

EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITY OF METHANOLIC EXTRACT OF *GUAZUMA ULMIFOLIA*

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ABSTRACT

Introduction: *Guazuma ulmifolia* (Sterculiaceae) is commonly used in folk medicine as a single drug remedy to treat various ailments like; skin diseases, headache, wounds, ulcers, asthma, kidney disease and malaria. However, the use of the herb for treatment of pain and inflammation has not been scientifically investigated. **Objective:** This study of 80% methanolic crude extract of the leaves of *Guazuma ulmifolia* was evaluated for its analgesic and anti-inflammatory properties using established animal models. **Material and methods:** Wister albino rats were randomly divided into four groups of six rats per group. The control group was orally given 2 ml/kg (*p.o.*) of distilled water. The positive control received standard drug (Diclofenac 10 mg/kg, Indomethacin 20 mg/kg). The rest of the groups were treated with 80% methanolic extract of *Guazuma ulmifolia* at doses of 250 and 500 mg/kg. Animals were then subjected to tests using hot plate and tail-clip for analgesic activity, for anti-inflammatory activity carrageenan induced paw edema test and histamine induced paw edema test were used. **Result and Discussion:** *Guazuma ulmifolia* extract showed a dose-dependent significant reduction of pain in analgesia models ($p < 0.001$) with 500 mg/kg dose producing the highest reduction. The extract significantly reduced carrageenan induced inflammation in a dose independent manner, in which the highest reduction of inflammation was observed at 500 mg/kg. **Conclusion:** The data collectively indicate that 80% methanolic extract of *Guazuma ulmifolia* leaves have potential analgesic and anti-inflammatory activities.

Keywords: Analgesic, Anti-inflammatory, Inflammation, Carrageenan, paw edema, Histamine.

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INTRODUCTION

Today the use of most commonly prescribed drugs for clinical management of analgesia and inflammation has been limited, because of the development of potential side effects including sedation, respiratory depression and dermatological reactions. [1] Inflammation or phlogosis is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can be induced, maintain or aggravate many diseases. [2] However, studies have been continuing on inflammatory diseases and the side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical use. [3] Therefore, development of newer and more powerful anti-inflammatory drugs with lesser side effects is necessary.

Guazuma ulmifolia (Synonym: *Guazuma tomentosa*) belongs to family Sterculiaceae and is a native to India, and tropical America. Despite of its ethnopharmacological uses, presently it is proven to have many therapeutic application because of the presence of many Phytoconstituents like colistin, colatannins, catechins, caffeine, kaempferol, procyanidin B-2, procyanidin B-5,

procyanidin C-1, tartaric acid, theobromine, xanthan gum, etc. Preclinical reported activities include, hepatoprotective, anti-diabetic activity, anti-hypertensive activity, anti-microbial activity, anti-oxidant activity, anti-ulcer activity, as a hair growth promoter, neurological activity, anti-secretory activity, cytotoxic activity and uterine stimulating activity [4], [5], [6]. Leaves have been uses for Alopecia, asthma, bruises, dermatitis, dysentery, erysipelas, fevers, inflammation, kidney diseases, liver diseases, skin eruptions, skin diseases, sores, ulcers, wounds. [7] Because this plant contains flavonoids (kaempferol), alkaloids (caffeine), glycosides, phytosterols and saponins which were confirmed by phytochemical test, therefore it may show analgesic and anti-inflammatory activities. The present study was undertaken to find out the possible actions of leaves parts of *Guazuma ulmifolia* for its analgesic and anti-inflammatory activity.

Material and Methods

Collection of plant

The leaves of plant *Guazuma ulmifolia* were collected directly from the plant in the month of August 2017 from Nehru Hostel

Sec.3, Udaipur. The leaves of the plant were identified by Botanist Prof. Karan Soampura (Principal at higher secondary school, Kherwara). The leaves were washed with tap water and then dried under the shade at room temperature.

Drugs and chemicals

Methanol, Normal Saline, Diclofenac, Indomethacin, Carrageenan, Histamine.

Preparation of the leaves extract

The dried leaves powder of plant was extracted successively in methanol by cold percolation method. About 250 g of dried leaves powder was dissolved in 600 ml of 80% methanol taken in a conical flask and exhaustively extracted for a period of two days (48 hours) with accompanying occasional shaking and stirring. Plugged with cotton wool and then kept on a rotary shaker at 190–220 rpm for 24 h. After 24 h, the extract was filtered with eight layers of muslin cloth, the supernatant was collected and the solvent was evaporated. The dried extract of was stored at 4°C in airtight bottles.

