obtained from the Institutional Animal Ethics Committee (IAEC)

Experimental Design:

Acute toxicity study of hydroalcoholic extract of *Aristolochia bracteolata* leaves as per OECD guideline 425.

The animals were randomly divided into five groups, each comprising six rats:

Carrageenan-Induced Paw Edema Model:

The test was carried out in healthy Female Wistar albino rats weighing 150-250 g. After 12 h fasting, 30 animals were randomly divided into 5 groups of 6 animals each. Carrageenan-induced paw edema served as the experimental model²³.

- Group I (Normal Control): received of normal saline (control group)
- Group II (Induction Control): received carrageenan in normal saline on 7th day
- Group III (Standard Control): received Indomethacin 10 mg/kg (standard group) orally for 7 days. + received carrageenan in normal saline on 7th day
- Group IV (Test-1): received extract of *Aristolochia bracteolata* 200mg/kg orally for 7 days + received carrageenan in normal saline on 7th day
- Group V (Test-2): received extract of

Aristolochia bracteolata 400mg/kg orally for 7 days + received carrageenan in normal saline on 7th day

- Group VI (Test-3): received extract of *Peganum harmala* 200mg/kg orally for 7 days + received carrageenan in normal saline on 7th day
- Group VII (Test-4): received extract of *Peganum harmala* 400mg/kg orally for 7 days + received carrageenan in normal saline on 7th day

On the 7th day, all the standard and test drugs were given orally 1 h before the injection of 0.1ml of 1% w/v carrageenan in normal saline solution sub-Plantar region in the left hind paw. Fluid displacement in Plethysmometer was measured at 0th, 1st, 3rd, and 5th hrs after carrageenan injection. The percentage inhibition of edema was calculated using following formula.

$$\% \text{ inhibition of edema} = 100 \left(1 - \frac{V_t}{V_c} \right)$$

Where, V_c: Edema volume in control and V_t is edema volume of test or standard compound in respective time intervals.

After 5th hours of carrageenan injection, animals were anaesthetized by ether and blood was withdrawn by retro orbital plexus for Erythrocyte sedimentation rate (ESR) and C-Reactive Protein (CRP) estimation.

RESULT:

The effect of extracts of *Aristolochia bracteolata* and *Peganum harmala* on carrageenan-induced paw edema in rats is shown in Fig. 1.

The carrageenan injection induced inflammation indicated as paw volume in all the rats at all intervals. The inflammation was less ($p < 0.05$) at 30 minutes as compared to rest time intervals ($p < 0.001$).

This paw volume was significantly reduced by both the dose of *Aristolochia bracteolata* but onset and potency was different.

The *Aristolochia bracteolata*, 200 mg/kg showed significant reduction at 3 hours and 5 hours in dose dependent manner while *Aristolochia bracteolata* 400 mg/kg showed significant reduction from 1 hours onwards at all intervals in dose dependent manner.

On the other hand, *Peganum harmala* was effective only at higher dose i.e. 400 mg/kg and effect was highest at 5 hours interval. Surprisingly the effect was more ($p < 0.01$) at 1 hours than effect was

recorded at 3 hours ($p < 0.05$).

The reference standard Indomethacin was significant from 01 hour onwards. The effect was lesser at 01 hour ($p < 0.05$) than rest 02 times lines which were equipotent ($p < 0.001$) in this regards.

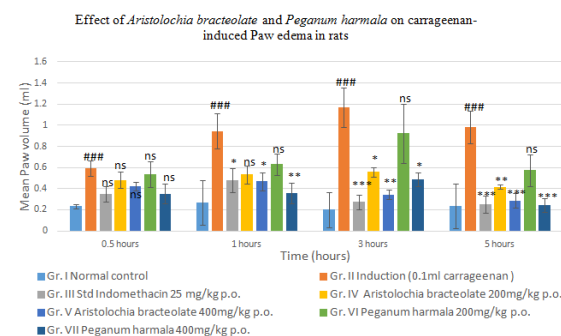


Figure 1 Effect of *Aristolochia bracteolata* and *Peganum harmala* on carrageenan-induced Paw edema in rats

The effect of *Aristolochia bracteolata* and *Peganum harmala* on percentage inhibition of edema in carrageenan-induced Paw edema in rats is shown in Fig. 2

The maximum percentage inhibition of inflammation exhibited by *Aristolochia bracteolata* (400 mg/kg b. w.) was 70.67% after 03 hours and remained same till after 5 hours whereas maximum percentage inhibition of inflammation exhibited by *Peganum harmala* treatment group (400 mg/kg b. w.) was 75.26%, after 5 hours.

