

**ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF HEARTWOOD OF  
*AQUILARIA AGALLOCHA* IN LABORATORY ANIMALS**

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**Summary**

Ethyl acetate extract of heartwood of *Aquilaria agallocha* (EAA) (Family: Thymelaceae) was tested for analgesic and anti-inflammatory activities using established methods and models reported in literature. Inhibition of the Acetic Acid induced abdominal constriction was observed at the doses of EAA 50 (34.51 %), 100 (55.65 %) and 200 (65.29 %) mg/kg as compared to the control group. EAA reduced the pain in the early (neurogenic) phase at doses of 50 (26.68 %), 100 (39.49 %) and 200 (59.87 %) mg/kg and late (inflammatory) phase at doses of 50 (21.9 %), 100 (58.1 %) and 200 (80.2 %) mg/kg of formalin induced paw licking in mice. EAA 50 (34.3 %), 100 (44.44 %) and 200 (82.68 %) mg/kg showed significant increase in latency time for thermal stimulation in tail flick test. On the anti-inflammatory front, EAA 50 (51.38 %), 100 (55.09 %), 200 (56.25 %) showed a significant decrease in edema induced by carrageenan in the third hour of the assay (edema peak) when compared to the normal control. In cotton pellet granuloma formation, EAA 50 (43.46 %), 100 (68.24 %), 200 (77.18 %) showed a significant reduction in the weight of granuloma in rats. The potential to cause ulcers by EAA (50, 100 and 200 mg/kg, p.o.) was comparatively less than that of diclofenac. In conclusion, heartwood of *Aquilaria agallocha* has analgesic as well as anti-inflammatory activities.

**Keywords:** *Aquilaria agallocha*, Analgesic, Anti-inflammatory

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## Introduction

Inflammation is a chronic disease, which involves (1) increase of vascular permeability resulting in exudation of fluid from blood into the interstitial space (2) infiltration of leucocytes from the blood into the tissues and (3) granuloma formation [1]. Currently drugs like opioid analgesics, corticosteroids and non-steroidal drugs like NSAID's and immunosuppressive agents are used to control the symptoms of inflammation and pain [2]. The use of these drugs cause unwanted effects like respiratory depression, sedation, constipation, tolerance, spasm, gastrointestinal disturbances, renal and hepatic damage, bone marrow depression, suppression of response to infection or injury, osteoporosis, development of Cushing's syndrome etc [3].

Phytomedicines offer an alternative source of therapy for inflammation treatment and also provide some information about the pathogenesis of inflammation. Also there is evidence regarding phytomedicines reducing the risk of serious toxic reactions compared to those produced by synthetic anti-inflammatory agents [4].

*Aquilaria agallocha* (Thymelaceae) Roxb, is a native evergreen plant of India, China and Tibet. It is of 20 m height and 1.5 to 2.4 m in girth. It is commonly described as eaglewood, aloe wood or agarwood. Traditionally bark, root and heartwood are used for their medicinal properties. It is reported to contain sesquiterpenes [5,6]. Agarwood is highly charged with resinous matter, and contains 48% of alcohol soluble matter. After saponification of the alcoholic extract, benzyl acetone, an unidentified ketone, molecular formula,  $C_{14}H_{20}O_2$ , a sesquiterpene alcohol, and some acids (including hydrocinnamic acid) are obtained. The sesquiterpene alcohol possesses the characteristic odor of the wood. [7]. It is reported for the treatment of anaphylactic reactions and also possesses anti-histaminic properties [8]. The plant is traditionally used to treat inflammation, arthritis, vomiting, cardiac disorders, cough, asthma, leprosy and anorexia [9]. Since the plant has been used in folk medicines in treatment of headache, inflammation, gout and arthritis [10,11], an investigation was undertaken to explore analgesic and anti-inflammatory activities of ethyl acetate extract of the heartwood of *Aquilaria agallocha*.

## Methods

### Plant material

Fresh heart-wood of *Aquilaria agallocha* obtained from commercial source in Pune, was identified and authenticated by Dr. H.B. Singh, Head, Raw materials Herbarium and Museum Division, New Delhi, India. Specimen voucher is preserved in Department of Pharmacognosy, Sinhgad College of Pharmacy, Pune.

