

In-Vivo and *In-Vitro* Anti-Inflammatory Activity of *Aquilaria agallocha* Oil

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Abstract – The present study aimed for evaluation of *Aquilaria agallocha* oil obtained by hydro-distillation from the woods for *in-vivo* and *in-vitro* anti-inflammatory activity. The oil was screened for *in-vivo* anti-inflammatory activity by carrageenan-induced paw edema in rat model and *In-vitro* anti-inflammatory activity by human red blood cell membrane stabilization method. The potency of the oil was compared with standard Diclofenac (10 mg/kg). The oil showed significant reduction of edema in carrageenan induced rat paw edema model maximum at 3 hr for AAO 50 mg/kg, AAO 100 mg/kg and diclofenec 10 mg/kg (% reduction in paw volume 58.59%, 62.11% and 68.94% respectably) and membrane stabilizing action on human red blood cell membrane at concentration of 100, 250 and 500 mcg/ml showed 39.66%, 62.94% and 78.50% which are comparable with standard diclofenec.

Keywords – *Aquilaria agallocha*, anti-inflammatory activity, carrageenan-induced paw edema, human red blood cell membrane.

1. Introduction

Inflammation is a complex biological response of vascular tissue to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain. Prolonged inflammation leads to the rheumatoid arthritis, atherosclerosis, hay fever, ischemic heart diseases etc and inflammation is a common manifestation of infectious diseases like leprosy, tuberculosis, syphilis, asthma, inflammatory bowel syndrome, nephritis, vasculitis, celiac diseases, auto-immune diseases etc.

Anti-inflammatory drugs like NSAIDs used to reduce the swelling and pain of inflammation. But these agents carry the risk of gastro-intestinal toxicity, cardiovascular and other toxicity for prolonged use. For this reason, there is a need for anti-inflammatory drugs having less severe side effects to use for chronic inflammatory disease as well. Therefore, in recent time, more interest is shown in alternative and natural drugs for treatment of various diseases, but there is lack of scientific evidence.

Agar wood (*Aquilaria agallocha* of family Thymelaeaceae) is extremely rare and precious oil available in North Eastern India, Bhutan and parts of South East Asia. The plant has reported to possess anti nociceptive [1], anti-microbial [2], lower hypersensitivity reactions [3], laxative [4], anti oxidant activity [5], CNS activity [6], sedative effect [7] and anti-hyperglycaemic activity [8]. So, present study aimed for anti-inflammatory activity for *In-Vivo* and *In-Vitro* models.

2. Material and Methods

2.1. Collection and extraction of oil

The oil was obtained by n-hexane extraction from the woods of *Aquilaria agallocha* plants of family Thymelaeaceae. The extraction was carried at temperature 50-60 °C in Soxlet Apparatus and percentage yield of oil was 2.3 % w/w. *Aquilaria agallocha* woods were obtained from market of Hojai and identified by Dr. M. Chinna Eswaraiah,

and the voucher specimen was deposited at the Herbarium of the Department of Pharmacognosy and Phytochemistry, Anurag Pharmacy College.

2.2. Physicochemical studies of oil

The oil obtained from *Aquilaria agallocha* were tested for qualitative tests for organoleptic characters, solubility, specific gravity, refractive index, saponification value, iodine value and chemical tests for oils.

2.3. Drugs and chemicals

Diclofenac (Symed Pharm. Pvt. Ltd, Hyderabad) used as the standard anti-inflammatory drug, Carrageenan (Type-1, Sigma Chemicals) and all others laboratory reagents were obtained from the institute store and are analytical grade.

2.4. Animals

Rats of either sex weighing 150-200 g were used in experiment. Animals were obtained from Anurag Pharmacy College, Kodad. Animals were kept under standard conditions at 23-25°C 12 hr light/dark cycle and given standard pellet diet and water. Before performing the experiment the ethical clearance was obtained (177/99/CPCSEA)

2.5. Acute oral toxicity studies

Acute oral toxicity study was carried out for *Aquilaria agallocha* oil (AAO) using Acute Toxic Class Method as described in OECD (Organization of Economic Co-operation and Development) Guidelines No. 423. The *Aquilaria agallocha* oil was safe up to a dose of 2,000 mg/kg body weight.