The results showed that *Aristolochia bracteolata* extracts inhibited the paw inflammation in dose dependent manner whereas *Peganum harmala* only at a dose of 400 mg/kg b.w

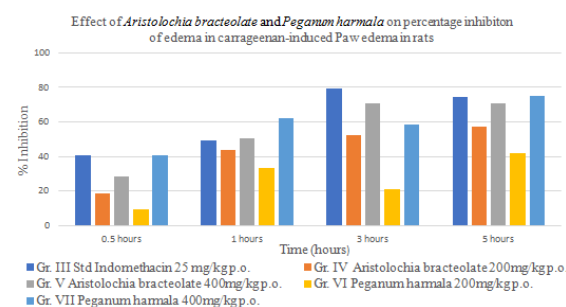


Figure 2 Effect of *Aristolochia bracteolata* and *Peganum harmala* on percentage inhibition of edema in carrageenan-induced Paw edema in rats

Erythrocyte Sedimentation Rate (ESR) and C-Reactive Protein (CRP) were found elevated significantly ($p < 0.001$ and $p < 0.01$) in induction group. It is shown in shown in Fig. 3 and Fig. 4 respectively.

There was significant reduction in ESR when compared to induction control group was recorded after treatment with *Aristolochia bracteolata*

($p < 0.05$) and *Peganum harmala* ($p < 0.01$). The CRP level was found reduced in both extract treated groups but results were not-significant.

The reference standard treated group showed significant ($p < 0.01$, $p < 0.05$) decrease in the levels of ESR and CRP when compared to induction group.

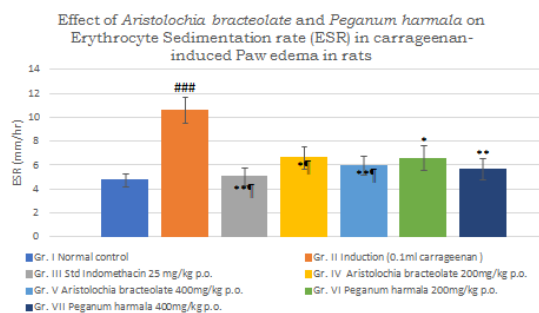


Figure 3 Effect of *Aristolochia bracteolata* and *Peganum harmala* on Erythrocyte Sedimentation rate (ESR) in carrageenan-induced Paw edema in rats

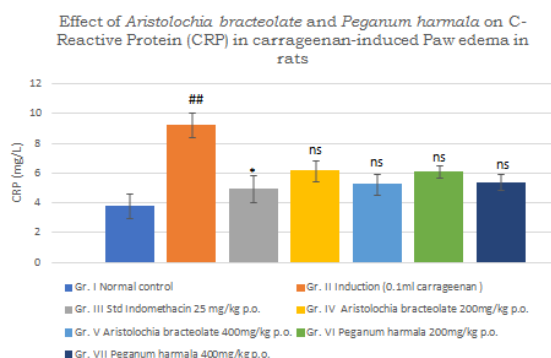


Figure 4 Effect of *Aristolochia bracteolata* and *Peganum harmala* on C-Reactive Protein (CRP) in carrageenan-induced Paw edema in rats

Statistical analysis:

Results were expressed as mean + SEM ($n=6$). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's Kramer test. $^{ns}p > 0.05$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ when compared to control group.

DISCUSSION:

Inflammation is a multifaceted biological reaction of tissues of the body to harmful stimuli like pathogens, injured cells, or irritants. Carrageenan-induced rat paw edema is an established model for the evaluation of anti-inflammatory action of pharmacologic agents because it simulates acute inflammation by inducing the release of pro-inflammatory mediators like histamine, serotonin, bradykinin, and prostaglandins^{22,23}. The current research assessed the anti-inflammatory activities of *Aristolochia bracteolata* and *Peganum harmala* extracts on carrageenan-induced paw edema in rats and their influence on inflammatory markers, such as erythrocyte sedimentation rate (ESR) and C-

reactive protein (CRP).