### Preparation of extract

The heartwood were dried under shade and powdered by mechanical grinder. The powdered heartwood (100 g) was extracted with ethyl acetate (60-80°C) using Soxhlet apparatus for 72 hours. The extract was concentrated under vacuum and dried at room temperature. The extract (5.8 g) thus obtained was resinous in nature and of brownish black color. Qualitative

tests (Table 1) were performed for EAA [12]. The extract showed presence of triterpenoids, tannins, phenolic compounds and glycosides.

**Table 1. Phytochemical Tests**

Sr.No.	Chemical group	Chemical Test	Standard Result	Result for Ethyl Acetate Extract of EAA
1	Triterpenoids	Lieberman Burchard Test	Brown color	Brown color
2	Tannins and Phenolic compounds	Ferric chloride test	Dark blue color	Dark blue color
3	Glycosides	Molisch's Test	Violet ring at the juncture of two layers.	Violet ring at the juncture of two layers

### Animals

Male albino mice (20-25 g) and wister rats (170-200 g) were used in this study. Mice or rats were housed in separate cages (six per cage). All animals were kept under standard 12: 12 h light/dark cycles (lights on 07 h) in a temperature controlled ( $23 \pm 2$  °C) environment with a relative humidity of 45-55 % and *ad libitum* access to rodent chow (Amrut®, Chakkan Oil Mills, Sangli, India) and tap water. Animals were habituated to laboratory conditions for a week prior to beginning of experimental protocol to minimize, if any non-specific stress. All the protocols were approved by the Institutional Animal Ethics Committee.

### Drugs and chemicals

Carrageenan (Sigma Chemical Co., St. Louis MO, USA), acetic acid (Ranbaxy laboratories Ltd. Punjab), formalin (S.D. Fine chemicals, Mumbai, India) and Diclofenac sodium (Voveran® injection, Novartis) were used in this study. Other chemicals used for extraction purpose were of laboratory grade.

### Pharmacological experimentation

Mice or rats were divided into 5 groups consisting of 6 animals each. Group I received vehicle (2 % Tween 80 solution in water), Group II, III and IV received per oral route ethyl acetate extract of 50, 100 and 200 mg/kg respectively. Group V received diclofenac (10 mg/kg p.o.).

### Analgesic activity

#### Acetic acid induced writhing in mice

Mice were treated with 0.6 % v/v aqueous acetic acid 10 ml/kg intraperitoneally (i.p.), 1h after oral administration of extracts. The mice were placed individually in glass observation chambers and 5 minutes were allowed to elapse. The number of writhes was counted for next

30 minutes. A significant reduction in number of writhes by treatment as compared to vehicle treated animals was considered as a positive analgesic response. The percentage inhibition of writhing was calculated [13].

#### **Formalin induced paw licking in mice**

The procedure was similar to that described by Hanskaar and Hole [14] and Gorski et al., [15]. The formalin test possesses two distinctive phases, which possibly reflecting different types of pain. Mice were treated orally with EAA (50, 100 or 200 mg/kg) or 2 % Tween 80 solution in water or diclofenac solution (10 mg/kg). One h later, 20  $\mu$ l of 1 % formalin was injected subcutaneously under the dorsal surface of hind paw. Mice were observed in chambers. The number of licks in the injected paw was counted till 5 minutes (early phase) and from 20 to 30 minutes (latter phase) after formalin injection. The early phase represents neurogenic pain while latter phase is of inflammatory pain.

#### **Tail flick method in mice**

The prescreened animals (reaction time: 6-7 seconds) were divided into group I-V as described above. After oral administration of extract or vehicle or standard, the tail flick latency was assessed at 0, 1, 2 and 3 h by analgesiometer (INCO, Ambala, India). The strength of current passing through naked nichrome wire was kept constant at 4 amps. The site of application of the radiant heat in the tail was maintained at 2.5 cm, measured from the root of the tail. The cut off time was fixed 15 seconds to avoid any tissue damage [16].

