

Anti-Inflammatory Activity of Crude Extract of Aesculus Indica in Comparison with Aspirin on Carrageenan Induced Paw Edema in Rats

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ABSTRACT

Objective: To determine the anti-inflammatory activity of crude extract of Aesculus Indica in the rat model.

Study Design: Laboratory-based experimental study.

Place and Duration of Study: Pharmacy Department, Bahauddin Zakria University, Multan Pakistan, from May to Oct 2018.

Methodology: Twenty-four healthy male and female Wistar rats (200-220gm) were included in the study. Each rat was given a single injection of 0.1 ml of 1% Carrageenan mixed in normal saline in the sub-planter region of the right paw for oedema induction. The rats were randomly divided by lottery method into four groups; Group-1 served as positive control; Group-2 and 3 received a crude extract of Aesculus Indica 100 mg/kg and 300mg/kg, respectively; Group-4 was given Aspirin at the dose of 12 mg/kg. The measurement of paw volume was made by displacement technique using the plathysmometer before and at 1st, 2nd, 3rd and 4th hour after the Carrageenan injection.

Results: The results confirmed the anti-inflammatory action of crude extract of Aesculus indica both at 100 mg/kg and 300 mg/kg. When Group-1 was compared with Groups 2, 3 and 4, it revealed a significant difference with a *p*-value of 0.002, 0.04 and 0.002, respectively. Similarly, after 3 hours of Carrageenan-induced oedema, the comparison of Group-2 with Group-3 showed a significant difference with a *p*-value of 0.03, while an insignificant difference was found between Groups-3 and 4. An insignificant difference of *p*-value of 0.07 was found when groups 2 and 4 were compared.

Conclusion: Crude extract of the Aesculus Indica showed significant anti-inflammatory properties, which at high doses were almost comparable with that of Aspirin.

Keywords: Aesculus indica, Aspirin, Carrageenan, Edema, Inflammation.

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INTRODUCTION

Inflammation is a defensive response of the body to stimuli. Its local reactions include pain, oedema and loss of function, which may be partial or complete, while the increase in neutrophil level and fever are considered systemic responses.¹ Since ancient times, medicinal plants have been used immensely to treat many diseases. Plants possess the ability to form secondary metabolites such as flavonoids, proteins, alkaloids, phenolic substances, and steroids, making them integral in the treatment of many diseases. Almost 70% population of remote areas depends on medicinal plants to treat numerous ailments.^{2,3} Therapeutic use of medicinal plants has been reported for over 5000 years.⁴ Various medicinal plants and their active ingredients have a broad spectrum of health benefits which include the antioxidant,⁵ antimicrobial,^{6,7} antiinflammatory, hepato-protective activities, and so on.⁸

The plant *Aesculus indica* (Linn.) (Ai Cr), which belongs to the family Sapindaceae, bears reddish

brown smooth fruits and is found abundantly in the Northern Western Himalayas. It is also known as Barkhor. It has many pharmacological uses, including anti-bacterial, immune-modulatory, spasmolytic, anti-inflammatory and antioxidant properties. The crude extracts of the different parts of the plant are used for various medicinal purposes. The oil is used for the treatment of various skin ailments and rheumatism, whereas the seeds are used as an astringent. The plant's fruit helps treat colic disorders, and its roots are used to treat leucorrhoea.⁹

Aspirin is an allopathic medicine which belongs to a group of drugs known as non-steroidal anti-inflammatory drugs (NSAIDs). It is widely used to relieve mild to moderate pain and inflammation. However, when used for a prolonged period, Aspirin may lead to adverse effects like gastrointestinal ulcerations, abdominal pain, drowsiness, headache, and cramping. Similarly, other anti-inflammatory drugs are also available, but they are also associated with multiple adverse effects.¹⁰ Therefore, there is a strong need to go for such a drug/plant extract with good efficacy but fewer adverse effects. Currently, various researches are

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carried out on medicinal plants in this regard; *Aesculus indica* is one of them. Flavonoids of *Aesculus indica* are considered the most important group of polyphenols which possess significant anti-inflammatory activities for which the plant is famous. Keeping in view the beneficial effects of *Aesculus indica*, the present study aimed to evaluate and compare its effect on the subsidence of oedema associated with inflammation in the models of standard lab animals.

