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Investigation of Anti-inflammatory and Analgesic Properties of Methanolic Extract of *Lophopetalum javanicum* Leaves

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ABSTRACT

We aimed to investigate and evaluate the anti-inflammatory and analgesic effectiveness of common tree in the locality of Bangladesh, *Lophopetalum javanicum*. Our study includes; the investigation of the phytochemical composition and *in-vivo* analgesic and anti-inflammatory activity of Methanolic Extract of *Lophopetalum javanicum* (MELJ). On that point are four methods we applied to investigate the effect, including; Formalin induced paw edema and Carrageenan induced paw edema for anti-inflammatory assay and to access analgesic effect we used the Hot plate method and Tail immersion method. At the dose of 500 mg kg⁻¹ the extract shows the considerable inhibitory effect on paw increase 1 h after carrageenan administration, by inhibiting over 50%. The maximum inhibition (52.27±0.017%; p<0.0001) elicited by the methanolic extract at 3 h after carrageenan injection. In the assay of hotplate method, *L. javanicum* increases pain tolerance, time up to 17.83±0.085 min, where the compared standard lengthened response time to 21.68±0.427 min. In tail immersion method the response time was elongated at 600 mg kg⁻¹ by *L. javanicum* 4.49±0.214 (p<0.0001) after 2 h of extract induction. This study reflects that the plant is effective in inhibiting pain mediators, thus can be developed as analgesic and anti-inflammatory agent through proper isolation and adjustment.

Key words: Analgesic, anti-inflammatory, carrageenan, Eddy's hot plate method, inflammation, *L. javanicum*

INTRODUCTION

Plants have provided humans with many of their essential needs, including life-saving pharmaceutical agents. Recently the World Health Organization estimated that 80% people worldwide rely on herbal medicines for some facial expression. There are more than 270,000 higher plants existing on this planet but exclusively a few has been explored scientifically for pharmacological use. Thus, it is anticipated that plants can provide potential bioactive compounds for the

development of new 'leads' to combat various diseases. *Lophopetalum javanicum* is the most common herb in Bangladesh and we investigate this plant as part of the search for good anti-inflammatory and analgesic medicinal plant. Besides its local and traditional use, it has also been studied for *in-vitro* anti-inflammatory property (Reyad-ul-Ferdous *et al.*, 2014) in recent years.

The inflammatory response by biological system requires many complex arrays of enzyme activation, mediator release, fluid extravasations, cell migration and tissue breakdown

and repairs all these issues are aimed at host defense and usually activated in most disease conditions (Vadivu and Lakshmi, 2008) including; heart attack and Alzheimer's disease (McGeer and McGeer, 2002; Woodward *et al.*, 2005; Howes and Houghton, 2003) and cancer (Mueller, 2006). This response towards harmful pathogen or stimuli or irritants, often characterized by pain, swelling, redness temperature rising (Palladino *et al.*, 2003; Ferrero-Miliani *et al.*, 2007). Several phenomena alter the antigenicity of endogenous proteins, including; protein denaturation and glycosylation. Protein denaturation may occur during chronic inflammatory phenomena *in vivo* and albumin denaturation was observed in patients with rheumatic diseases and in rats with inflammatory lesions (Saso *et al.*, 1999). Lysosomal enzymes released during inflammation have been implied in acute or chronic inflammation. Many of the NSAIDs, such as; diclofenac act by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane. Since, the membrane of RBC is structurally similar to lysosomal membrane, the issue of any substance on stabilization of RBC membrane may be generalized to the stabilization of lysosomal membrane (Rajurkar *et al.*, 2009; Chatterjee and Das, 1996). It has been reported that leukocyte proteinases play an important role in the development of tissue damage during inflammatory reactions and significant level of protection was provided by proteinase inhibitors. Hence inhibition of albumin denaturation, RBC membrane stabilization and protease inhibition afford protection against chronic inflammatory conditions (Ilavarasana *et al.*, 2005).

