

Evaluation of Anti-Inflammatory and Analgesic Activities of Methanolic Extract of *Aitchisonia Rosea* in Albino Mice

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ABSTRACT

Objective: Current analysis aims at *in-vivo* investigation of analgesic and anti-inflammatory activities of *Aitchisonia rosea* methanolic extract.

Methods: Albino mice (20-30 g) were used in the study. Extract at 200, 400 and 800 mg/kg doses was tested against egg albumin and carrageenan induced mice paw edema along with formalin persuaded paw licking and acetic acid prompted writhing for analgesic activity.

Results: Results depicted dose reliant and significant ($p < 0.001$) inhibition of carrageenan as well as egg albumin brought paw edema, abdominal constrictions/ hind limb stretching tempted by acetic acid and formalin instigated paw licking. Capability of crude extract to halt inflammatory and analgesic response was akin to that of ibuprofen in all models.

Conclusion: The current findings support the use of *Aitchisonia rosea* for inflammatory conditions, which might be attributed to its previously proven high alkaloid, flavonoids, phenol, tannins content and free radical scavenging activity.

Key Words: *Aitchisonia rosea*, analgesic, ibuprofen, anti-inflammatory.

INTRODUCTION

Inflammation, a protective reaction yields an array of inflammatory intermediaries to infection, irritation and tissue injury to exterminate the irritant and uphold tissue repair [1]. Moreover, free radicals also contribute towards the pathogenesis of inflammation. During inflammation, reactive oxygen species (ROS) increase unusually owing to the action of free radicals on molecular oxygen, which causes an imbalance among oxidizing agents and anti-oxidant system of body. The oxidative stress ensues in inflammatory processes that impair the cellular constituents [2]. Hence, pharmacological agents are required to

relieve the damage caused by inflammatory response and associated algosia, for instance, NSAIDs (non-steroidal anti-inflammatory drugs), opiates and corticosteroids. Nevertheless, because of frequent use of these drugs, unpleasant effects (GI bleeding, peptic ulcer etc) occur repeatedly, particularly when higher doses are used for prolonged duration. Hence, natural products obtained from medicinal plants are becoming ideal alternative therapies [3]. In this aspect, secondary metabolites of numerous medicinal plants have been shown to be active in the management of pain and inflammation [2].

Aitchisonia rosea Hemsl. ex Aitch (Family: Rubiaceae) plant is a native to Pakistan

(Baluchistan), Afghanistan and Iran [4]. Traditionally it is used for the treatment of dermal infections in Baluchistan. The aerial parts of *Aitchisonia rosea* have been reported to possess anti-convulsant, anti-microbial and hemolytic effect [4]. Indeed, it has been confirmed that *Aitchisonia rosea* extract exhibit robust anti-oxidant potential against reducing power assay and DPPH free radical scavenging assay [5], mainly attributed to its polyphenolic phytoconstituents, identified through HPLC technique [4]. A large number of flavonoids and other phenolics have been proved their noteworthy effects on inflammatory processes [6]. Phenolic compounds exert anti-inflammatory property by impeding pro-inflammatory mediators production, inhibiting triggered immune cells, altering eicosanoid synthesis, preventing cyclooxygenase-2/nitric oxide synthase and acting as free radical scavengers [7]. Since, ROS and free radicals are prime mediators that sustain or incite inflammatory procedures, their deactivation by anti-oxidants and radical scavengers can mitigate inflammation so, based upon the reported anti-oxidant nature of *Aitchisonia rosea* and imperative role of polyphenolic constituents in prevention of inflammatory processes, the existing investigation was conducted to appraise its potential against inflammation.

MATERIALS AND METHODS

Chemicals

In the current study, carrageenan (Sigma, USA), fresh hen egg-albumin, formalin (Merck, Germany), acetic acid (Sigma, USA) and ibuprofen were used.

