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### EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF GUAZUMA TOMENTOSA L. LEAVES IN WISTER ALBINO RAT.

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#### ABSTRACT

*Guazuma TomENTOSA L.* a member of the Sterculiaceae family, is used in folk medicine because of its antimicrobial, antioxidant, and antihypertensive properties. Most of the research work carried out on this plant has focused on the leaves because of its high concentration of antioxidant, kaempferol, proanthocyanidins. The leaves and flowers of *Guazuma tomentosa*, though less studied, are also used as a remedy for different conditions, such as inflammation, kidney and gastrointestinal diseases, fever and diabetes. The aim of this study was to assess the anti-inflammatory effects of the ethanolic extract from leaves of *Guazuma tomentosa* in a model of carrageenan paw edema and leukocyte migration method by carrageenan as inflammatory agent, using the indomethacin as a reference. Therefore, the extract was administered single dose orally to three groups of Wistar rats at doses of 500, 250 and 125 mg/kg, with a indomethacin (10 mg/kg) was given of the extract. Pretreatment with *Guazuma TomENTOSA* or indomethacin decreased the inflammation area in a dose-dependent way. In the present study, leaves extract of *Guazuma tomentosa L.*, were studies for anti-inflammatory activity by in-vivo method. The extracts were found to possess significant activity at all selected doses i.e. 125, 250 and 500 mg/kg body weight, *Guazuma tomentosa L.* showed comparable results with the standard drug. It showed maximum percentage inhibition of 53.25%, which were comparable with the Standard, indomethacin, which showed 51.08% inhibition. In leukocytes migration method by exudate volume standard shows 56.96% inhibition, while 125, 250, and 500 mg/kg ethanolic extract of *Guazuma tomentosa L.* The probable mechanism for the acute anti-inflammatory action could be due to anti-bradykinin activity and inhibition of synthesis of prostaglandins and other inflammatory mediators like histamine and serotonin in early hours of inflammation.

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## INTRODUCTION

The inflammatory process is the response to an injurious stimulus. It can be evoked by noxious agents, infections, antibodies, physical injuries. The ability to mount an inflammatory response is essential for survival in the face of environmental pathogens and injury; in some situations and diseases, the inflammatory response may be exaggerated and sustained without apparent benefit and even with severe adverse consequences. Many molecules are involved in the promotion and resolution of the inflammatory process. Histamine, bradykinin, 5-HT, prostanooids, leukotrienes (LTs), and platelet-activating factor are important mediators of inflammation. Most currently available traditional NSAIDs (tNSAIDs) act by inhibiting the prostaglandin (PG) G/H synthase enzymes, colloquially known as the cyclooxygenases. The inhibition of cyclooxygenase-2 (COX-2) is thought to mediate, in large part, the antipyretic, analgesic, and anti-inflammatory actions of tNSAIDs, while the simultaneous inhibition of cyclooxygenase-1 (COX-1) largely but not exclusively accounts for unwanted adverse effects in the GI tract. Selective inhibitors of COX-2 (celecoxib, etoricoxib, lumiracoxib) are a sub class of NSAIDs that are also discussed. Aspirin, which irreversibly acetylates COX, is discussed, along with several structural subclasses of tNSAIDs, including propionic acid derivatives (ibuprofen, naproxen), acetic acid derivatives indomethacin, and enolic acids (piroxicam), all of which compete in a reversible manner with the arachidonic acid (AA) substrate at the active site of COX-1 and COX-2. Acetaminophen (paracetamol) is a weak anti-inflammatory drug; it is effective as an antipyretic and analgesic agent at typical doses that partly inhibit COXs. Acetaminophen has fewer GI side effects than the tNSAIDs. The world presently is shifting towards organic products. In the current scenario organic medication or herbal medication is need of the hour. So, in this case herbal drugs with multiple therapeutic activities are being encouraged greatly. One such herbal plant is *Guazuma tomentosa* L. commonly known as Rudrakshi<sup>1</sup> is a plant with many therapeutic applications and most of them target the prevailing disorders like Inflammation, Diabetes, Asthma, Anti-Oxidant, Antiulcer Action, Hair growth promoter etc. The following review emphasizes on various therapeutic uses of plant parts of *Guazuma* highlighting its potential and scope in the field of herbal medicine.