2.6. Experimental design

The animals were divided into four groups of six animals each as follows:

Group I: Control: received 1 % aqueous solution of 2% Tween-80, p.o (1 hr before carrageenan injection)

Group II: Drug treated: received AAO 50 mg/kg, p.o (1 hr before carrageenan injection)

Group III: Drug treated: received AAO 100 mg/kg, p.o (1 hr before carrageenan injection)

Group IV: Drug treated: Diclofenac 10 mg/kg, p.o (1 hr before carrageenan injection)

2.7. In-vivo anti-inflammatory activity

Carrageenan-induced paw edema in rats- The acute hind paw edema in Wister rats (150-200 gm) was produced by Carrageenan-induced paw edema in rats [9]. 0.1 ml of carrageenan (prepared as 1% w/v suspension in saline) locally injected into sub plantar region of the left hind paw of rats. *Aquilaria agallocha* oil (AAO) (50 and 100 mg/kg, p.o. and Diclofenac 10 mg/kg, p.o.) were given orally 1 hour prior of carrageenan injection. Other group served as control in this experiment which were administered with carrageenan and vehicle. The rat paw volume up to the ankle joint was measured at 0 hr (30 min before carrageenan injection), 1 hr, 2 hr, 3 hr and 4 hr after the injection of carrageenan using plethysmometer. Increase in the paw edema volume was considered as the difference between 0 and 3 h as maximum of inflammation shown in 3 hr. Percent inhibition of paw volume between treated and control groups were calculated as follows:

$$\text{Percent inhibition} = \frac{V_c - V_t}{V_c} \times 100$$

Where V_c and V_t represent the mean increase in paw volume in control and treated groups, respectively.

2.8. In-vitro anti-inflammatory activity

In-vitro anti-inflammatory activity of AAO was performed by using human red blood cell membrane stabilization method [10-11]. The blood was collected from healthy human volunteer who was not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of extracts were prepared (100, 250, 500 mcg/ml) using distilled water

and to each concentration 1 ml of phosphate buffer, 2 ml hypo saline and 0.5 ml of Human red blood cells (HRBC) suspension were added. It is incubated at 37°C for 30 min and Centrifuged at 3,000 rpm for 20 min. the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (50, 100 and 200 mcg/ml) were used as reference standard and a control was prepared omitting the extracts.

2.9. Statistical Analysis

The data were expressed as mean \pm standard error mean (SEM). The data were analyzed by using Graph pad software Prismversion5 by one way analysis of variance (ANOVA). The test was followed by Dunnett's t -test, p values less than 0.05 were considered as significance.

3. Results

3.1. Preliminary physicochemical screening

The AAO was screened for various Physicochemical test as per the reported methods and found the oil Colour appeared as dark brown to bark yellow, Odour occurs as aromatic sweet, spicy fresh odour., specific gravity contains 0.952 at 25°C. The oil is soluble in organic solvents such as ethanol but insoluble in water. Saponification value 195, iodine value 186 and refractive index 1.52. Chemical tests also performed and confirmed as an essential oil.

Preliminary

3.2. In-vivo anti-inflammatory activity of *Aquilaria agallocha* oil (AAO)

The results of *in-vivo* anti-inflammatory activity of *Aquilaria agallocha* oil (AAO) on carrageenan induced paw edema in rats were given in Table 1.

Anti-inflammatory effect of *Aquilaria agallocha* oil was evaluated after sub plantar injection of carrageenan in rats. Sub plantar injection of carrageenan results in significant increased in paw edema after one hour in control rats as compared to normal rats which was subsequently increased up to 3 hrs. Rat treated with AAO (50mg/kg and 100mg/kg) showed significant decrease in paw edema in paw edema on 3 hr and 4hr and was comparable with standard drug Diclofenac (10 mg/kg). The percentage inhibition at 3rd hour.

