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ANTI-INFLAMMATORY ACTIVITY OF STEM AND LEAF JUICE OF *ACACIA ARABICA* WILD

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ABSTRACT: Herbal drugs constitute a major part in all the traditional systems of medicine. From the literature review, it comes to know that *Acacia arabica* stem is used as a chewing stick and claimed to be useful for bleeding gums and pain related to it. In some communities use of leaves is also known. The components are taken from live parts as they are, without allowing them to dry for a long period, which may lead to enzymatic degradation over the process of drying. The juice is dried at room temperature to avoid degradation of heat sensitive moiety if any, that may be present in the juice. This dried juice is expected to have properties which will be useful for the treatment of inflammation. The properties analyzed here are the anti-inflammatory activity of stem and leaf juice. The objective of the proposed study is to perform the phytochemical studies on the fresh juice of babul stem and leaves. It is further envisaged to study anti-inflammatory properties of the dried fresh juice.

Keywords: Anti-Inflammatory, *Acacia Arabica* Wild, Carrageenan Induced Rat Paw Oedema

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INTRODUCTION: Inflammation is an initial response of a tissue or any living cell to injury or damage. It is characterized by increased blood flow to the tissue, causing, increased temperature, Redness, Swelling, and Pain. Both *in-vivo* and *in-vitro* methods are available for the evaluation of anti-inflammatory agent, but among the *in-vivo* method the carrageenan-induced rat paw edema assay is believed to be one of the most reliable and also the most widely used. Carrageenan is a mixture of polysaccharides composed of sulfated galactose units and is derived from Irish Sea Moss. Its use as an endogen was introduced by C.A. Winter *et al.*, in 1932.

The edema which develops in rat paw after carrageenan injection is a biphasic event. The initial phase is attributed to the release of histamine and serotonin; the edema maintained between the 1st and 2nd phase to release of kinins like substances and the 2nd phase to release of prostaglandins like compound.

The carrageenan assay method is advantageous

1. The edema is specifically inhibited by anti-inflammatory compounds.
2. Single oral dose of drugs at non-toxic levels are effective.
3. Low variability.
4. Better reproducibility.
5. Carrageenan itself is neither antigenic nor causes any systemic effects.

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MATERIALS AND METHOD:

Collection of Plant Material: The plant material was collected from wildy grown mature trees along roadsides and boundaries of farmyards. The full-grown trees of heights more than 3-4m are chosen. All material collected from the fields around Nagpur city. Leaves and stem were collected separately.

Preparation of Juices: The plant parts collected are immediately washed, and stem was cut into small pieces. The stem pieces are weighed and transferred to the mixer to make a fine powder and to that measured quantity of water was added and again allowed the mixer to run for a few minutes. The juice was separated by squeezing the material through clean muslin cloth. The juice so obtained

was filtered by vacuum filtration through sintered glass funnel so that all the green and suspended material gets removed.

This clear liquid was then allowed to dry in trays at normal temperature to avoid degradation of heat sensitive constituents that might be present in the juice, till all the water got evaporated and the complete dry powder was formed showing brown color. The dry juice was transferred to airtight glass container. This container was placed inside a vacuum container to avoid the attack of moisture. The same procedure was applied for leaf juice. The percentage yield of dry juices was determined. **Table 2** indicates the extractive value of stem juice and leaf juice.

TABLE 1: EXTRACTIVE VALUES OF JUICES

| Sample code | Weight of plant part taken (g) | Quantity of water added | Quantity of juice obtained (ml) | Weight of dry juice obtained (g) | % practical yield | Average |
|-------------|--------------------------------|-------------------------|---------------------------------|----------------------------------|-------------------|---------|
| AASJ-1 | 300 | 700 | 400 | 12.3 | 4.8 | 3.2175 |
| AASJ-2 | 300 | 700 | 340 | 6.7 | 2.23 | |
| AASJ-3 | 476 | 700 | 300 | 14 | 2.94 | |
| AASJ-4 | 250 | 500 | 350 | 9 | 3.6 | |
| AALJ-1 | 310 | 500 | 310 | 12 | 3.87 | 4.5675 |
| AALJ-2 | 400 | 600 | 350 | 14 | 3.5 | |
| AALJ-3 | 200 | 500 | 330 | 13 | 6.5 | |
| AALJ-4 | 240 | 500 | 300 | 11 | 4.4 | |

AASJ- *Acacia arabica* stem juice; AALJ- *Acacia arabica* leaf juice

Procurement of Experimental Animals: Male Swiss albino rats (100-150 g) of the approximate same age are used in the present studies were procured from Institute of Pharmaceutical Education and Research, Boargaon (Wardha), India. The animals were fed standard pellet diet and water *ad libitum*. All the animals were housed in polypropylene cages. The animals were kept under an alternate cycle of 12 h of darkness and light.

The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. The animals were fasted for at least 12 h before the onset of each activity. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC No.- 648/02/c/CPCSEA) after scrutinization. The animals received the drug treatments by oral gavage tube.

Acute Toxicity Studies:

Principle: The determination of ED₅₀ (the dose effective in producing certainly expected response

in 50% of the animal group) value helps in ascertaining the potency of drugs in terms of the reference standard. Calculation of ED₅₀ value is done when a drug is showing a graded response. But when the response is quantal or all or none, the ED₅₀ value becomes LD₅₀ (the dose lethal to 50% of the animal group) both these values, *i.e.*, ED₅₀ and LD₅₀ are important for knowing the safety of the drug. The ratio between ED₅₀ & LD₅₀ (LD₅₀/ED₅₀) represents the therapeutic index. Greater the therapeutic index, safer is the drug.