#### **Anti-inflammatory activity**

##### **Carrageenan induced paw edema in rat**

Acute inflammation was produced by sub-plantar injection of 0.1 ml of 1 % w/v solution of carrageenan in normal saline, in left hind paw of rats, one hour after oral administration of drugs. Right paw served as a control. The paw volume was measured by micrometer at 0, 1, 2, and 3 h after carrageenan injection. The difference between two readings was taken as volume of edema and % inhibition of paw edema was calculated as described by Winter et al., [17].

##### **Cotton pellets induced granuloma**

After shaving the hairs on its back, rats were anesthetized with light ether and granulomatous lesions were induced by surgically implanting two cotton pellets ( $10 \pm 1$  mg) subcutaneously in the dorsal region of the rats, one near each axilla as described by Winter and Poster [18]. Ethyl acetate extract (50, 100 and 200 mg/kg), diclofenac sodium (10 mg/kg, p.o.) or vehicle (10 ml/kg body wt.) was given orally once daily for 7 days at fixed time of day. On 8<sup>th</sup> day, the rats were sacrificed by over dose of anesthetic ether, and the pellets covered by granulomatous tissue were dissected and dried to a constant weight at 50 °C for 20 h. The mean weights for different groups were determined, and compared to the control group. Ulcer scores were also calculated by examining the changes observed at the stomach, microscopically.

**Statistical analysis**

The data were expressed as mean  $\pm$  SEM. Parametric data were assessed by the method of analysis of One-way ANOVA followed by Dunnett's test. Ulcers scores were statistically tested using Kruskal- Wallis test followed by Dunn's test.  $P < 0.05$  was considered as statistically significant.

**Results****Analgesic activity**

ANOVA revealed that pretreatment with EAA (50, 100 and 200 mg/kg) showed significant ( $p < 0.05$ ) reduction in number of writhes in dose dependent manner (Table 2). EAA at doses of 50, 100 and 200 mg/kg caused a significant inhibition of the neurogenic (early phase) and inflammatory (late phase) phases of formalin induced licking in mice (Table 3). EAA showed significant increase in latency time for thermal stimulation in tail flick test (Figure 1 ). The standard drug, diclofenac sodium (10 mg/kg) also significantly inhibited all the responses evoked by the noxious stimuli except early phase of formalin induced paw licking.

**Table 2. Analgesic effect of EAA in writhing induced by acetic acid in mice**

Treatment	Dose mg/kg, <i>p.o.</i>	Number of writhes	% Inhibition
Control (2 % Tween 80)	10 ml/kg	63.88 $\pm$ 3.82	
EAA	50	41.83 $\pm$ 3.77**	34.47
	100	28.33 $\pm$ 3.06***	55.61
	200	22.17 $\pm$ 2.12***	65.27
Diclofenac sodium	10	18.50 $\pm$ 1.54***	71.03