METHODOLOGY

This laboratory-based experimental study was conducted at the Pharmacy Department, Bahauddin Zakria University, Multan Pakistan, from May 2018 to October 2018 after Ethical Approval (EC/10/18).

Inclusion Criteria: Wistar Rats of either gender, weighing 200-220gm were included in the study.

Exclusion Criteria: Animals with any obvious injury or disease were excluded from the study.

Twenty four male and female Wistar rats were obtained from the National Institute of Health, Islamabad. They were acclimatized for one week before the actual procedure.^{11,12} They were kept in an animal house according to the guidelines outlined in the "Animals Bye-Laws 2008 of the University of Malakand (Scientific Procedures Issue- 1).¹³ The rats were deprived of food 12 hours before the experiment and water during the experiment. Water deprivation was to ensure uniform hydration and minimize variability in edematous response.

For induction of oedema, each rat was given a single injection of 0.1 ml of 1% Carrageenan, a phlogistic agent, in normal saline in the sub plantar region of the right paw through 26-gauge needle.¹⁴ Edema was induced in each rat after 30 min. Carrageenan-induced oedema is widely used to check the anti-

each. Group-1 (Positive control): This group received 10 mg/kg of normal saline as a vehicle; Group-2 (Aspirin treated group): This group was given Aspirin at the dose of 12 mg/kg; Group-3: This group was treated with 100 mg/kg dose of crude extract of *Aesculus indica*; Group-4: This group received a crude extract of *Aesculus indica* at the dose of 300 mg/kg.

For evaluation of anti-inflammatory activity, Winter *et al*, method was used. The measurement of paw volume was made immediately by displacement technique using the plathysmometer before and at 0, 1, 2, and 3 hours after the Carrageenan injection. All the animals in Groups-2 and 3 were treated with *Aesculus indica* extract and Group-4 with Aspirin 1 hour before the Carrageenan injection. Percent inhibition of the test drug was calculated in comparison with positive control.¹⁶

Statistical Package for Social Sciences (SPSS) version 24.0 was used for the data analysis. Data was presented as Means \pm SD deviation (SD). For statistical analysis, data was analyzed by the one-way ANOVA followed by a Duncan post-hoc for pair-wise comparisons between groups. The p -value ≤ 0.05 indicated a significant difference between the groups.

RESULTS

The result of the study confirmed the anti-inflammatory action of crude extract of *Aesculus indica* at both 100 mg/kg and 300 mg/kg. The mean and standard deviation for different groups after 3 hours of Carrageenan-induced oedema were as follows: Group-1: 2.76 ± 0.13 ; Group-2: 0.99 ± 0.04 ; Group-3: 0.57 ± 0.14 ; Group-4: 0.055 ± 0.27 . In addition, a significant difference was found when paw volumes of Groups 1, 2, 3 & 4 were compared at different timings (Table-I).

When Group-1 was compared with Groups-2, 3 and 4 after 3 hours of Carrageenan-induced oedema, it

Table-I: Effects of crude extract of aesculus indica and aspirin on paw volume in carrageenan induced edema in rats at different time intervals.

Paw Volume (ml)	Group-1 (n=6) (Mean \pm SD)	Group-2 (n=6) (Mean \pm SD)	Group-3 (n=6) (Mean \pm SD)	Group-4 (n=6) (Mean \pm SD)	p -value
0 hour	2.27 ± 0.031	2.06 ± 0.02	1.56 ± 0.38	1.49 ± 0.027	0.02
1 hour	2.39 ± 0.043	1.80 ± 0.10	1.15 ± 0.21	1.05 ± 0.17	0.01
2 hour	2.53 ± 0.06	1.47 ± 0.21	0.86 ± 0.09	0.79 ± 0.06	0.001
3 hour	2.76 ± 0.13	0.99 ± 0.04	0.57 ± 0.14	0.05 ± 0.27	0.001

inflammatory activity of various agents and is considered a valid tool for exploring new anti-inflammatory drugs.¹⁵

Twenty four rats (24) were then divided randomly by lottery method into four groups of 6 rats

revealed a significant difference with p -value 0.002, 0.042 and 0.002, respectively. Similarly, Group-2 with Group-3 showed a significant difference with a p -value of 0.03, while an insignificant difference was found between Groups-2 and 4. An insignificant difference of

p-value of 0.06 was found when Groups-3 and 4 were compared (Table-II).

effects on the activities of Phospholipase A2 and the 15-lipoxygenase pathway. Blockage of COX-2 enzyme

Table-II: Pair wise comparison of different groups after 3h of Carrageenan induced edema.