Collection and Extraction of Plant

The aerial parts (stems and branches) of *Aitchisonia rosea* were acquired from Quetta, Pakistan. The plant was recognized and verified by taxonomists, Prof. Zaheer Ahmad Khan, GC University Lahore, and Prof. Rasool Bakhsh Tareen, University of Balochistan, Quetta. For further citation, voucher sample (No. 1911) was kept at Sultan Ayub Herbarium, GC University Lahore. The plant were shade dried at room temperature. The powdered plant (5 kg) was dipped in 6 L methanol (100%) for three days with frequent stirring every day. This procedure was repeated thrice. It was then filtered using Whattmann filter paper. The *Aitchisonia rosea* methanol extract (ARME) was concentrated and kept at 4 °C in a refrigerator [4].

Experimental Animals

Albino mice (20-30 g) were employed in each test. Mice were kept in propylene cages under controlled environment of 24 ± 1 °C and 12 h light/dark cycles and ventilation. Appropriate feed and water were provided *ad libitum* unless otherwise specified. All mice were treated as per standard protocols directed by National Research Council, 1996 [8]. The animal studies were permitted by Animal Ethics Committee, University of Sargodha.

Carrageenan Induced Paw Edema of Inflammation

Mice were divided into 5 groups (n=5). Group I served as control and given distilled water (2 ml/kg p.o). Group II, III, IV were administered 200, 400, 800 mg/kg ARME orally, respectively whereas, group V was given 40 mg/kg ibuprofen p.o. The edema was induced into sub-planar surface of right hind paw by inserting 0.1 ml carrageenan (1% w/v in saline), 1 hour after treatment. Diameter of injected paw was assessed at 0, 1st, 2nd and 3rd h of treatment using digital vernier calliper [9].

Egg Albumin Induced Paw Edema of Inflammation

Mice were segregated into 5 groups (n=5). Group I (control) was given orally 2 ml/kg distilled water. Group II, III, IV were administered ARME orally at 200, 400 and 800 mg/kg doses, in that order. Group V was given ibuprofen (40 mg/kg) orally. The fresh hen's egg albumin (0.1 ml) was administered into right hind paw of all mice after 1 h of treatment. Then, injected paw diameter was monitored at 0, 1st, 2nd and 3rd h of administration of test material via digital vernier calliper [9].

Acetic acid Induced Writhing Model of Analgesia

The mice were segregated into 5 groups (n=5). Group 1 i.e. control was administered 2 ml/kg distilled water orally. The groups II, III, IV received ARME at 200, 400, 800 mg/kg doses orally, correspondingly. Group V was given ibuprofen (40 mg/kg, p.o.). The solution of acetic acid (10 ml/kg, 0.6% in normal saline) was administered i.p. to each mice, 30 min after administering the test substance. Number of writhings described by abdominal contractions and stretching with hind limb jerk was calculated for 5-15 min subsequent to acetic acid inoculation. The reaction of mice in treatment groups was equated with control group mice [10].

Formalin Induced Paw Licking Model of Analgesia

The 5 groups of mice (n=5) were kept on overnight fasting. Group I i.e., control group was given 2 mg/kg distilled water orally. Whereas, groups II, III, IV mice were administered orally 200, 400 and 800 mg/kg doses of ARME, correspondingly. Whereas, group V was given ibuprofen (40 mg/kg, p.o.). 20 µl of formalin solution (2.5%) was inoculated into sub-plantar region of left hind paw of all mice after 1 h of treatment. The time taken to lick formalin injected paw was observed for 30 min immediately after injecting formalin [10].

Statistical Analysis

The data was accessed as mean \pm SEM and statistically evaluated with one-way and two-way ANOVA accompanied by Bonferroni posttest via Graph Pad Prism 5. The statistical significance was taken at $p < 0.05$.

RESULTS

Effect of ARME on Carrageenan Induced Paw Edema

In mice administered 200, 400 and 800 mg/kg doses of ARME, constant dose reliant and considerable

($p < 0.001$) decline in paw diameter ($3.17 \text{ mm} \pm 0.25$, $3.00 \text{ mm} \pm 0.14$ and $2.90 \text{ mm} \pm 0.19$, respectively) was noted at 3rd h of treatment, compared to control group mice ($4.60 \pm 0.09 \text{ mm}$). Furthermore, ibuprofen (40 mg/kg) significantly ($p < 0.001$) decreased paw diameter ($3.32 \text{ mm} \pm 0.14$), when compared to control mice, but reduction in edema formation was observed to be less in contrast to ARME groups (Table 1).