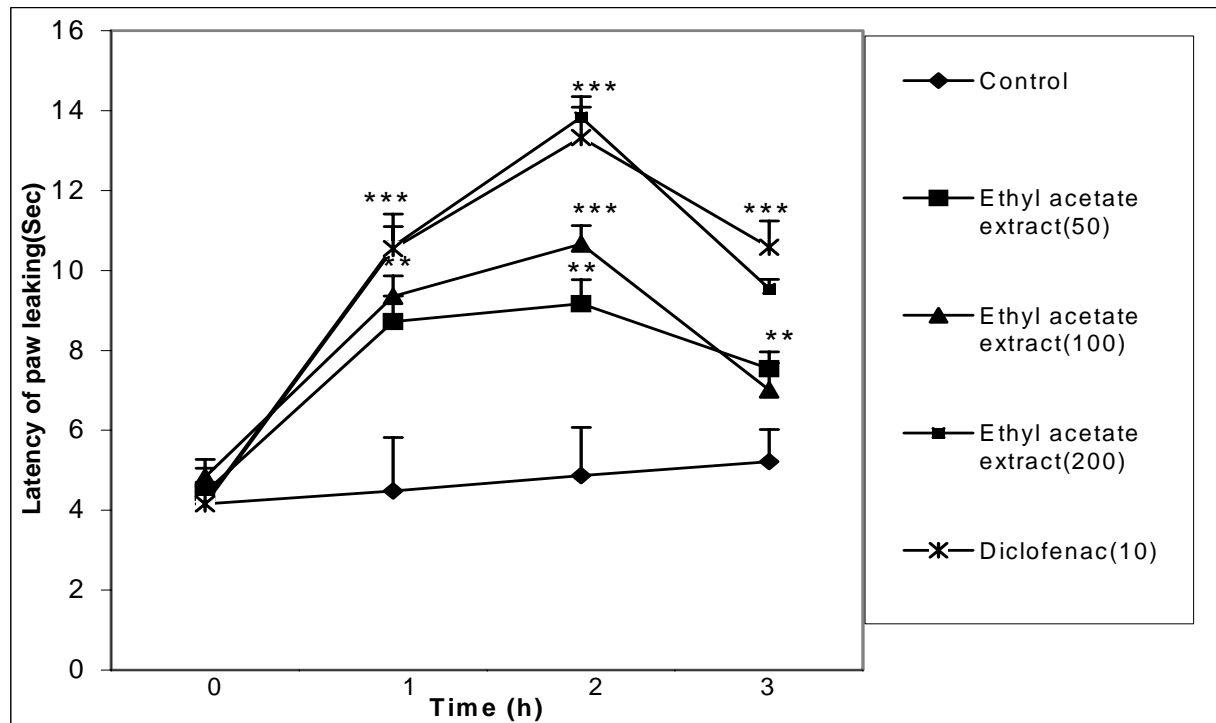
The results are mean  $\pm$  SEM obtained from 6 animals; \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ; compared to vehicle control.

**Table 3. Analgesic effect of EAA on formalin induced pain in mice**

Treatment	Dose mg/kg, <i>p.o.</i>	Total time in spent in licking (s)			
		0 –10 min	% Inhibition	15–30 min	% Inhibition
Control (2 % Tween 80)	10 ml/kg	79.3 $\pm$ 5.4		113.3 $\pm$ 5.6	
EAA	50	58.2 $\pm$ 4.4	26.68	88.5 $\pm$ 5.2*	21.9
	100	48.0 $\pm$ 4.8**	39.49	47.5 $\pm$ 4.5**	58.1
	200	31.8 $\pm$ 4.1***	59.87	22.5 $\pm$ 1.5***	80.2
Diclofenac sodium	10	73.3 $\pm$ 6.8	7.56	28.8 $\pm$ 2.6***	74.6

The results are mean  $\pm$  SEM obtained from 6 animals; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ; compared to vehicle control.