## MATERIALS AND METHODS

### Collection and authentication of plant<sup>5</sup>:

The leaves of *Guazuma tomentosa* were collected in the month of July, 2016 from the Veer Mata Jijabai Bhosale Udyan, Byculla. Mumbai and get authenticated at St Xavier's College, Mumbai from the specimen match with the blatter herbarium specimen number R 24030 of R.R. Fernandez. The leaves were washed with tap water followed by drying under shade (temperature 30-40°C). Dried leaves were grinded to coarse powder and packed in suitable container.

### Preparation of Extract's<sup>3,4,5,6,7</sup>

Ethanollic extract of leaves was prepared using Soxhlet apparatus. The leaves of *guazuma tomentosa* were sundried for 7 days and later dried in drier at 40°C for about an hour. The dried leaves were then ground into powder using high capacity grinding machine and stored in airtight plastic container and kept in cool, dark and dry place. The sun dried and powdered plant leaves (500 gm.) of *guazuma tomentosa* was successively extracted in a Soxhlet extractor at 50°C-60°C temperature using 250 ml of ethanol. Poured on Petri dishes to evaporate the liquid solvents from the extract to get dry extracts. The dry crude extracts were weighed and stored in air-tight container and kept in refrigerator (0-4)°C until use.

### Phytochemical screening<sup>6,7,8</sup>:

The ethanollic extract of leaves of *Guazuma tomentosa* showed the presence of various phytoconstituents like alkaloids, terpenoids, saponins, tannins, flavonoids and steroid. Leaves contain octacosanol, taraxerol, friedelin-3, sitosterol, Friedelinol-3-acetate, and kaempferol.

### Procurement and acclimatization of animals:

Animals required for the research work were approved from the Oriental college of pharmacy, Sanpada Institutional Animal Ethics Committee (IAEC) Proposal No. OCP/IAEC/2016-2017/01. Animals procured were female Swiss Albino mice (25 to 30 gm.) and Wistar albino rats (150 to 180 gm.) were obtained from Bombay Veterinary College, Pare 1, Mumbai - 411012 (REG NO. 230/99/CPCSEA). The animals were housed in well ventilated, air conditional animal house at a constant temperature of 23±20°C, with the relative humidity of 55-60%. Wistar rats (150-180gm) kept in standard laboratory conditions were kept fasting overnight in single wire net floor cages with free access to tap water, and were randomly assigned to groups of animals. All experiments followed a protocol approved by animal Ethics Committee. Acclimatize for a period of 7 days.<sup>10,15</sup>

### Experimental animals:

#### Acute oral toxicity<sup>18, 19</sup>:

The method followed to perform the AOT was the Acute Toxic Class method i.e. OECD – 423. Three doses were chosen from the Annex II of OECD 423 i.e. Minimum, Medium and Maximum i.e. 500 mg, 1000mg and 2000mg after sighting study. Total of 9 animals were chosen i.e. 3 animals per group and three groups were taken namely *guazuma tomentosa* (GT) Extract -300 mg, GT Extract - 500 mg and GT Extract - 2000 mg. A single dose was administered and animals were observed for a period of 14 days for clinical signs and mortality.

### Carrageenan induced rat paw edema:

A total number of rat were randomly divided into five groups (no=6 per group), namely the normal control (group 1), positive control (group 2) and 3 test groups (125 mg/kg, 250 mg/kg and 500 mg/kg), which were administered normal saline 1ml, 10 mg/kg indomethacin and estimated doses 125, 250 and 500 mg/kg of Ethanolic extract of *Guazuma tomentosa* leaves. One hour later, 0.1 ml of 1% w/v carrageenan solution was injected into the plantar side of the right hind paw of the rat. Prior to the injection of the carrageenan solution, the right hind paw of each rat was measured by plethysmograph. Measurements were repeated at hourly interval maximum of five hours after carrageenan injection. The difference between the initial paw size and the paw size at each post treatment time point was used to estimate the degree of edema.