Group Comparison	Group-1 Vs. Group-2	Group-2 Vs. Group-3	Group-1 Vs. Group-3	Group-2 Vs. Group-4	Group-1 Vs. Group-4	Group-3 Vs. Group-4
<i>p</i> -values	0.002	0.03	0.04	0.07	0.002	0.06

DISCUSSION

In the present study, *Aesculus indica* showed anti-inflammatory activity at 100 mg/kg and 300 mg/kg. The significant difference was found when positive control (Group-1) was compared with low dose *Aesculus* treated group (Group-2), high dose *Aesculus* treated group (Group-3) and Aspirin-treated group (Group-4) with *p*-value 0.002, 0.042 and 0.002 respectively. Similarly, a comparison of low dose *Aesculus* treated group (Group-2) with high dose *Aesculus* treated group (Group-3) showed a significant difference with a *p*-value of 0.03. In contrast, an insignificant difference was found between the high dose *Aesculus* treated group (Group-3) and Aspirin-treated group (Group-4). Furthermore, a significant difference in a *p*-value of 0.03 was found when low dose *Aesculus* treated (Group-2) and Aspirin-treated group (Group-4) were compared.

The human body's natural response to injury results in inflammation-induced swelling, pain, and erythema. For the reduction of pain, many anti-inflammatory agents, such as NSAIDs, are used, which work on multiple inflammatory pathways, and are very effective and useful, but at times these result in many undesirable side effects.^{15,16} Chronic pain alone costs almost \$100 billion annually in healthcare, lost work and medication.¹⁷ A meta-analysis study has shown that NSAIDs use causes an increase of almost 5 mmHg in systolic blood pressure, thus should be used with great caution in hypertensive patients.¹⁸ There is evidence of Aspirin-induced peptic ulcers, especially stomach ulcers. On the other hand, perforation has also been a common adverse effect of Aspirin. Therefore, scientists are shifting towards organic anti-inflammatory agents like *Aesculus indica*, which work similar to Aspirin, but have fewer adverse effects.¹⁹

Sato *et al.*, performed a study to determine the anti-inflammatory effects of *Aesculus* family on croton oil-induced swelling in animal models. The extract of *Aesculus* reduced the swelling induced by croton oil. The anti-inflammatory effects of the *Aesculus* family are usually believed to be due to inhibition of the cyclooxygenase (COX) 1 and 2 enzymes. They have no

usually requires low dose than COX-1.²⁰

Yang *et al.* 2020 conducted a study that confirmed the *Aesculus* family's anti-inflammatory activity by downregulating the gene expression of complement factor 3, CD 40 tumour necrosis factor- α (TNF- α), upregulation of the expression of granulocyte-macrophage colony-stimulating factor.²¹ Similarly, in another study, it decreases the levels of other mediators involved in inflammation like nitric oxide (NO), interleukin-1 β (IL-1 β) and IL-6 (Du Y). It also increases the expression of glucocorticoid receptors and increases the actions of superoxide dismutase (SOD) and glutathione peroxidase (GPx). It also augments the endogenous antioxidant capacity.¹

The results of the mentioned studies are similar to our study. However, as the results of this study are based on a single-centre, more studies are required to generalize it.

CONCLUSION

Crude extract of *Aesculus indica* showed significant anti-inflammatory activity both at low (100 mg/kg) and high doses (300 mg/kg). Furthermore, its anti-inflammatory effect was almost equal to that of Aspirin at a high dose.

Conflict of interest: None.

Author's Contribution

RF: Substantial contributions to conception and design, or acquisition of data, or analysis and interpretation of data, Drafting the article or revising it critically for important intellectual content and final approval of the version to be published, AA: Design, or acquisition of data, or analysis and interpretation of data and revising it critically, AK: Analysis and interpretation of data, Drafting the article or revising it critically for important intellectual content, AS: Acquisition of data, or analysis and interpretation of data, Drafting the article or revising it critically for important intellectual content, AJ: Design, or acquisition of data, or analysis and interpretation of data and revising it critically, AJ: Acquisition of data, or analysis and interpretation of data, Drafting the article or revising it critically for important intellectual content.

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