PHARMACOLOGICAL INVESTIGATION

Experimental Animals

Wistar albino rats of weighing between 100-250 g were obtained from Geetanjali Medical College & Hospital, Udaipur and kept at the Laboratory Animal centre of the college. The animals maintained under standard environmental conditions had free

access to standard diet and water *ad libitum*. Rats were housed in groups of six per cage. All the animals were maintained under standard conditions; that is room temperature 26±1°C, relative humidity 45-55% and 12:12 h light-dark cycle. The cages were maintained clean, and all experiments were conducted between 9 am and 4 pm.

Approval of Animal Ethical

The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) and all the experiments were conducted according to the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Acute Toxicity Study

Wistar albino rats of both sexes weighing between 100-250 g were used for acute oral toxicity study. The animals were fasted overnight and divided into 4 groups with 6 rats in each group. Extract were administrated at dose of 2000 mg/kg, *p.o.* body weight. The rats were observed continuously for behavioral and autonomic profiles for 2 h. and for any signs of toxicity or mortality up to 48 h. The study was carried out as per the guidelines set by OECD 423. [8]

Animal Grouping

Rats were randomly divided into four groups with each group consisting of six rats as seen in Table 1.

Table 1: Animal Grouping

Group I	Control (Distilled Water)	2 ml/kg (<i>p.o.</i>)
Group II	Methanolic Extract of <i>Guazuma ulmifolia</i> (MEGU-250)	250 mg/kg
Group III	Methanolic Extract of <i>Guazuma ulmifolia</i> (MEGU-500)	500 mg/kg
Group IV	Standard drug (Diclofenac for analgesic activity)	10 mg/kg
	Standard drug (Indomethacin for anti-inflammatory activity)	20 mg/kg

DETERMINATION OF ANALGESIC ACTIVITY

Hot plate method

Evaluation of analgesic activity of the plant extract was carried out using hot plate method. The rats were placed on a hot plate maintained at 55 ± 1°C within the restrainer. The reaction time (in sec.) or latency period was determined as the time taken for the rats to react to the thermal pain by licking their paws or jumping. [9] The reaction time was recorded at 0, 30, 60, 90 and 120 min after drug administration, with a cut-off time of 15 sec. as to avoid lesions to the animals' paws analgesic and anti-inflammatory properties. [10]

Tail clip method

A metal artery clip was applied to the root of animals tail (1cm from the body) to induce pain. [11] A sensitivity test was carried out and animals that did not attempt to dislodge the clip within 15 sec. were discarded. Analgesic activity was evaluated at 0, 30, 60, 90, and 120 min. after oral administration of the extracts and controls. An artery clip is placed at the root of tail and a positive analgesic response was indicated if animal attempt to dislodge the clip by biting the clip or tail within 5 sec. in any of the consecutive trials. The reaction time between application of the clip and response is noted by a stop watch. The mean value was evaluated. [12]

DETERMINATION OF ANTI-INFLAMMATORY ACTIVITY

Carrageenan induced rat paw oedema method

The anti-inflammatory activity of the test compounds were evaluated in Wistar rats. Anti inflammatory activity of methanolic extracts in the dose of 250 and 500 mg/kg was evaluated against Carrageenan induced paw edema model in rats. Twenty four animals were divided into four groups consisting of six animals each. Group I served as control group and received distilled water (2 ml/kg *p.o.*), Groups II and III received methanolic extract of *Guazuma ulmifolia* at 250 and 500 mg/kg dose through oral route, respectively. Group IV received standard drug Indomethacin in a dose of 20 mg/kg *p.o.* Edema was induced by injection of Carrageenan (0.1 ml, 1%, w/v in saline) into the subplantar tissue of the right hind paw. The linear paw circumference was then measured at 0, 30, 60, 90 and 120 min of the administration of phlogistic agent, using the Vernier calipers. The following formula was used to calculate percentage of inhibition.

$$\text{Inhibition (\%)} = \frac{V_c - V_t}{V_c} \times 100$$

Where Vc and Vt represent average paw volume of control and treated animals, respectively [13], [14].