### Carrageenan induced Peritonitis (leukocytes migration) in rats:<sup>13,14,15</sup>

A total number of rat were randomly divided into five groups (no=6 per group), namely the normal control (group 1), positive control (group 2) and 3 test groups (125 mg/kg, 250 mg/kg and 500 mg/kg), which were administered normal saline 1ml, 10 mg/kg indomethacin and estimated doses 125, 250 and 500 mg/kg of Ethanolic extract of *Guazuma tomentosa* leaves. <sup>16</sup>one hour before intraperitoneal injection of 0.1 ml of carrageenan, 1%w/v in normal saline. Three hours later the rats were sacrificed by using CO<sub>2</sub> Desicator<sup>23,25</sup>. An incision was made on the peritoneal cavity of each rat and 5 ml of phosphate buffered saline (containing 1.38 g/l of disodium hydrogen orthophosphate 0.19 g/l of potassium dihydrogen orthophosphate; 8.0 g/l of sodium chloride; 5.0 iu /ml of heparin and 3% egg albumin) was injected. After gentle massage of the peritoneum, the exudate was aspirated with a sterile syringe. The exudate volume (minus the wash fluid, 5 ml) as well as the total leucocytes count was determined.

### STATISTICAL ANALYSIS:

Data Obtained is analyzed in One way Annova using Prism Graph pad. This experiment employed three different doses of *Guazuma tomentosa* for anti-Inflammatory screening namely minimum dose (125 mg), Medium dose (250 mg) and Maximum Dose (500 mg). All the three doses showed significant result. From the p values and the statistical analysis, the test – 3 (500mg/kg dose) is found to be most effective when compared to test -1 and test 2 while screening for Anti -Inflammatory effect.

## RESULT

### Results of Acute Oral Toxicity:

Acute oral toxicity studies for the test extract were carried out in mice as per the OECD Guideline No. 423. Oral acute toxicity was studied in overnight fasted mice with water. The Mice were randomly allocated into three groups each containing three animals. The observed parameters such as muscular tones, tremors, convulsions, feed and water intake, breathing patterns and presence of mouth secretions were observed for the first 12 hrs. and for further 14 days. Mortality: The extracts were found to be safe till a dose of 2000mg/kg since no mortality was observed. Signs and Symptoms of toxicity:- Signs of intoxication were not observed 24 hrs post treatment as dose did not produce any significant changes in behavioral pattern and failed to elicit any clinical abnormality. LD<sub>50</sub> was considered as more than 2000mg/kg.

### Results of Phytochemical screening:

Table 1:-Phytochemical screening.

| Phytochemicals      | Present (+)/Absent (-) in Ethanolic extract of the leaves of <i>Guazuma tomentosa</i> L. |
|---------------------|------------------------------------------------------------------------------------------|
| Alkaloids           | +                                                                                        |
| Flavonoids          | +                                                                                        |
| Saponins            | +                                                                                        |
| Phenols             | +                                                                                        |
| Terpenoids          | +                                                                                        |
| Glycosides          | +                                                                                        |
| Phytosterols        | +                                                                                        |
| Proteins            | +                                                                                        |
| Fixed oils and fats | +                                                                                        |

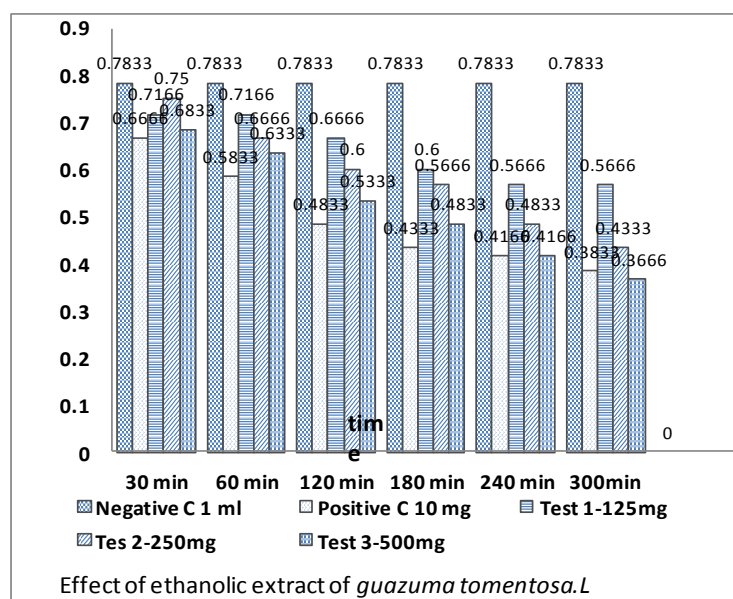
**Anti-Inflammatory Evaluation:**

Carrageenan induced paw edema was calculated the results:

| Treatment (Group) | Paw edema (ml) |                          |             |             |             |             |             |
|-------------------|----------------|--------------------------|-------------|-------------|-------------|-------------|-------------|
|                   | Normal Paw     | After giving carrageenan |             |             |             |             |             |
|                   |                | 0 hrs                    | 1 hrs       | 2 hrs       | 3 hrs       | 4 hrs       | 5 hrs       |
|                   | ME±SD          | ME±SD                    | ME±SD       | ME±SD       | ME±SD       | ME±SD       | ME±SD       |
| NC 1 ml           | 0.383± 0.05    | 0.783± 0.04              | 0.783± 0.04 | 0.783± 0.04 | 0.783± 0.04 | 0.783± 0.04 | 0.783± 0.04 |
| PC 10 mg/kg       | 0.366± 0.05    | 0.666± 0.05              | 0.583± 0.04 | 0.483± 0.04 | 0.433± 0.05 | 0.416± 0.07 | 0.383± 0.07 |
| GTEE- 125 mg/kg   | 0.366± 0.05    | 0.716± 0.04              | 0.716± 0.04 | 0.666± 0.05 | 0.6± 0.06   | 0.566± 0.05 | 0.566± 0.05 |
| GTEE- 250 mg/kg   | 0.383± 0.04    | 0.75± 0.05               | 0.666± 0.05 | 0.6± 0.06   | 0.566± 0.05 | 0.483± 0.04 | 0.433± 0.05 |
| GTEE- 500 mg/kg   | 0.366± 0.05    | 0.683± 0.04              | 0.633± 0.05 | 0.533± 0.05 | 0.483± 0.04 | 0.416± 0.04 | 0.366± 0.05 |

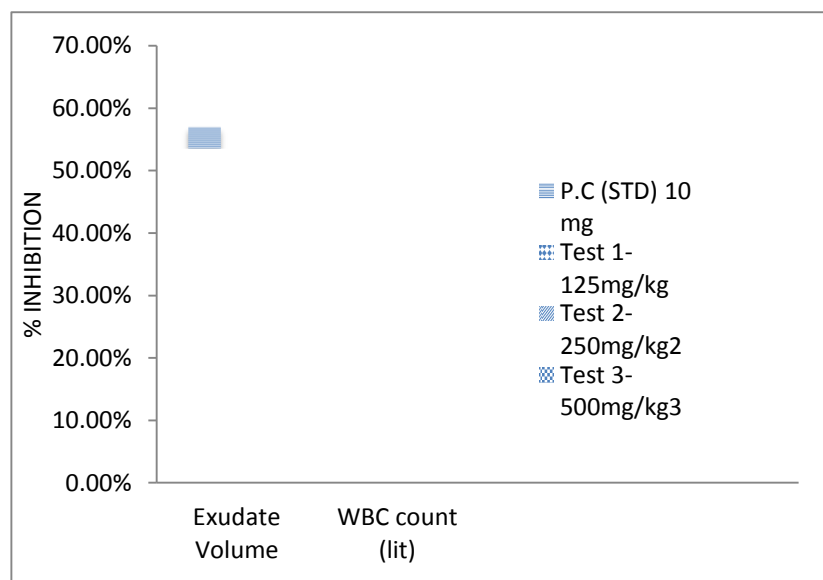
NC-Normal control, PC-Positive control, GTEE-*Guazuma tomentosa* Ethanolic Extract

The leukocytes migration was calculated the results.