Effect of ARME on Egg Albumin Induced Paw Edema

The methanolic extract of plant at all dose levels showed a considerable ($p < 0.001$) decline in paw edema at 3rd h, particularly with 800 mg/kg dose, compared to control group. Nonetheless, reduction in paw diameter of mice with ibuprofen treatment was significantly ($p < 0.001$) lesser than extract treated mice at 3rd h of treatment. The paw diameter of mice treated with 200, 400, 800 mg/kg ARME and 40 mg/kg of ibuprofen were found to be $2.88 \text{ mm} \pm 0.12$, $2.80 \text{ mm} \pm 0.06$, $2.66 \text{ mm} \pm 0.31$ and $3.19 \text{ mm} \pm 0.31$ respectively as compared to control (3.92 ± 0.14) at 3rd h (Table 2).

Table 1. Effect of ARME on carrageenan induced paw edema.

Animal Groups	Change in paw diameter (mm)			
	0 h	1 st h	2 nd h	3 rd h
Control (2 ml/kg)	3.62 ± 0.1	4.11 ± 0.08	4.39 ± 0.12	4.60 ± 0.09
ARME (200 mg/kg)	3.97 ± 0.10	$3.77 \pm 0.53^{***}$	$3.41 \pm 0.17^{***}$	$3.17 \pm 0.25^{***}$
ARME (400 mg/kg)	3.84 ± 0.13	$3.59 \pm 0.15^{***}$	$3.28 \pm 0.16^{***}$	$3.00 \pm 0.14^{***}$
ARME (800 mg/kg)	3.64 ± 0.13	$3.47 \pm 0.17^{***}$	$3.22 \pm 0.16^{***}$	$2.90 \pm 0.19^{***}$
Ibuprofen (40 mg/kg)	3.90 ± 0.03	$3.69 \pm 0.13^{***}$	$3.54 \pm 0.22^{***}$	$3.32 \pm 0.14^{***}$

Values are stated as mean \pm SEM (n=5). ***= $p < 0.001$, compared to control. ARME= *Aitchisonia rosea* methanol extract

Table 2. Effect of ARME on egg albumin induced paw edema.

Animal Groups	Change in paw diameter (mm)			
	0 h	1 st h	2 nd h	3 rd h
Control (2ml/kg)	3.56±0.14	3.72±0.15	4.71±0.16	4.92±0.18
ARME (200 mg/kg)	3.82±0.13	3.55±0.1***	3.10±0.06***	2.88±0.12***
ARME (400 mg/kg)	3.66±0.15	3.36±0.13***	3.09±0.14***	2.80±0.06***
ARME (800 mg/kg)	3.52±0.13	3.29±0.22***	3.02±0.34***	2.66±0.31***
Ibuprofen (40 mg/kg)	3.70±0.12	3.50±0.34**	3.38±0.11***	3.19±0.31***

Values are stated as mean ± SEM (n=5). **= $p<0.01$, ***= $p<0.001$, compared to control. *Aitchisonia rosea* methanol extract

Table 3. Effect of ARME on acetic acid induced writhing.

Animal Groups	No. of Writhing	Inhibition (%)
	Mean ± SEM	
Control (2 ml/kg)	48.6±1.41	—
ARME (200 mg/kg)	25.4±0.92***	47.73
ARME (400 mg/kg)	20.2±0.68***	58.43
ARME (800 mg/kg)	15.4±0.20***	68.31
Ibuprofen (40 mg/kg)	18.2±0.90***	62.55

Values are stated as mean ± SEM (n=5). ***= $p<0.001$ compared to control. *Aitchisonia rosea* methanol extract

Table 4. Effect of ARME on formalin induced paw licking.