Histamine induced rat paw oedema method

Male albino rats weighing between 150-250 g were used for the study and fasted overnight prior and during the experiment but have free access to water. Anti inflammatory activity of methanolic extracts in the dose of 250 and 500 mg/kg was evaluated against Histamine induced paw edema model in rats. The rats were divided into 4 groups of 6 animals each. Group I served as control group and received distilled water (2 ml/kg *p.o.*), Groups II and III received methanolic extract of *Guazuma ulmifolia* at 250 and 500 mg/kg dose through oral route, respectively. Group IV received standard drug Indomethacin in a dose of 20 mg/kg *p.o.* Edema was induced by injection of histamine (0.1 ml, 1%, w/v in saline) into the subplantar tissue of the right hind paw. The linear paw circumference was then measured at 0, 30, 60, 90 and 120 min of the administration of phlogistic agent, using the Vernier calipers. The following formula was used to calculate percentage of inhibition.

$$\text{Inhibition (\%)} = \frac{V_c - V_t}{V_c} \times 100$$

Where V_c and V_t represent average paw volume of control and treated animals, respectively [15], [16].

Statistical analysis: The results are expressed as the means \pm standard error of mean (SEM). Parametric data were compared to

control group and were assessed by the method of one-way ANOVA followed by Dunnett's tests. Values $p < 0.05$ was considered as statistically significant.

RESULTS

ANALGESIC ACTIVITY

Hot plate test in rat

In the hot-plate method the extract 500 mg/kg and diclofenac 10 mg/kg prolonged the reaction time significant ($p < 0.05$) as compared to the control group throughout the observation period as shown in Table 2 and Figure 1. The reaction time was taken as the parameter for the evaluation of analgesic activity. The effect produced by 250 mg/kg of the extract does not show any significance. However, with 500 mg/kg significance difference was noted at 0, 30, 60, 90 and 120 min. respectively, in relation to the control group. Comparing different doses of the extract revealed that there is positive relationship between reaction time and increase dose of the extract in which, protection against thermal stimuli with 500 mg/kg was significant compared to control.

Table 2: Effect of 80% methanolic extracts of *Guazuma ulmifolia* on hot plate test.

Treatment (mg/kg)	Latency (sec) \pm SEM				
	0 min	30 min	60 min	90 min	120 min
Control	3.05 \pm 0.15	3.10 \pm 0.12	3.10 \pm 0.13	3.17 \pm 0.08	3.28 \pm 0.11
MEGU-250 mg/kg	3.32 \pm 0.17 ^{n.s}	6.25 \pm 0.40 ^{n.s}	7.96 \pm 0.17 ^{n.s}	9.69 \pm 0.28 ^{n.s}	11.06 \pm 0.16 ^{n.s}
MEGU-500 mg/kg	3.85 \pm 0.10*	7.90 \pm 0.76*	9.75 \pm 0.30*	10.55 \pm 0.28*	11.95 \pm 0.28*
Diclofenac 10mg/kg	3.93 \pm 0.13**	10.97 \pm 0.19**	12.45 \pm 0.13**	12.76 \pm 0.12**	13.51 \pm 0.13**

Values are represented as means \pm SEM (n=6 in each group) Data expressed by using one way ANOVA followed by Dunnett's Test. $p < 0.05$, * $p < 0.01$, ** $p < 0.001$ was considered as significant and n.s = non-significant as compared to control.

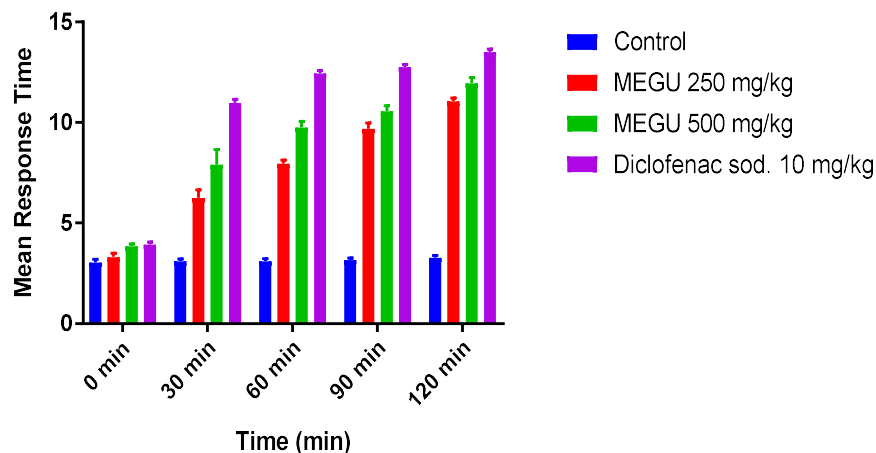


Figure 1: Effect of 80% methanolic extracts of *Guazuma ulmifolia* on hot plate test

Tail clip test in rat

The effect of *Guazuma ulmifolia* extract on tail clip test is shown in Table 3 and Figure 2. The extract at dose of 250 mg/kg dose not shown any significant, but at dose 500 mg/kg caused a significant

inhibition of pain. However, diclofenac sodium 10 mg/kg was effective than methanolic extract.

