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# Study on Analgesic property of Ligustrum Robustum leaves

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**Abstract:** The practices of using medicinal plants incorporated ancient beliefs and were passed on from one generation to another. Although effective in the treatment of various ailments, very often these drugs are unscientifically exploited and/or improperly used. This study aimed to evaluate the analgesic property of the aqueous extract of the leaves of Ligustrum robustum. The aqueous extract of Ligustrum robustum was obtained by the methods of Rao SK and Mishra SH (1997) and Khosla P *et al*., (2000) with slight modifications. Healthy albino mice weighing between 25-30gm were obtained for the study. The animals were divided into five groups with six animals in each group. The analgesic activity of the aqueous extract of Ligustrum robustum was tested on albino mice by the following methods: a) Acetic acid induced writhing method in mice as described by Witkin LB *et al*., 1961. b) Tail flick test in albino rats as described by Sheth UK et al., 1972. The results were compared with those of control, test & standard drugs for statistical significance by one-way ANOVA followed by Dunnett's't' test. The test drug (Ligustrum robustum) in concentrations of 100mg/kg, 200mg/kg and 400mg/kg produced 6.42% ($p>0.05$), 22.1% ($p<0.05$) and 34.03% ($p<0.001$) protection from writhing respectively. In the tail flick, the test drug (Ligustrum robustum) in concentrations of 100mg/kg, 200mg/kg and 400mg/kg increased the pain threshold significantly ($p<0.05$ to 0.01) after 30min, 1hr, 2hr and 3hr of administration. Aqueous Ligustrum robustum increased the pain threshold significantly on acetic acid induced writhing model, also showed to possess significant analgesic activity in the tail flick method by analgesiometer using albino mice.
**Keywords**: Ligustrum robustum, Analgesic property, Albino mice.

## INTRODUCTION

Good medicines prescribed by doctors will not cure the disease if the patient cannot afford them. If infections can be treated by easily available herbal medicines, it will be a boon to the economy of the general population. Drugs have been the most widely used form of therapeutic intervention available to physicians for many years. Reliance on natural products, mainly from plants, predominated until 1920s, when synthetic chemicals were first introduced and modern pharmaceutical industry began to develop [1].

The World Health Organization lays emphasis on the essentiality of drugs with low cost and easy availability [2]. But modern drugs or conventional medicines are often viewed as impersonal, emphasizing crisis intervention. It is not only expensive but many of them bring about side effects which are sometimes more dangerous than the disease itself [3].

Ligustrum robustum (Manipuri – Oo-yaanggun), is a common medium sized tree found abundantly in Manipur. The indigenous doctors (Maaiba) of Manipur use this plant for treatment of various ailments. Juice obtained from crushed leaves is applied to inflammatory gland swelling, skin infections and the leaves are boiled and watery extract is given orally for treatment of gouty arthritis.

About 50 species of Ligustrum trees or shrubs are distributed in Europe, Asia and Australia; about 16 species in India[4]. Common species known to us are Ligustrum compactum, Ligustrum nepalense, Ligustrum robustum [5], Lisustrum vulgaris, Ligustrum ovalifolium[6]. Ligustrum robustum is found abundantly in Manipur, also found scattered throughout the state of Tripura and distributed from Assam to Bangladesh, Myanmar & Malaysia [4].

Thorough pharmacological experiments on various plants used in traditional medicines are in progress in order to establish their effectiveness and safety. Keeping in view the above idea, the present study is undertaken on the plant Ligustrum robustum to explore its analgesic property in various animal experimental models.

**AIM OF THE STUDY**

This study aimed to evaluate the analgesic property of the aqueous extract of the leaves of Ligustrum robustum on Albino mice.

**MATERIALS & METHODS**

The leaves of Ligustrum robustum were collected from different parts of Imphal, during the months of June and July. The aqueous extract of Ligustrum robustum was obtained by the methods of Rao SK and Mishra SH[7] and Khosla P *et al*. [8], with slight modifications. 50gm of powdered leaves were extracted with soxhlet apparatus with 500ml of distilled water till the eluent was colorless. The water extract was evaporated, scraped out, weighed and stored in a glazed porcelain jar for use. The yield was 18.83% and percentage of solubility of the extract was 0.6%.

After obtaining ethical clearance from Animal Institutional Ethical Committee, The analgesic activity of the aqueous extract of Ligustrum robustum was tested on albino mice by the following methods: a) Acetic acid induced writhing method in mice as described by Witkin LB *et al*. [9], b) Tail flick test in albino rats as described by Sheth UK *et al*.[10].