Figure 1: Effect of EAA (50, 100 and 200 mg/kg, p.o.) on tail flick test in mice

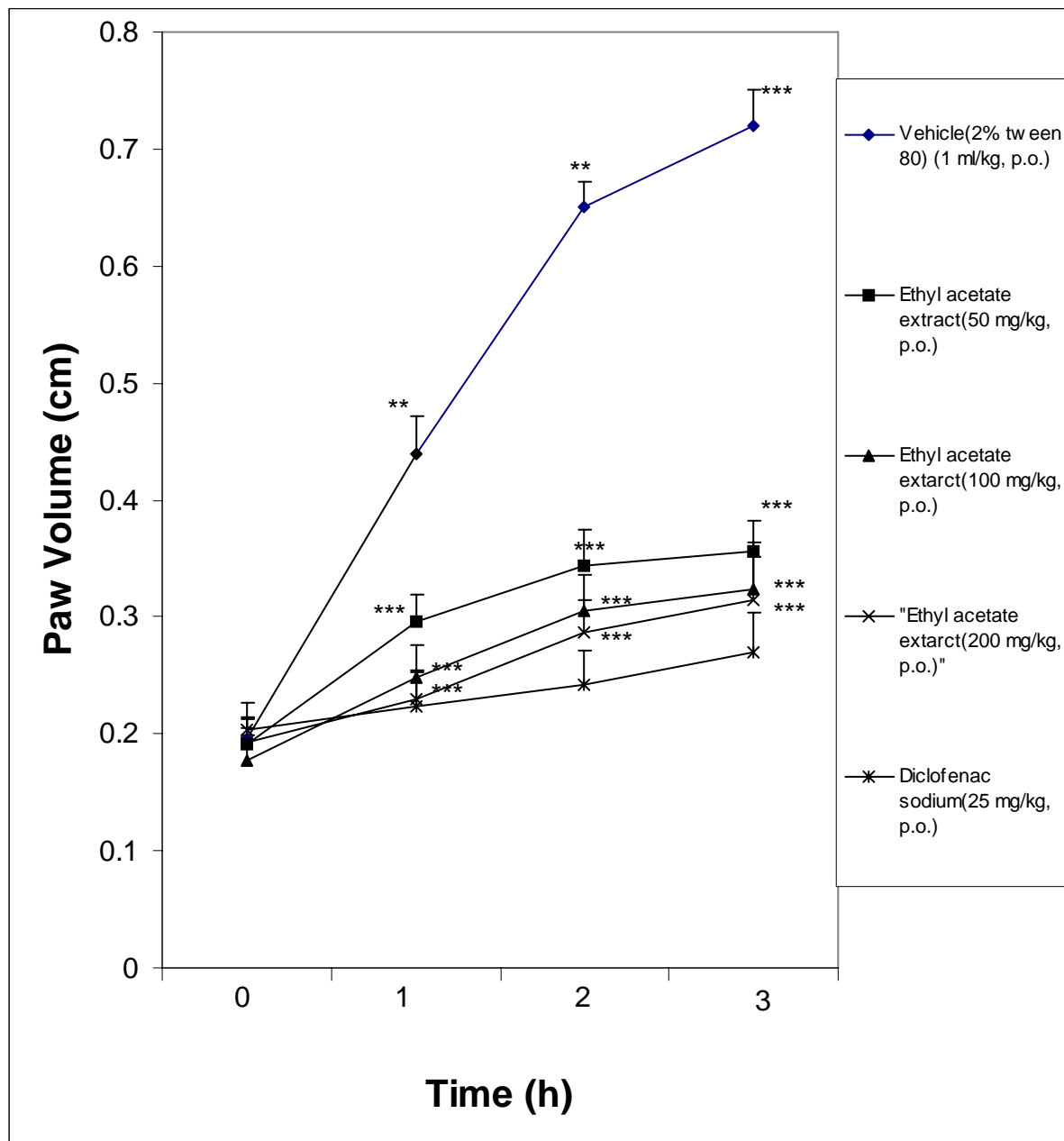


The results are mean  $\pm$  SEM obtained from 6 animals; \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ; compared to vehicle control.

### Anti-inflammatory activity

As shown in Figure 2 after injection, carrageenan induced edema in rats (control group). Pretreatment with EAA (50 mg/kg, 100 mg/kg and 200 mg/kg, p.o.) significantly reduced edema at 1, 2 and 3 hr after carrageenan injection. The reduction in edema produced by EAA (800 mg/kg, p.o.) is similar to that of diclofenac (10 mg/kg, p.o.). In cotton pellet granuloma model, there was a statistically significant reduction in the dry weight of granuloma in EAA (100 and 200 mg/kg, p.o.) as well as diclofenac sodium (10 mg/kg, p.o.) treated rats as compared to control group. EAA treated animals showed reduced ulcer scores as compared to diclofenac treated group (Table 4). The potential to cause ulcers by EAA (50, 100 and 200 mg/kg, p.o.) was comparatively less than that of diclofenac.

Figure 2: Effect of EAA (50, 100 and 200 mg/kg) carrageenan edema



Each data mg/kg, p.o.) in represents mean  $\pm$  SEM from 6 animals. \*\* $p < 0.01$ , \*\*\*  $p < 0.001$ ; compared to vehicle control.

Table 4. Effect of EAA on cotton pellet granuloma

Treatment	Dose mg/kg, p.o.	% Increase in granuloma weight (mg/100g)	% Inhibition	Ulcer scores
Control	10 ml/kg	27.37 ± 2.79		No ulcer
Ethyl acetate extract	50	15.48 ± 1.57**	43.46	0.3 ± 0.2*
	100	8.69 ± 1.35***	68.24	1.7 ± 0.98*
	200	6.25 ± 1.19***	77.18	2.2 ± 0.77*
Diclofenac sodium	10	5.23 ± 0.69***	80.87	6.4 ± 0.97**

The results are mean ± SEM obtained from 6 animals; \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ ; compared to vehicle control.