Table 1. Effect of *Aquilaria agallocha* oil (AAO) on carrageenan induced paw edema in rats

Treated groups	Difference in paw edema volume (ml)							
	1 hr		2 h		3h		4h	
	PV	% RPV	PV	% RPV	PV	% RPV	PV	% RPV
Control	0.266±0.03	-	0.316±0.01	-	0.483±0.03	-	0.333±0.02	-
AAO 50mg	0.216±0.01	18.79	0.233±0.02**	26.26	0.200±0.03***	58.59	0.133±0.02***	60.06
AAO 100 mg	0.233±0.03	12.40	0.233±0.02**	26.26	0.183±0.03***	62.11	0.133±0.02***	60.06
Diclofenac 10 mg	0.200±0.03	24.81	0.200±0.0***	36.70	0.150±0.02***	68.94	0.183±0.03***	45.04

Values are in Mean \pm S.E.M (n=6); ^{ns} -Non Significant, * p <0.05, ** p <0.01, *** p <0.001 when compared with Control using One way ANOVA followed by Dunnett's multiple " t " test

PV= Paw Volume

%RPV= Percentage Reduction in Paw Volume

3.3. *In-vitro* anti-inflammatory activity of *Aquilaria agallocha* oil (AAO)

The results of *in-vitro* anti-inflammatory activity of *Aquilaria agallocha* oil (AAO) on carrageenan induced paw edema in rats were given in Table 2.

In-vitro anti-inflammatory activity of AAO was performed by using human red blood cell membrane stabilization method. AAO showed significant anti-inflammatory activity in a concentration dependent manner. AAO at concentration of 100, 250 and 500 mcg/ml showed 39.66%, 62.94% and 78.50% protection of HRBC in hypotonic solution respectively. All the results were compared with standard Diclofenac at 50, 100 and 200 mcg/ml which showed 43.74%, 63.93% and 86.73% protection of HRBC in hypotonic solution respectively.

Table 2. In-Vitro anti-inflammatory activity of *Aquilaria agallocha* oil (AAO) by HRBC method

Treatment	Conc. mcg/ml	Absorbance 560 nm	Percentage inhibition
Control	---	2.526	-
AAO	100	1.521	39.66%
AAO	250	0.936	62.94%
AAO	500	0.543	78.50%
Diclofenac	50	1.421	43.74%
Diclofenac	100	0.911	63.93%
Diclofenac	200	0.335	86.73%

4. Discussion

The edema and inflammation induced by Carrageenan is shown to be mediated by histamine and serotonin during first 1 h. After which increased vascular permeability is maintained by the release of kinins up to 2.30 h, followed by the release of kinins and finally through the release of bradykinin, prostaglandin and lysosomes from 2.30 to 6 h. The later phase is reported to be sensitive to most of the clinically effective anti-inflammatory agents. The mediators appear to be prostaglandins, the release of which is closely associated with migration of leucocytes into the inflamed site [12]. The Carrageenan induced paw edema model in rats is known to be sensitive to cyclo-oxygenase (COX) inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents [13-14]. Though AAO (50 and 100 mg/kg, p.o.) significantly reduced the paw edema in rats but the effect was of less intensity, when compared with Diclofenac (10 mg/kg, p.o.).

The *Aquilaria agallocha* oil exhibited membrane stabilization effect by inhibiting hypo tonicity induced lyses of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes [15]. Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release [16].

5. Conclusion

Aquilaria agallocha oil possesses potent anti-inflammatory activity both *in-vivo* and *in-vitro* studies which is comparable to standard Diclofenac. Since, serotonin, histamine and prostaglandins are the major mediators of inflammation, anti-inflammatory effect of *Aquilaria agallocha* oil either due to inhibition of their synthesis or inhibition of prostaglandin synthesis at third stage of inflammation. Based on the present study, it can be concluded that *Aquilaria agallocha* oil have potent anti-inflammatory activity and by further studies it can be possible to formulate natural anti-inflammatory drug of *Aquilaria agallocha*.

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