Table 3: Effect of 80% methanolic extracts of *Guazuma ulmifolia* on tail clip test

Treatment (mg/kg)	Latency (sec) ±SEM				
	0 min	30 min	60 min	90 min	120 min
Control	3.00±0.14	3.16±0.23	3.20±0.16	3.50±0.18	3.50±0.13
MEGU- 250 mg/kg	3.20±0.12 ^{n.s}	4.54±0.90 ^{n.s}	6.50±0.84 ^{n.s}	7.00±0.74 ^{n.s}	6.40±1.10 ^{n.s}
MEGU-500 mg/kg	5.00±0.16 ^{**}	7.70±0.99 ^{**}	9.50±1.24 ^{**}	10.54±1.17 ^{**}	9.40±0.62 ^{**}
Diclofenac 10mg/kg	4.00±0.24 ^{***}	8.99±1.50 ^{***}	11.00±1.74 ^{***}	13.50±1.42 ^{***}	12.90±0.84 ^{***}

Values are represented as means±SEM (n=6 in each group) Data expressed by using one way ANOVA followed by Dunnett's Test. **p*<0.5, ***p*<0.01, ****p*<0.001 was considered as significant and n.s= non-significant as compared to control.

Data 1

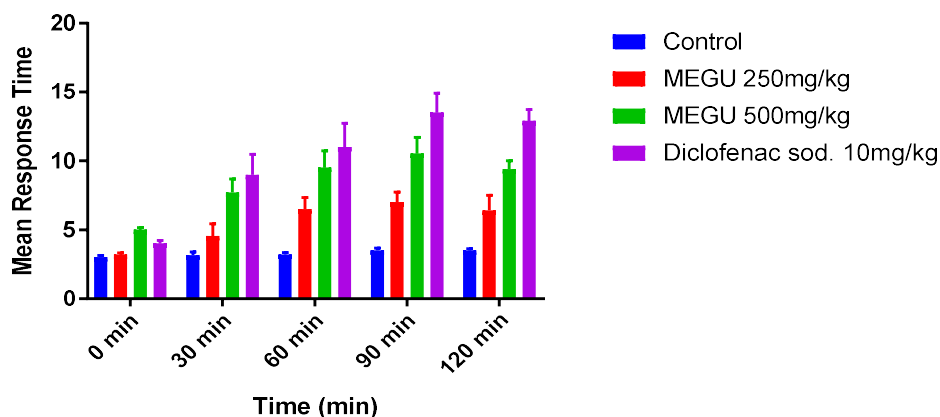


Figure 2: Effect of 80% methanolic extracts of *Guazuma ulmifolia* on tail clip test

ANTI-INFLAMMATORY ACTIVITY

Carrageenan induced paw edema

The result shown by methanolic extract of *Guazuma ulmifolia* against carrageenan induced paw edema are given in Table 4 and Figure 3. methanolic extract at dose of 250 mg/kg showed non-

significant reduction of paw oedema (13.28%), but in methanolic extract administered at a dose of 500 mg/kg, the paw volume were reduced by (5.81%). The result demonstrated dose time related significant reduction by methanolic extract. Indomethacin 20mg/kg similarly produced significant inhibitory effect of the paw edema (8.30%) as compared to normal control group.

Table 4: Effect of methanolic extract of *Guazuma ulmifolia* at 250 and 500 mg/kg doses against carrageenan induced paw edema in rat.