The most common drugs in the treatment of inflammatory condition are NSAIDs, but they have many drawbacks. All NSAIDs works on similar pathways, they inhibit either both COX-1 and COX-2 or some selected drugs only blocks COX-2 enzyme activity. Equally, they are interrupting enzyme activity long term usage of these drugs causes serious adverse events like gastric or peptic ulcer (Wallace, 2001; Shih and Chang, 2007), cardiovascular disease and renal failure (Huerta *et al.*, 2002). That's why, researchers are looking for novel drugs that will be less or no adverse towards body mechanism and also more potent (Murugesan and Deviponnuswamy, 2014). Plant based medicine have gained great attention, because they deliver less or no side-effect, readily available, cost effective (Adeyemi *et al.*, 2004).

The phytochemical study reports following constituents of *L. javanicum*: alkaloids, steroids, tannins, triterpenes, flavonoids, glycosides, phenols, saponin. The bearing of the flavonoids, phenols, etc., encourages us more to study this plant, because these contents are responsible for a broad scope of anti-oxidant property that means, they aid protection against ROS and reduce the impairment of cells and so they can be able to protect against the release of inflammatory mediators.

MATERIALS AND METHODS

Collection and extraction: The plant was gathered from the Botanical Garden of Bangladesh, where this plant is harvested widely. The herbarium number of this collected plant was made by National Herbarium Bangladesh and it is Accession no. 36570. It's a growing tree, up to 56 m tall, leaves are opposite and simple we worked with those leaves. The leaves were separated and dried at room temperature, then extracted using, a cold extraction process, it is thus called, because no heat is applied during this procedure. Methanol was used as solvent; we have got extract weighing 31.8 g.

Anti-inflammatory testing

Animal selection: Healthy male rats were selected weighing between 120-180 g. They were retained at room temperature with 12 h light and dark cycle. Beasts in this study were selected from the animal farm house of North South University (NSU) and their utilization of this protocol was approved by the ethical committee of NSU.

Acute oral toxicity study in rats: The acute toxicity of methanolic extract of *L. javanicum* was determined using Wistar albino rat model. The survey was held out by adopting the guidelines of the OECD (Organization for Economic Cooperation and Development). Agreeing to their guidelines rats were fasted overnight and were randomly divided into groups of five rats per group. About 250 and 1000 mg kg⁻¹ were selected dose and they were meted out individually to the scum bags in each of the groups by means of per oral route. All the animals were, then allowed free access to food and water and observed over a point of the first 2 h for whatever signs of acute toxicity. The observation was elongated to 48 h with a periodic interval of 4 h; the number of deaths and manifestation of toxicity signs like behavioral change (Cathrine and Prabavathi, 2011) within this full point was recorded.

Carrageenan induced paw edema (Hemamalini *et al.*, 2010): Carrageenan (lambda form, FMC Marine Colloids Division, NJ or type IV, Sigma Aldrich, Poole, UK) was prepared as a 1% W/V solution in 0.9% saline, no more than 24 h before exercise. The lambda form does not gel strongly at room temperature and is injectable to induce an inflammatory response. Inflammation induced by carrageenan, originally described by Winter *et al.* (1962). Animals were divided into 4 groups (n = 5), all animals received 0.1 mL carrageenan injection in their right hind paw. About 30 min after carrageenan injected, control groups received 0.5% of Tween 80 (p.o.), without drug substances. Indomethacin (5 mg kg⁻¹) was applied as standard drug and was injected after 30 min, p.o. The MELJ was also introduced p.o. route

after 30 min at the dose 250 and 500 mg kg⁻¹. The full work period was designed for 4 h. The paw volume was measured by Plethysmometre, before and after 4 h with 1 h observation interval. The percentage of inhibition was calculated by the following equation:

$$\text{Inhibition (\%)} = \frac{\text{PIC} - \text{PITD}}{\text{PIC}} \times 100$$

Where:

PIC = Paw inflammation of control

PITD = Paw inflammation of treatment drug

Formalin induced paw edema: Formalin was injected into swiss albino mice for the assay of anti-inflammatory activity following formalin induced paw edema method. There were also four groups, each containing 5 animal models. All animals received 0.1 mL carrageenan injection in their right hind paw. 30 min after carrageenan injected, control groups received 0.5% of Tween 80 p.o., without drug substances. Indomethacin (5 mg kg⁻¹) was applied as, standard drug and was induced after 30 min (p.o.). The MELJ was also introduced by p.o., route after 30 min at the dose 200 and 400 mg kg⁻¹. The total study period was designed for 4 h. The paw volume was measured by Plethysmometre, before and after 4 h with 1 h observation interval. The percentage of inhibition was calculated by comparison to control group, where the control group resembled as, 100%.

Analgesic activity evaluation

Hot plate method: The analgesic effect of MELJ was assessed by the Eddy's hot plate in Swiss albino mice. Mice were divided into 4 groups, each group containing 5 mice. The control group received aqueous suspension in 0.5% of Tween 80 (0.5 mL) p.o., 15 min after the beast was set and tried for its onset of response in hot plate. The reference group was designed using, Aspirin. Animals received 25 mg kg⁻¹ of aqueous (p.o.) and 15 min later placed on the hot plate. In group 3rd and group 4th MELJ was placed at the dose of 250 and 500 mg kg⁻¹, respectively, both of them were suspended in 0.5% of Tween 80 solvent and they respectively, induced to model animal p.o. The reaction time was put down at 15 min interval and whole study was projected for 60 min.

Tail immersion method: The tail immersion assay was made method described by Di Stasi *et al.* (1988). Mice were divided into 4 groups, each containing 5 animals. The rear end of the animal, up to 5 cm, was dipped into hot water, temperature maintained at 55±0.1°C. The time taken from mice in response, which means withdrawal of the tail, was recorded in seconds. Reaction time was counted before and 15 min after oral administration (p.o.) of control group, the test agents MELJ at 200 and 400 mg kg⁻¹ (p.o.) and intra peritoneal (i.p.)

Table 1: Phytochemical screening of methanolic extract of *L. javanicum*

Test of constituents	Intensity of presence
Alkaloid	+++
Flavonoids	+++
Carbohydrate	+++
Glucoside	+++
Glycoside	+++
Phenol	+++
Saponin	+++
Steroid	+++
Tannic acid	+++

injection of morphine (reference group) at 5 mg kg⁻¹. The reaction time was evaluated every 15 min interval over a 90 min period of this designed study.

Phytochemical screening: Different phytochemical tests were done to discover the presence of alkaloids, steroids, triterpenoids, flavonoids, saponins, tannins, glycosides and reducing sugars in extracts (Trease, 1992) (Table 1).

Statistical analysis of data: Values for analgesic and anti-inflammatory activity was expressed as Mean±SEM. The significance of divergence between the means was analyzed by one-way ANOVA followed by Bonferroni multiple comparison tests. The difference was considered significant when p<0.05. All statistical analysis was carried out through GraphPad Prism-6 software.

RESULTS AND DISCUSSION

Acute anti-inflammatory effect: This study was held out on the methanolic extract of *L. javanicum* and it was found that this extract is better and potent in inhibition of acute inflammation with a significant decrease as compared to the standard drug Indomethacin (Bhadauria *et al.*, 2011) after carrageenan injection. In case of the control group, paw swelled up to 10.33±0.169 mL (Table 2 and Fig. 1) at 3 h, after the carrageenan injection. Mice treated with the methanolic extract of *L. javanicum* showed a significant anti-inflammatory activity with 52.27% of inflammation reduction at 3 h (Table 3, Fig. 2) and in a dose dependant manner when compared with standard drug Indomethacin.

