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ANTI-INFLAMMATORY AND ANALGESIC ACTIVITIES OF LIGUSTRUM ROBUSTUM IN EXPERIMENTAL ANIMAL MODELS

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ABSTRACT

Ligustrum robustum is an indigenous medicinal shrub that belongs to the family, Oleaceae. It is a major component of Chinese tea, Ku-Ding-Cha, which is used to prevent common cold, rhinitis, itching eyes, and headache. The present study was undertaken to evaluate the anti-inflammatory and analgesic activities of the aqueous extract of *Ligustrum robustum* (ALR) in experimental animal models. ALR was evaluated for anti-inflammatory action by carrageenan induced rat paw edema. The analgesic activity was assessed by tail flick response in albino rats and writhing response in albino mice. ALR in dose of 200 mg/kg showed 39% inhibition of paw edema. In the tail flick model, ALR increased the pain threshold significantly after 30 min and 1h of administration. The percentage protection of writhing with ALR was 46.9% which indicates that ALR has significant anti-inflammatory and analgesic properties.

KEY WORDS

Ligustrum robustum, anti-inflammatory, analgesic, carrageenan, tail flick, writhing

INTRODUCTION

Ligustrum robustum is an indigenous medicinal shrub that belongs to the family Oleaceae. It grows throughout the world[1]. It is traditionally used as local medicine in European, Chinese, and Japanese communities for centuries. Various species of the plant are commonly used by traditional Chinese physicians to cure hepatitis and ageing-associated symptoms. Recent pharmacological studies indicate that *Ligustrum* species possess anti-oxidative, anti-mutagenic, hepatoprotective, and neuroprotective activities[2]. *Ligustrum robustum* is a major component of Chinese tea, Ku-Ding-Cha, which is used to prevent common cold, rhinitis, itching eyes, and headache[3]. However, there are no scientific studies to validate the folklore claim of using *Ligustrum robustum* in the above conditions. Therefore, this study was undertaken to evaluate the a) anti-inflammatory potential of the aqueous extract of *Ligustrum robustum* (ALR) on carrageenan-induced rat paw edema and b) analgesic activity using tail flick response in albino rats and acetic acid- induced writhing test in albino mice.

MATERIALS AND METHODS

Preparation of the test drug: Fresh aerial parts of *Ligustrum robustum* were collected, identified and authenticated. The plant parts were cleaned, dried under shade and powdered by a mechanical grinder. Sixty grams of the pulverized plant was extracted with distilled water using a soxhlet apparatus. The yield was 11.5% in powder form. The extract was administered as a suspension in 2% gum acacia to the animals.

Phytochemical studies: Freshly prepared ALR extract was subjected to phytochemical screening tests for the detection of various constituents using conventional protocol[4].

Chemicals: The following chemicals and drugs were used: carrageenan (Sigma-Aldrich), aspirin (Vikash Pharma, Mumbai), pethidine (Bengal Immunity, Kolkata), acetic acid (Ranbaxy Laboratories Ltd., Punjab).

Instruments*:* Analgesiometer, Plethysmometer, Stop watch.

Animals*:* Albino Wistar rats weighing between 150 to 200 g were procured from the central animal house of the institute. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 20C; relative humidity 60-70%) in a 12 h light-dark cycle. The rats were given standard laboratory diet and water ad libitum. Food was withdrawn 12 h before and during the experimental hours. All experimental protocols were approved by the institutional animal ethics committee.

Acute toxicity study: No adverse effect or mortality was detected in albino rats up to 3 gm/kg, p.o. of ALR during the 24 h observation period.

Anti-inflammatory study*:* The animals were divided into groups as shown in Table 1. Acute inflammation was produced by sub-plantar injection of 0.1 ml of 1% suspension of carrageenan with 2% gum acacia in normal saline, in the right hind paw of the rats, one hour after oral administration of the drugs. The paw

volume was measured plethysmometrically (Ugo Basile, Italy) at '0' and '3' hours after the carrageenan injection. The difference between the two readings was taken as the volume of edema and the percentage anti-inflammatory activity was calculated[5]. Aspirin 100 mg/kg, p.o. suspended in 2% gum acacia was used as the standard drug.

Analgesic study (Tail flick method)*:* The prescreened animals (reaction time: 3-4 sec) were divided into groups as shown in Table 2. Pethidine 5 mg/kg acted as the standard drug. The drugs were administered intra-peritoneally. The tail flick latency was assessed by the analgesiometer (Inco, India). The strength of the current passing through the naked nicrome wire was kept constant at 6 Amps. The distance between the heat source and the tail skin was 1.5 cm. 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 reaction time was fixed at 10 sec to avoid tissue damage[6, 7].