- Acetic acid induced writhing test: - Healthy albino mice weighing between 25-30 gm were obtained from the Central Animal House, R.I.M.S., Imphal, and used for the study. The animals were screened beforehand. Animals which failed to exhibit writhing within 10min were discarded. The pre-screened animals were fasted overnight but allowed free access to water during the experiment. The albino mice were divided into five groups with six animals in each group.

| Group | Drug |
|---|---|
| Con trol | Distilled water |
| Test A | *L. Robustum (100mg/kg)* |
| Test B | L. Robustum (200mg/kg) |
| Test C | *L. Robustum (400mg/kg)* |
| Standard | *Aspirin (100mg/kg)* |

The drugs were suspended in distilled water using 2% gum acacia and administered orally. The volume of medicaments was kept constant at 10ml/kg body weight of the animals.

Writhing was induced 20 minutes later in each mouse by intraperitoneal injection of 10ml/kg body weight of 3% acetic acid in distilled water. The number of writhes was counted for 30 minutes. The data were subjected to statiscal analysis using one way "ANOVA" followed by Dunnetts' 't' test for significant difference between different groups.

The percentage of protection at each dose-level is calculated as follows for each group of 6 mice:

$$\% \text{ Protection} = 100 - \{\frac{\text{Experimental}}{\text{Control}} \times 100\}$$

- Tail flick test:- Healthy young albino rats of either sex weighing between 100-150gm were obtained from the central animal house R.I.M.S., Imphal with normal reaction time of 4-5 seconds were used for the study. The animals were fasted overnight and had water ad libitum. They were divided into five groups with six animals in each group.

| Group | Drug |
|---|---|
| Con trol | Distilled water |
| Test A | *L. Robustum (100mg/kg)* |
| Test B | L. Robustum (200mg/kg) |
| Test C | *L. Robustum (400mg/kg)* |
| Standard | *Aspirin (100mg/kg)* |

The drugs were suspended in 2% gum acacia and administered orally. The volumes of all the medicaments were kept constant at 10ml/kg body weight of the animals.

The tail flick latencies (reaction time) of the animals were 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 sources and tail skin was 1.5cm. The site of application of the radiant heat in the tail had been maintained at 2.5cm, measured from the root of the tail for all rats. The time taken by the animals to withdraw (flick) their tail from the hot wire were noted and taken as the 'reaction time'. The cut off reaction time was fixed at 10 sec. To avoid tissue damage. The reaction time is recorded from 30min, 1hr, 2hr and 4hr.

The results of the test drug groups were compared with other groups and analyzed for statistical significance using one way 'ANOVA' followed by Dunnett's 't' test.

## RESULTS & TABLES

Acetic acid induced writhing test:- Table I shows that analgesic effect of the aqueous extract of *Ligustrum robustum* on acetic acid induced writhing test in albino mice. The analgesic activity was determined by observing the number of writhes in the various treated groups.

**Table-I: Analgesic activity of the aqueous extract of Ligustrum robustum on acetic acid induced writhing response in albino mice**

| Group | Drug Dose (mg/kg), p.o. | No. Of writhing movement (mean ±SEM) | Percentage of protection |
|---|---|---|---|
| Control | 10ml/kg | 70.16 ± 2.77 | - |
| Test A | 100 | 65.66 ± 3.05 | 6.42% |
| Test B | 200 | 54.66 ± 2.13* | 22.1% |
| Test C | 400 | 46.16 ± 3.42$^{\psi}$ | 34.03% |
| Aspirin | 100 | 40.50 ± 2.60$^{\phi}$ | 42.28% |
| One-way ANOVA | F(df)<br>*P* | 15.22 (5,30)<br><0.0001 | |

n= 6 in each group. *p <0.05; ψp<0.001; ϕp<0.0001;

The mean number of writhing movements with the control drug was 70.16 ± 2.77, Test A (100mg/kg) 65.66 ± 3.05, Test B (200mg/kg) 54.66 ± 2.13 (p<0.01), Test C (200mg/kg) 46.16 ± 3.42 (p<0.001), Aspirin (50mg/kg) 48.16 ± 3.68 (p<0.01); Aspirin (100mg/kg) 40.50 ± 2.60 (p<0.0001) respectively.

The test drug in concentration of 100mg/kg, 200mg/kg produced 42.28% inhibition of writing movements. The number of writhing was significantly reduced in both the test and standard group in comparison to control group.

- Tail flick response:- Table II shows the effect of the aqueous extract of *Ligustrum robustum* leaves on the tail flick response in albino rats. There was no significant difference between the mean pre-drug reaction time of the different groups (p>0.05). After 30 minutes of the administration of the various drugs, there was significant increase in reaction time for all the groups when compared to the pre drug reaction time.