Carrageenan induced bioassay is well established for investigation and evaluation of anti-inflammatory agents. In this study, the edema developed in animal model is a biphasic process (Crunkhorn and Meacock, 1971). First phase occurs within an hour and the pain is intervened by the increase of histamine, serotonin and prostaglandin at the damaged tissue site. At the late phase, which is approximately at 3 h prostaglandin sustained and it is influenced by bradykinin, leukotrienes etc. The maximum inhibition shown by methanolic extract of *L. javanicum* after 3 h (52.27%) at 500 mg kg⁻¹ dose. The compared standard also shows its better inhibition effect after 3 h (56.73%). The highest

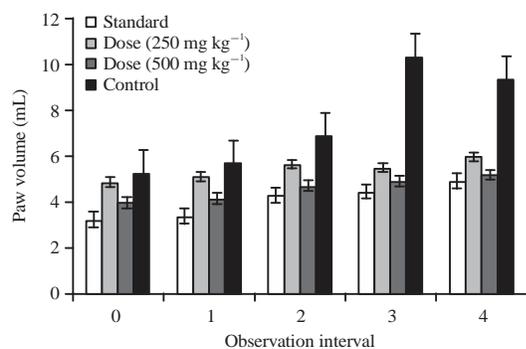


Fig. 1: Paw volume ratio decreased by methanolic extract of *L. javanicum*

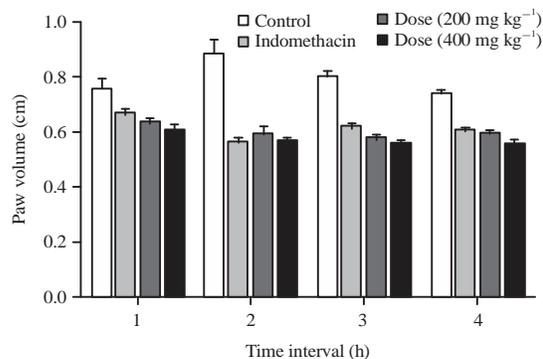


Fig. 3: Paw volume at different time interval and with different dose treatment

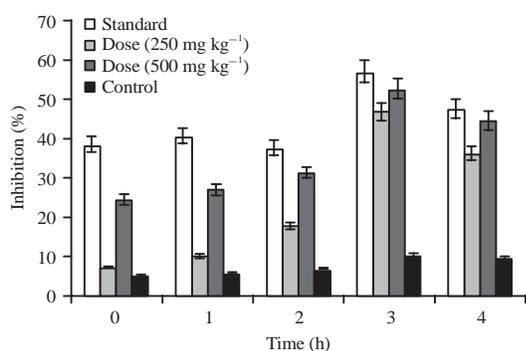


Fig. 2: Percent of inflammation inhibited by methanolic extract of *L. javanicum* compared to the standard

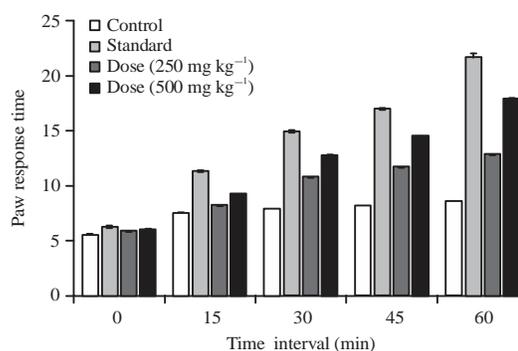


Fig. 4: Analgesic effect of Eddy's hot plate method

Table 2: Paw volume in different groups

Time (h)	Standard	Dose (250 mg kg ⁻¹)	Dose (500 mg kg ⁻¹)	Control	p-value
0	3.25±0.071	4.88±0.028	3.97±0.026	5.26±0.176	<0.0001
1	3.40±0.050	5.13±0.025	4.17±0.025	5.71±0.320	<0.0001
2	4.31±0.102	5.66±0.030	4.74±0.035	6.89±0.217	<0.0001
3	4.47±0.048	5.51±0.015	4.93±0.017	10.33±0.169	<0.0001
4	4.93±0.055	5.98±0.013	5.20±0.018	9.35±0.185	<0.0001

inhibition of inflammation at late phase indicates the sensitivity of our extract towards cyclooxygenase (COX) inhibition, because as we know carrageenan induced paw edema assay is used to evaluate the effect of non-steroidal anti-inflammatory agents, which inhibit cyclooxygenase enzyme induced prostaglandin synthesis. Therefore, from our present study, we can assume that the anti-inflammatory effect presented by *L. javanicum* leaves extract may be due to inhibition of COX enzyme induced prostaglandin synthesis. The similar trend was shown by methanolic extract of *Euphorbia heyneana* Spreng (Battu *et al.*, 2011).