Analgesic study (Acetic acid induced writhing test): The prescreened animals were divided into groups as shown in Table 3. Aspirin, in dose of 100 mg/kg, suspended in 2% gum acacia was used as the standard drug. The drugs were autoclaved at 121˚C for 30 min and administered subcutaneously. Writhing was induced 30 min later by intra-peritoneal injection of 10ml/kg of 0.6% acetic acid in distilled water. The number of writhes was counted for 30 min immediately after the acetic acid injection. The percentage protection was calculated[8, 9].

Statistical analysis*:* The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test. A P value of <0.05 was considered significant.

RESULTS

The results of the animal experiments are shown in Tables 1, 2 and 3.

Anti-inflammatory study: The result of the effect of ALR on carrageenan-induced rat paw edema is shown in Table 1. In acute inflammation model, ALR in dose of 200 mg/kg, p.o. and aspirin in dose of 100 mg/kg produced significant inhibition of paw edema as compared to the control. The inhibition was, however, less than that of the standard drug, aspirin.

Table 1
Effects of ALR on carrageenan-induced rat paw edema

Group	Dose (mg/kg, p.o.)	Increase in paw volume (mean ± SEM) in ml	% inhibition of paw edema
Normal saline	10 ml	0.52 ± 0.08	–
ALR	200	0.32 ± 0.05	39*
Aspirin	100	0.22 ± 0.01	58**

(n=6; One-way ANOVA: df, 2,15: *p<0.02; **p,0.001 when compared to control)

Tail flick test*:* In the tail flick model, there was no significant difference in the mean predrug reaction time between the different groups. Thirty min after drug administration, reaction time increased significantly for the test and standard groups when compared to the predrug reaction time. ALR and Aspirin produced a significant increase in the reaction time after 30 min and one hr time intervals of observation as shown in Table 2.

Table 2
Analgesic activity of ALR on tail flick response in albino rats

Group	Dose (mg/kg, p.o.)	Pre drug reaction time	Reaction time after 30 minutes	Reaction time after 1 hour
Normal saline	10 ml	3.3 ±0.21	4.25±0.38	4.16±0.27
ALR	200	3.5 ± 0.22	8.0±0.77*	8.60±0.71**
Pethidine	5	3.8±0.17	9.16±o.54**	9.33±0.27**

(n=6; One-way ANOVA: df, 2,15 ; *p<0.01;*P<0.001 when compared to control)

Acetic acid induced writhing test: ALR significantly suppressed the acetic acid induced writhing when compared to control. However, Aspirin produced increased percentage inhibition of writhing movements as shown in Table 3.

Table 3
Analgesic activity of ALR on writhing response in albino mice

Group	Dose (mg/kg, p.o.)	Number of writhing movements	% of protection
Normal saline	8 ml	80.30±0.95	
ALR	200	40.20±7.48	46.9**
Aspirin	100	16.33±6.70	79.7**

(***n=6; One-way ANOVA: df, 2,15 ; **P<0.001 when compared to control)***

DISCUSSION

Carrageenan-induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effects. Moreover, the experimental model exhibits a high degree of reproducibility[5]. Carrageenan-induced edema is a biphasic response. The first phase is mediated through the release of histamine, serotonin and kinins whereas the second phase is related to the release of prostaglandin and slow reacting substances which peak at 3 h[10]. The increase in the paw volume following carrageenan administration in the control (0.52 ± 0.08 ml) and aspirin treated group (0.22 ± 0.01 ml) corresponds with the findings of previous workers[11, 12]. ALR extract produced significant inhibition of carrageenan-induced paw edema. The inhibition was, however, less than that of the standard drug, aspirin.

In the tail flick model, the test drug increased the pain threshold significantly during the period of observation and this indicates the involvement of a higher center. The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. The response involves the local peritoneal receptors. The number of writhing movement during 30 min observation process in the control group was 80.30±0.95 which corresponds to the findings of previous workers[13, 14]. ALR produced significant inhibition of writhing response which was, however, less than that of the standard drug, aspirin. The results of the present study suggest that ALR in dose of 200 mg/kg significantly suppressed carrageenan-induced paw edema in rats and demonstrated significant analgesic activity in the tail flick and writhing model.

On preliminary phytochemical screening ALR was found to contain flavonoid compounds. Flavonoids are known to target prostaglandins which are involved in the late phase of acute

inflammation and pain perception[15]. Hence, the presence of flavonoids may be contributory to the anti-inflammatory and analgesic activities of ALR. Further studies with multiple doses and purified extracts may reveal the exact mechanisms of action responsible for the analgesic and anti-inflammatory activities of ALR.

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