### Discussion

*Aquilaria agallocha* is used to treat inflammation in folklore medicine. The purpose of the study was to establish scientific evidences for the usage of this plant in inflammatory conditions. The EAA was studied for its modulatory effects on pain and inflammation induced by chemical and thermal stimuli.

The analgesic effect of EAA was tested in three different models of analgesia: the acetic induced writhing test, formalin induced paw licking model and tail flick model in mice. In our study it was found that EAA treatment had reduced the intensity of acetic acid induced abdominal constriction in mice. Acetic acid causes an increase in peritoneal fluid level of prostaglandins involving in part peritoneal receptor [19] and inflammatory pain by capillary action [20]. Although the test is nonspecific model (e.g. Anticholinergic and antihistaminic and other agents show activity in this test). It is widely used for analgesic screening and predominately involves induction of prostaglandins. The mechanism of analgesic effect of EAA is probably due to a blockade of capillary permeability or release of endogenous substances like prostaglandins.



Formalin test has advantage that it involves biphasic pain, with an early pain representing neurogenic and late phase of that of inflammatory reaction [14]. The drugs that affect the central nervous system for pain modulation pain produced by formalin [14]. In the present study EAA was more efficacious in inhibiting neurogenic pain (early phase), while standard drug did not inhibit the neurogenic exhibited comparable potency. Inhibition of both these phases in formalin induced licking by EAA.

Drugs that act centrally inhibit pain produced by thermal stimuli [21]. The EAA produced anti-nociceptive effect against thermal induced pain stimuli in mice in tail flick method at various points. In the present study, diclofenac also inhibited the pain produced by tail flick method. Although, this model is specific for centrally inhibited pain there are evidences that support that NSAID's also inhibit the pain induced by thermal stimuli [22, 23]. The observations from writhing test; formalin test and tail flick model suggests that EAA inhibited the pain induced by chemical and thermal stimuli.

In addition, to the analgesic models the EAA was investigated in acute and subacute inflammatory models for their anti-inflammatory activity. Acute inflammation in rats was induced by sub-plantar injection of carrageenan (phlogistic agent). Various mediators are released by carrageenan in the rat paw. Mediators like histamine and serotonin (initial phase); kinins (middle phase) and prostaglandin's (final phase after 3 to 5 h of carrageenan injection) play an important role in the development of inflammation [24, 25]. Figure 1 showed that EAA inhibited all the three phases of edema equally suggesting that the extract has nonselective inhibitory effect on release of these endogenous mediators.

Cotton pellet granuloma is a chronic model of inflammation, which is widely used to evaluate the proliferative components of the inflammations. The dry weight of the pellets correlates with the amount of granulomatous tissue [26]. EAA (200 mg/kg) appears to be equally effective to that of diclofenac (10 mg/kg) in inhibiting the dry weight of the cotton pellet. These results suggest that EAA is effective in inhibiting the granuloma formation.

Mucosal erosion and ulceration are produced by most NSAID'S of varying degree. Inhibition of synthesis of gastric protective prostaglandin's ( $PGE_2$  and  $PGI_2$ ) is clearly involved [27, 28]. To assess the mucosal erosion and ulceration produced the animals that underwent sub chronic therapy with the drug are autopsied and the stomachs are examined for mucosal injury and ulcer formation. Treatment with EAA increased the ulcer scores dose dependently indicating that the drug possesses prostaglandin inhibitory activity. Further, it also suggests that the risk of production of ulcer increases as the dose increase with little change in the anti-inflammatory activity. However, the damage produced to gastric mucosa by EAA is comparatively less than diclofenac at dose showing similar anti-inflammatory activity.

Thus the study confirms analgesic and anti-inflammatory mechanism of the EAA. In addition, this study also highlights optimization of dose of this extract to avoid the side effects. Further studies need to be done to identify and characterize the active constituents responsible for analgesic and anti-inflammatory activity from ethyl acetate extract of *Aquilaria agallocha*.

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