Treatment (mg/kg)	Mean paw volume in ml					% Inhibition
	0 min	30 min	60 min	90 min	120 min	
Control	1.17±0.007	1.29±0.009	1.37±0.006	1.25±0.006	1.24±0.0082	
MEGU-250 mg/kg	1.10±0.0040 ^{n.s}	1.22±0.0040 ^{n.s}	1.30±0.0044 ^{n.s}	1.19±0.0029 ^{n.s}	1.16±0.0116 ^{n.s}	13.28%
MEGU-500 mg/kg	1.18±0.012 ^{**}	1.10±0.050 ^{**}	1.08±0.001 ^{**}	1.20±0.0118 ^{**}	1.070±0.002 ^{**}	5.81%
Indomethacin 20 mg/kg	0.82±0.009 ^{****}	0.98±0.005 ^{****}	0.89±0.003 ^{****}	0.77±0.003 ^{****}	0.90±0.009 ^{****}	8.30%

Values are represented as means±SEM (n=6 in each group). Data expressed by using one way ANOVA followed by Dunnett's Test. **p*<0.5, ***p*<0.01, ****p*<0.001 was considered as significant and n.s= non-significant as compared to control.

Histamine induced paw edema

In this method, histamine was used to induce paw oedema in rats. The animals were treated with the extracts of the leaves of *Guazuma ulmifolia* at the dose of 250 mg/kg, 500 mg/kg and Indomethacin 20 mg/kg respectively and result are tabulated in Table 5 and Figure 4. Among the two doses of *Guazuma ulmifolia* extract, 500 mg/kg showed maximum reduction in the paw volume

(2.28%), while methanolic extract at 250 mg/kg dose not shown any significant reduction in the paw volume (4.23%) induced by histamine as compared to control group. Indomethacin 20 mg/kg produced significant inhibitory effect of the paw edema (13.55%) as compared to normal control group.

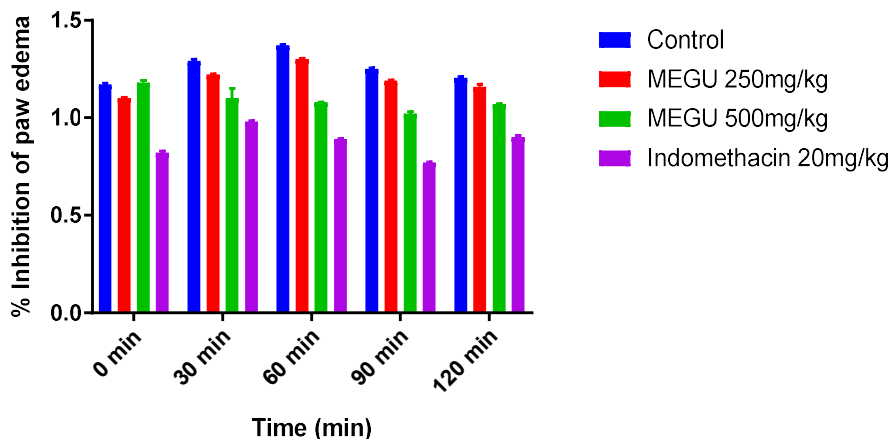


Figure 3: Effect of methanolic extract of *Guazuma ulmifolia* at 250 and 500 mg/kg doses against carrageenan induced paw edema in rat.

Table 5: Anti-inflammatory activity of 80% methanolic extract of the leaves of *Guazuma ulmifolia* on histamine induced paw edema in rats

Treatment (mg/kg)	Mean paw volume in ml					% Inhibition
	0 min	30 min	60 min	90 min	120 min	
Control	1.133±0.009	1.200±0.006	1.315±1.188	1.188±0.006	1.180±0.004	
MEGU-250 mg/kg	1.030±0.015 ^{n.s}	1.123±0.015 ^{n.s}	1.226±0.015 ^{n.s}	1.110±0.015 ^{n.s}	1.050±0.006 ^{n.s}	4.23%
MEGU-500 mg/kg	0.926±0.034 ^{***}	1.003±0.021 ^{***}	1.070±0.015 ^{***}	0.983±0.012 ^{***}	0.973±0.003 ^{***}	2.28%
Indomethacin20 mg/kg	0.793±0.009 ^{****}	0.866±0.012 ^{****}	0.996±0.009 ^{****}	0.920±0.012 ^{****}	0.841±0.006 ^{****}	13.55%

Values are represented as means± SEM (n=6 in each group) Data expressed by using one way ANOVA followed by Dunnett's Test. **p* <0.5, ***p*<0.01, ****p*<0.001 was considered as significant and n.s= non-significant as compared to control.