On the other hand, formalin induced paw edema (Table 4) bioassay in rat model is best suit to evaluate anti-arthritis and anti-inflammatory agent as it resemble produce localized inflammation and pain similar to human arthritis. Formalin induced paw edema is also biphasic event; an initial neurogenic response monitored and in later tissue mediators responded to that stimuli. Thus, this model can evaluate

Table 3: Percent of paw volume reduction by methanolic extract of *L. javanicum* compared with control and standard for significance

Time (h)	Control	Standard	Dose (250 mg kg ⁻¹)	Dose (500 mg kg ⁻¹)
0	5.26	38.21	7.22	24.52
1	5.71	40.46	10.16	26.97
2	6.89	37.45	17.85	31.20
3	10.33	56.73	46.66	52.27
4	9.35	47.27	36.04	44.39

anti-proliferative activity, as it is associated with the proliferation phase of inflammation. The decrease in paw volume from 1 h by the extract means, it may have better pharmacokinetic property than Indomethacin (standard). From phytochemical study (Table 1), the leaves of *L. javanicum* contains flavonoids, steroids and some other anti-oxidant content, the anti-inflammatory effect we observed in this study may be due to their presence.

Paw volume significantly reduced by our studied methanolic extract of *L. javanicum* at 4th h $0.55±0.019$ which was more than standard compared ($0.60±0.013$) (Table 4, Fig. 3).

Analgesic activity: The analgesic activity of methanolic extract of *L. javanicum* was found from both Eddy's hot plate and Tail immersion method that this extract is effective against analgesia. Extract at the dose of 500 mg kg^{-1} increases the response time up to $17.83±0.085$, which was close enough to reference group ($21.68±0.417$) at 60 min (Table 5, Fig. 4).

Table 4: Paw volume at different dose and group in formalin induced edema

Groups	Paw volume cm ³			
	1 h	2 h	3 h	4 h
Control	0.75±0.039	0.88±0.050	0.79±0.028	0.73±0.019
Indomethacin	0.66±0.023	0.56±0.015***	0.61±0.017***	0.60±0.013*
Dose 200 mg kg ⁻¹	0.63±0.015*	0.59±0.027***	0.57±0.017***	0.59±0.011**
Dose 400 mg kg ⁻¹	0.60±0.022**	0.56±0.017***	0.55±0.018***	0.55±0.019***

*p<0.01, **p<0.001, ***p<0.0001, p<0.05 was hypothetically considered as significant limit

Table 5: Onset of reaction time recorded at different dose and expressed

Groups	Observation time intervals (min)					p-value
	0	15	30	45	60	
Control	5.43±0.138	7.45±0.104	7.75±0.065	8.18±0.048	8.50±0.041	-
Standard	6.20±0.041	11.35±0.065	14.93±0.131	16.93±0.111	21.68±0.427	<0.0001
Dose (250 mg kg ⁻¹)	5.85±0.065	8.14±0.069	10.70±0.108	11.65±0.096	12.78±0.063	<0.0001
Dose (500 mg kg ⁻¹)	5.93±0.085	9.18±0.075	12.75±0.065	14.48±0.063	17.83±0.085	<0.0001

Recorded at different dose and expressed as Mean±SEM, p<0.0001 resembles that the result is statistically significant