The outcome proved that injection of carrageenan caused great inflammation in the experimental groups with maximum edema at different intervals. Interestingly, the severity of inflammation was found to be smaller at 30 minutes ($p < 0.05$) than at the later time periods ($p < 0.001$). It is consistent with the biphasic character of carrageenan-induced inflammation involving histamine and serotonin in the initial phase (0–1 hour) followed by the later phase (more than 1 hour) through prostaglandins and cytokines³.

Both the doses of 200 mg/kg and 400 mg/kg of *Aristolochia bracteolata* extract significantly decreased paw edema with varying onset and potency. The lower dose (200 mg/kg) had a significant anti-inflammatory effect at 3 and 5 hours in a dose-dependent manner. On the other hand, the higher dose (400 mg/kg) had an earlier onset of action, which decreased edema significantly from 1 hour onward at all points measured, signifying greater efficacy. This implies that *Aristolochia bracteolata* could potentially act against inflammation by regulating both early and late-phase inflammatory mediators¹³.

Conversely, *Peganum harmala* extract was observed to show anti-inflammatory activity only at a high dose (400 mg/kg). The peak inhibition of paw edema by *Peganum harmala* was at 5 hours, with significant reduction also seen at 1 hour ($p < 0.01$), which was relatively higher than at 3 hours ($p < 0.05$). This delayed but prolonged effect indicates that *Peganum harmala* might mainly exert its effect on late-phase inflammation, possibly through the modulation of prostaglandin synthesis or cytokine release¹⁰.

The control reference drug, Indomethacin, showed substantial inhibition of inflammation from 1 hour onward ($p < 0.05$) with maximum efficacy at subsequent time intervals ($p < 0.001$), showing its well-established nonsteroidal anti-inflammatory effect through inhibition of cyclooxygenase (COX)²¹. The equipotent activities of Indomethacin at subsequent time points consolidate its efficacy in inhibiting prostaglandin-mediated inflammation.

The percentage inhibition of inflammation further consolidated the efficacy of these extracts. *Aristolochia bracteolata* (400 mg/kg) showed the highest inhibition of 70.67% at 3 hours, which was sustained until 5 hours. *Peganum harmala* (400 mg/kg) showed a slightly increased inhibition of 75.26% but at a later time interval (5 hours), which suggests a different mechanism or speed of action. These results indicate that *Aristolochia bracteolata*

can have more sustained anti-inflammatory activity in the long term, while *Peganum harmala*'s activity is more evident at later times.

Aside from paw edema, the inflammatory response was also evaluated through the measurement of ESR and CRP levels, which are significant biomarkers of systemic inflammation. The induction control group had significantly increased ESR ($p < 0.001$) and CRP ($p < 0.01$), validating the systemic inflammatory response induced by carrageenan⁹. Following treatment, *Aristolochia bracteolata* and *Peganum harmala* decreased ESR levels considerably ($p < 0.05$ and $p < 0.01$, respectively), indicating their potential to suppress systemic inflammation. Although CRP levels were lowered in the extract-treated groups, the reduction was not significant, showing a moderate effect on acute-phase proteins. The control Indomethacin-treated group was found to have a highly significant decrease in both ESR ($p < 0.01$) and CRP ($p < 0.05$), establishing its high anti-inflammatory activity.

The reported anti-inflammatory activity of *Aristolochia bracteolata* could be due to its phytochemical components such as flavonoids, alkaloids, and terpenoids, which were found to have anti-inflammatory and antioxidant activities¹¹. *Peganum harmala* also possesses β -carboline alkaloids that have been shown to influence inflammatory processes, likely by inhibiting pro-inflammatory cytokines and modifying oxidative stress¹⁵.

CONCLUSION:

The current research presents robust evidence for the anti-inflammatory activity of *Aristolochia bracteolata* and *Peganum harmala* in carrageenan-induced paw edema in rats. *Aristolochia bracteolata* showed dose-dependent anti-inflammatory activity, with the higher dose (400 mg/kg) showing a faster and longer-lasting effect. *Peganum harmala* was effective at the higher dose (400 mg/kg), with its maximum effect at 5 hours. The decrease in ESR levels further corroborates their anti-inflammatory activity, even though the decrease in CRP was not significant statistically. These results imply that both plant extracts have promising anti-inflammatory activity and are candidates for further studies regarding their mechanism and possible clinical utility.

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