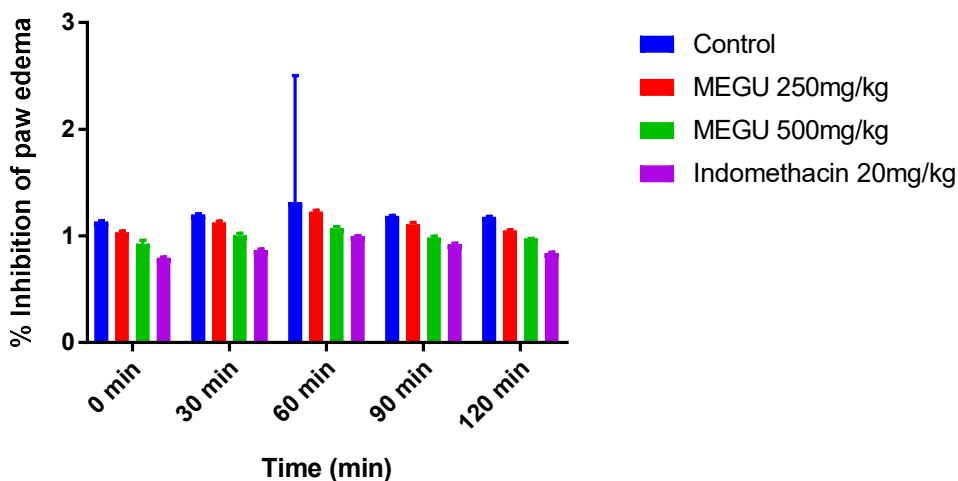


Figure 4: Anti-inflammatory activity of 80% methanolic extract of the leaves of *Guazuma ulmifolia* on histamine induced paw edema in rats.

DISCUSSION

Pain and inflammation are associated with pathophysiology of various diseases like arthritis, cancer and vascular diseases. A number of natural products are used in various traditional medicinal systems to relief symptoms of pain and inflammation.^[17]

Inflammation is well orchestrated response to deleterious stimuli including tissue injury, and infection.^[18] It is elicited to restore normal condition of tissue or body. Classically inflammation is characterized by increase in the blood flow, reddening of the affected part due to increased erythrocyte accumulation and edema.^[19]

In both methods *i.e* hot plate and tail clip, the methanolic extract of *Guazuma ulmifolia* does not show significant ($p < 0.05$) analgesic activity at the dose of 250 mg/kg. However, the extract at dose of (500 mg/kg) and diclofenac prolonged the reaction time significantly ($p < 0.01$) as compared the control throughout the observation period but the standard drug (diclofenac) showed the maximum reaction time (Table 2 & 3). The result indicated that the extract significantly ($p < 0.001$) raised pain threshold as compared to control and the activity was persistent throughout the entire observation period of 120 min.

The anti-inflammatory activity of methanolic extract of *Guazuma ulmifolia* has showed effect in a dose dependent manner as comparable to those of the standard drug, Indomethacin. In both methods, we observed that methanolic extract at dose 500 mg/kg showed significant inhibition against carrageenan-induced paw edema and histamine induced paw edema, but in case of methanolic extract at dose 250 mg/kg failed to possess anti-inflammatory effect (Table 4 & 5). The percentage inhibition of the carrageenan-induced paw edema of the methanolic extract at dose of 500 mg/kg were reduced by (5.81%) and produced significant ($p < 0.01$) inhibition. In histamine-induced paw edema, the methanolic extract at dose of 500 mg/kg reduced edema by (2.28%) and produced significant ($p < 0.01$) inhibition in animals and continued during all phase of inflammation. Methanolic extract at 250 mg/kg does not show any significant reduction in the paw vol. (13.28%) induced by carrageenan and (4.23%) induced by histamine as compared to control group.

CONCLUSION

The present study concludes that the methanolic extract of *Guazuma ulmifolia* leaves possesses analgesic and anti-inflammatory activities in rats at the doses of 250 mg/kg and 500 mg/kg respectively. These activities in the methanolic extract of *Guazuma ulmifolia* may be due to the presence of flavonoids (kaempferol), alkaloids (caffeine), glycosides, phytosterols and saponins which were confirmed by phytochemical test.

The probable mechanism for the anti-inflammatory action could be due to inhibition of synthesis of prostaglandins and other inflammatory mediators like histamine and serotonin in early hours of inflammation.

This plant has a leading capacity for the development of new good efficacy drugs in future and further work should be done on the isolation and identification phytochemical compounds of *Guazuma ulmifolia*.

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CONFLICT OF INTEREST

The author declares that he has no competing interests.

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