Table 6: Analgesic effect assessed by the tail immersion method

Groups	0 min	15 min	30 min	45 min	60 min	90 min
Control	1.21±0.045	1.36±0.036	1.42±0.135	1.03±0.028	1.41±0.194	0.93±0.926
Morphine (5 mg kg ⁻¹)	1.04±0.029*	1.38±0.172**	1.76±0.147**	1.79±0.246**	1.83±0.238**	6.05±0.822**
Extract (200 mg kg ⁻¹)	1.03±0.074*	1.16±0.094**	1.03±0.042**	1.13±0.156**	1.56±0.194**	2.55±0.160**
Extract (400 mg kg ⁻¹)	1.11±0.043*	1.05±0.086**	1.13±0.030**	1.21±0.033**	1.57±0.079**	2.88±0.073**

Tail immersion method, *p<0.12: Not significant, **p<0.0001: Statistically significant

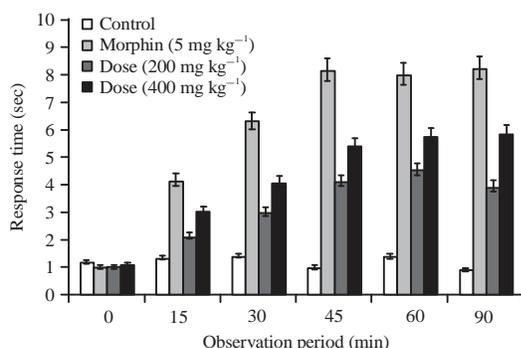


Fig. 5: Analgesic effect of Tail immersion method

The result of tail immersion assay in mice is presented in Table 6. It shows that the extract at the dose of 400 mg kg⁻¹ significant inhibition of analgesia (1.57±0.079, p<0.0001), whereas, the reference drug Morphine results 1.83±0.238 at 60 min. Result of analgesic effect is also discussed graphically in Fig. 5.

The tail immersion and hot plate models usually used to assess centrally acting analgesics. The tail immersion model is designed as it can evaluate the morphine like drugs, which act by inhibiting nociception centrally (Toma *et al.*, 2003). So, the drug that selectively prolong the reaction time of tail withdrawal reflex is supposed to be effective. However, the sensitivity of paws of mice at 50-55±2°C temperature are observed. From the above results, the extract showed analgesic actions in both models, it was found as, pronounced as seen in the carrageenan induced and formalin induced edema model. Therefore, we may suggest that the leave extract of *L. javanicum* may have central mechanism to minimize

analgesia. Both models, increase in pain reaction time or latency period indicates the level of analgesia of drug or extract and it is observed from the result of our investigation that the extract increases pain reaction time in both cases. In hot plate method, the extract at the dose of 500 mg kg⁻¹ was able to elongate the latency period up to 17.83±0.085, where standard did 21.68±0.427 and the dose dependency manner observed in the action of our extract. Same pattern also seen in the tail immersion models, extract at the dose of 400 mg kg⁻¹ prolonged latency period to 1.57±0.079, which was close to morphine 1.83±0.238 at 90 min.

CONCLUSION

The result from our present study allows us to conclude saying that the methanolic extract of *L. javanicum* has good analgesic and anti-inflammatory property. This property presents probably due to the presence of the antioxidant component like the flavonoid and the steroidal content in it. The mechanism of action is not corroborated but from the point of view of our methods, we applied, the mechanism of action can be predicted. The anti-inflammatory activity shown by extract is due to inhibition of COX enzyme followed by the inhibition of synthesis of prostaglandin and the significant analgesic effect is due to its action over nociceptors in CNS. Therefore, these findings of our present study demonstrate that the methanolic extract of *L. javanicum* is a potent anti-inflammatory and analgesic herbal extract and its use in traditional medicine to treat inflammatory and painful conditions is justified. The results also provide indication that the beneficial effects of this plant may be due to its free radical scavenging activity.

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The authors have declared that there no conflict of competing interest. We would like to thank North South University for permitting us to utilize their lab, unless we may not able to take this task. Whenever we use any animal model it is our obligation to get permission from the appropriate authority for certification. We took ethical consent from our university authority, when we were collecting animal and applying in our subject area. And then the whole survey was run in an honorable way.

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