**Table-II: Analgesic activity of the aqueous extract of *Ligustrum robustum* on tail flick response in albino rats**

| Group | Drug dose | Pre-drug reaction time | Reaction time in seconds (Mean ± SEM) | | | |
|---|---|---|---|---|---|---|
| | Mg/kg, p.o. | in sec. (Mean ± SEM) | 30min | 1hr | 2hr | 4hr |
| Control | 10ml/kg | 4.60±0.21 | 5.0±0.25 | 4.35±0.21 | 4.50±0.34 | 4.50±0.22 |
| Test A | 100mg | 4.83±0.16 | 5.5±0.2 | 6.34±0.21* | 6.00±0.36 | 6.16±0.30* |
| Test B | 200mg | 4.80± 0.16 | 6.0± 0.26 | 6.84±0.30Δ | 7.17±0.30$^{\Delta}$ | 6.84±0.30$^{\Delta}$ |
| Test C | 400mg | 4.60±0.21 | 5.3±0.21 | 6.17±0.47* | 6.67±0.21* | 6.84±0.30$^{\Delta}$ |
| Standard | 100mg | 5.00±0 | 6.33±2.10* | 6.70±0.51$^{\Delta}$ | 7.34±0.42$^{\Delta}$ | 7.34±0.33$^{\Delta}$ |
| One-way F(df) | | 0.692(4,25) | 5.183(4,25) | 11.08(4,25) | 11.63(4,25) | 14.91(5,25) |
| ANOVA | | *P* >0.05 | <0.01 | <0.0001 | <0.0001 | <0.0001 |

n= 6 in each group; **P* <0.05, $^{\Delta}$*P* <0.01, when compared to control at that hour
Note: Cut off time of the animals was fixed at 60 seconds.

The mean reaction time in seconds after 30min, 1st hour, 2nd hour, and 4th hour of drug administration for test drug (100mg/kg) was 5.5±0.22, 6.34±0.21 ($p<0.05$), 6.00±0.36, 6.16±0.30 ($p<0.05$).

The increase in mean reaction time in seconds with the test drug (200mg/kg) were 6.0±0.26, 6.84±0.30 ($p<0.01$), 7.17±0.30 ($p<0.01$), 7.17±0.30 ($p<0.01$) at 30 min, 1st hr, 2nd hr, and 4th hr respectively.

With the test drug (400mg/kg) the mean reaction time in seconds after drug administration at 30min, 1st, 2nd, and 4th hours were 5.3±0.21, 6.17±0.47($p<0.01$), 6.67±0.21($p<0.05$) and 6.84±0.30 ($p<0.01$) respectively.

For standard drug aspirin (100mg/kg) the mean reaction time were found to be increased by – after 30 min: 6.33±2.10($p<0.01$), 1hr: 6.70±0.51 ($p<0.01$), 2hr: 7.34±0.42 ($p<0.01$) and 4hr: 7.34±0.33 ($p<0.001$) respectively when compared to control at the respective hour. The degrees of significance are enclosed within parenthesis.

## DISCUSSION

Medicinal herbs are an indispensable part of traditional medicine practice all over the world due to low cost, easy excess and ancestral experiences[11]. Pain has been defined as "unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage[12]".

In the acetic acid induced writhing method, the number of writhes was counted for 30 minutes. The number of writhing movements during 30min observation in the control group was 2.33 per minute (70.16±2.77) which almost corresponds to the findings of Hajare SW *et al*. [13] and Effraim KD *et al*. [14] as 2.55 and 2.46 per minute respectively. The test drug in concentrations of 100mg/kg, 200mg/kg and 400mg/kg produced 6.42%, 22.1%, 30.03% protection from writhing respectively.

The standard drug, aspirin in concentrations of 100mg/kg produced 42.28% protection from writhing. The results obtained with the test and standard drugs were significant when compared to the control. The test drug was however, found to be less effective than the standard drug in increasing the pain threshold.

In the tail flick model, an analgesiometer was used. The animals which responded to the heat stimulus within 5sec were used for the study. It has been observed that the aqueous extract of Ligustrum robustum in concentrations of 100mg/kg, 200mg/kg and 400mg/kg increased the pain threshold significantly at 1hr, 2hr and 4hr of administration when compared to the control group at that particular hour. The standard drug, aspirin increased the pain threshold significantly. For the control group, the mean reaction time was maintained throughout the study.

Thus the aqueous extract of Ligustrum robustum on the models of writhing and tail-flick methods showed significant analgesic properties. The abdominal constriction response induced by acetic acid is a very sensitive procedure. This response is thought to involve, in part, local peritoneal receptors Bentley GA *et al.* [15]. Since prostaglandins are involved in the pain perception and are inhibited by flavonoids, it could be suggested that the reduced availability of prostaglandins caused by flavonoids of Ligustrum robustum might be responsible for its analgesic effect.

## CONCLUSION

Aqueous Ligustrum robustum increased the pain threshold significantly on acetic acid induced writhing model, also showed to possess significant analgesic activity in the tail flick method by analgesiometer using albino mice.

Our study reveals that the aqueous extract of Ligustrum robustum has significant analgesic effect concluded by above findings.

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