| Treatment (Group) | Exudate Volume (ml) | % Inhibition | WBC x 10 <sup>9</sup> /lit | % Inhibition |
|-------------------|---------------------|--------------|----------------------------|--------------|
|                   | Mean ± S.D          |              | Mean ± S.D                 |              |
| NC 1 ml           | 0.96 ± 0.19         | -            | 7.76 ± 0.09                | -            |
| PC 10 mg/kg       | 0.40 ± 0.12         | 56.96 %      | 5.30 ± 0.07                | 65.05 %      |
| GTEE- 125 mg/kg   | 0.67 ± 0.10         | 29.05 %      | 4.21 ± 0.35                | 34.66 %      |
| GTEE- 250 mg/kg   | 0.55 ± 0.13         | 41.67 %      | 2.25 ± 0.27                | 66.63 %      |
| GTEE- 500 mg/kg   | 0.44± 0.15          | 52.01 %      | 1.12 ± 0.15                | 54.42 %      |

NC-Normal control, PC-Positive control, GTEE-*Guazuma tomentosa* Ethanolic Extract  
Ethanolic extract of *Guazuma tomentosa* L. on carrageenan induced Leukocytes migration in rats



## DISCUSSION

The inflammatory response represents a complex biological and biochemical process involving cells of the immune system and a plethora of biological mediators. Cell-to-cell communication molecules known collectively as cytokines play an extremely important role in mediating the process of inflammation. During an inflammatory response, both the level and the profile of PG production change dramatically. PG production is generally very low in un-inflamed tissues but increases immediately in acute inflammation before the recruitment of leukocytes and the infiltration of immune cells.<sup>19</sup> The acute induced-inflammatory is a fast response to a harmful agent responsible to induce the defense mechanism of the body (leukocytes and antibody) to the lesion place. The defense mechanisms are present and move according to the bloodstream. In an inflammatory process the blood vessels suffer some alterations to facilitate the movement of the leukocytes and the antibody to the lesion place, whose rate depends on the level of inflammation<sup>20</sup>. Leukocyte recruitment to the site of inflammation is a fundamental event in the inflammatory process. Cell migration occurs as a result of much different process including adhesion and cell mobility. This experiment employed three different doses of *Guazuma tomentosa* L. for anti-Inflammatory screening namely minimum dose (125 mg), Medium dose (250 mg) and Maximum Dose (500 mg). In the present study, leaves extract of *Guazuma tomentosa* L., were studied for anti-inflammatory activity by in-vivo method. The extracts were found to possess significant activity at all selected doses i.e. 125, 250 and 500 mg/kg body weight, *Guazuma tomentosa* L. showed comparable results with the standard drug. Ethanolic extracts showed significant anti-inflammatory activity. This ethanolic extracts showed maximum anti-inflammatory activity at 500 mg/kg dose. It showed maximum percentage inhibition<sup>21</sup> of 53.25%, which were comparable with the Standard, indomethacin, which showed 51.08% inhibition. In leukocytes migration method by exudate volume standard shows 56.96% inhibition, while 125, 250, and 500 mg/kg ethanolic extract of *Guazuma tomentosa* L. leaves shows 29.05%, 41.67%, and 52.01% inhibition respectively.

In leukocytes migration method by WBC Count technique, standard shows 65.05% inhibition, while 125, 250, and 500 mg/kg ethanolic extract of *Guazuma tomentosa* L. leaves shows 34.66%, 66.63%, and 54.42% inhibition. This confirming that 500 mg/kg ethanolic extract of *Guazuma tomentosa* L. leaves shows comparable activity as of standard drug which is Indomethacin. Carrageenan when injected into rat hind Paw and peritoneal cavity irritates the peritoneum and paw induces acute inflammatory reaction producing aseptic peritonitis and hind paw. According to a previous study carrageenan induces at a low dose of 0.1 % induces an edema which mainly results from aneurogenic inflammation mediated by neuropeptides such as substance P. At higher dose of 5%, carrageenan induce edema which mainly depends on the release of substance prostanoids, 5-hydroxytryptamine and histamine. All the three doses showed significant result activity as that of standard Indomethacin under the present experimental conditions.<sup>23</sup>

Therefore *Guazuma tomentosa* L. showed a comparable anti-inflammatory activity as that of the reference drug. The data in our study showed that Indomethacin and *Guazuma tomentosa* L. were effective in reducing paw edema and peritonitis induced by carrageenan. The probable mechanism for the acute anti-inflammatory action could be due to anti-bradykinin activity and inhibition of synthesis of prostaglandins and other inflammatory mediators like histamine and serotonin in early hours of inflammation.

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All the authors have contributed equally to this paper.

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