Animal Groups	Licking time(s)	Inhibition (%)
	Mean ± SEM	
Control (2 ml/kg)	293.8±2.10	—
ARME (200 mg/kg)	123.8±2.23***	57.86
ARME (400 mg/kg)	98.2±2.65***	66.57
ARME (800 mg/kg)	74.2±1.47***	74.74
Ibuprofen (40 mg/kg)	104.5±2.74***	64.43

Values are stated as mean ± SEM (n=5). ***= $p<0.001$, compared to control. *Aitchisonia rosea* methanol extract

Effect of ARME on Acetic Acid Prompted Writhing

Treatment with ARME at 200, 400, 800 mg/kg doses significantly ($p<0.001$) and dose dependently subdued abdominal constrictions as 47.73%, 58.43% and 68.31%, respectively analogous to ibuprofen (62.55%) as demonstrated in Table 3.

Effect of ARME on Formalin Induced Paw Licking

The mice administered ARME unveiled a remarkably ($p<0.001$) shorter mean licking time for instance,

57.86%, 66.57% and 74.74% at 200, 400 and 800 mg/kg doses, in that order in contrast to control group mice. Besides, the mice treated with reference drug ibuprofen also exhibited a notable reduction (64.43%) in mean itching period (Table 4).

DISCUSSION

The existing study was intended to appraise anti-inflammatory and analgesic potential of methanolic

extract of *Aitchisonia rosea* in mice models of inflammation and pain.

Carrageenan and egg albumin induced mice paw edema are biphasic models of inflammation. Serotonin and histamine stimulates paw edema in 1st phase while, prostaglandins, leukotriene and bradykinin maintains inflammation in 2nd phase [1]. Besides, these inflammatory intermediaries prompt the efflux of edematous fluid, plasma proteins and neutrophils owing to increased vascular permeability and blood flow [11]. In the current study, paw edema was significantly reduced after ARME treatment. The study also established that ARME treatment is capable of reducing inflammatory reaction in either phase, which propose that ARME might impede the assembly of aforementioned inflammatory intermediaries similar to that produced by NSAIDs.

Acetic acid induced algesia in mice is a pertinent model to appraise analgesic ability of test substances. The discharge of inflammatory intermediaries (arachidonic acid, cyclooxygenase, prostaglandins, histamine, bradykinin and serotonin) upon injecting acetic acid produces algesia and abdominal writhing via peripheral nociceptive sensitization [1]. Results of the present study asserted that ARME dose dependently produced significant analgesic effect by reducing number of writhings and this response might be because of inhibition of arachidonic acid metabolite synthesis.

Furthermore, formalin persuaded hind paw licking test was also employed to evaluate analgesic effect in this study. A biphasic pain response is produced, in which 1st phase is ascribed to bradykinin and tachykinins, however, 2nd phase is owing to an inflammatory reaction instigated by tissue injury that prompts the discharge of serotonin, histamine, excitatory amino acids and prostaglandin. The first phase is normally inhibited by opioids and late phase is inhibited by NSAIDs and opioid both [12]. ARME significantly and dose dependently decreased the paw licking time in second phase that might be owing to the inhibition of inflammatory mediators, so it acts more like NSAIDs in reducing pain.

Accordingly, current findings have avowed that crude extract of *Aitchisonia rosea* possess preeminent potential to alleviate inflammation accompanied by algesia and may be allied to the presence of various phytoconstituents, as have been earlier reported in *Aitchisonia rosea* i.e., alkaloids, terpenoids, flavonoids, tannins and phenolics (quercetin, caffeic

acid, chlorogenic acid and syringic acid) [4]. In addition, free radical scavenging and reducing potential of *Aitchisonia rosea* has previously been measured by using DPPH and reducing power assay [5]. Phytochemicals like flavonoids and saponins are well known for reducing pain sensitivity owing to their free radical scavenging properties [13], together with exert anti-inflammatory properties via inhibition of enzymes involved in production of inflammatory mediators and arachidonic acid metabolism [14]. Flavonoids also suppress prostaglandin synthesis that lead to several immune reactions [15]. Likewise, phenols, tannins and alkaloids have also been explored to be bestowed with anti-inflammatory potential [16].

CONCLUSION

In a nutshell, anti-inflammatory and analgesic potential of *Aitchisonia rosea* could plausibly be owing to alkaloids, flavonoids, phenols, tannins along with anti-oxidant effect of flavonoids. Nevertheless, activity directed fractionation of ARME to separate active constituents from plant and further studies to explicate probable mode of action are mandatory to rationalize its use.

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