The therapeutic index of most of the drugs, which have a low margin of safety, is generally closed to unity. Ideally one would like to determine a dose that is effective in most of the animals (ED₉₉) and least toxic to most of the animals of the group (LD₁), these values can, however, be calculated from the graph.

For calculating LD₅₀ by either method, find out the least tolerated (smaller) dose (100 % Mortality) and most tolerated (highest) dose (0% Mortality) by hit

& trial method. Once these 2 doses are determined, to select at least 5 doses in-between the least tolerated and most tolerated dose and observe the mortality due to these doses. Generally, mice or rats are used for this purpose, and each dose group should consist of 6 animals.

Requirements:

Animal: Male albino rats (100-150 g).

Drug: Stem juice and leaf juice of *Acacia arabica Willd*

Procedure: The overnight fasted rats were weighed and divided into 6 groups of six in each. Stem and leaf juice of *Acacia arabica Willd* has been given in various doses (500 - 5000 mg/kg body weight) by the oral route. After administration of the juice suspension, the animals were observed continuously for the 24 hours for the death due to acute toxicity.

RESULTS: Mortality was not found till dose of 500-5000mg/kg body weight in stem juice and leaf juice both.

Carrageenan-induced Paw Edema: Male Swiss albino rats weighing 100- 150 gm were used. The animals were put on a standard diet, and water was provided *ad libitum*. The animals fasted overnight before the experimentation. The rats were divided into six groups (n=5). The anti-inflammatory activity of the drug was assessed by the method described by Winter *et al.*, Rats in group I were

given normal saline and were treated as control. Rats in group II were administered Diclofenac Potassium in normal saline at the dose of 10 mg/kg body weight given intraperitoneally and were kept as standard. Rats in Group III to Group V were administered orally with the leaf juice and stem juice at the dose of 50, 100, 200 mg/kg body weight respectively. Since, the LD₅₀ has not been determined during the acute toxicity study, the doses for this study were selected by trial and error method. The standard and drugs were given orally to the animal one hour before carrageenan injection acute paw edema was induced by injecting 0.1ml of 1% (w/v) carrageenan solution, prepared in normal saline in the sub-plantar region of the left hind paw of the rat. The perimeter of paw was measured by using Vernier caliper. Measurements were taken at 0, 1, 2, 3 h after the administration of the carrageenan.

Formula:

$$\% \text{ inhibition of Edema} = \{[C-T]\}/C \times 100$$

Where C-Control Paw Edema

T-Test Paw Edema

RESULTS AND DISCUSSION:

Statistical Analysis: The result is expressed as mean ± S.E.M. and difference in mean are determined by one-way ANOVA followed by post-hoc with Dunnett's t-test; p values < 0.05 were considered as statistically significant.

Anti-inflammatory Activity of Leaf Juice:

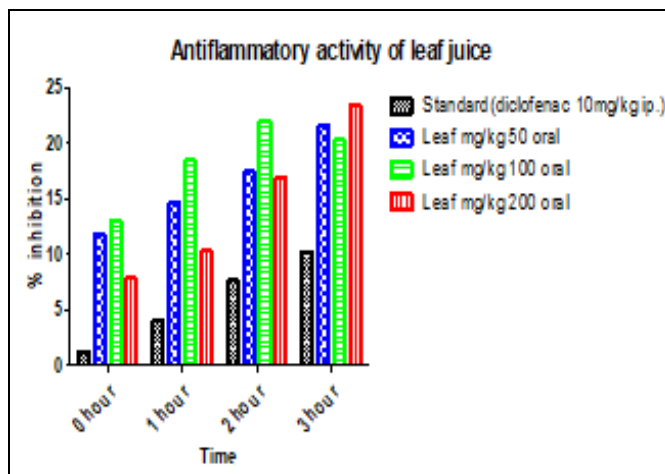


FIG. 1: ANTIINFLAMMATORY ACTIVITY OF LEAF JUICE

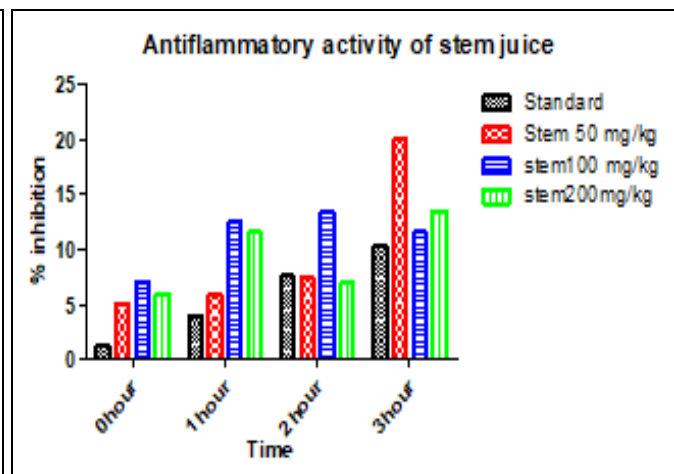


FIG. 2: ANTIINFLAMMATORY ACTIVITY OF STEM JUICE

TABLE 2: ANTI-INFLAMMATORY ACTIVITY OF LEAF AND STEM JUICE

| Groups | 0 h | 1 h | 2 h | 3 h |
|----------------|--------------|--------------|--------------|--------------|
| Control | 5.77 ± 0.057 | 5.27 ± 0.057 | 5.012 ± 0.04 | 4.59 ± 0.06 |
| Standard | 5.70 ± 0.09 | 5.06 ± 0.08 | 4.63 ± 0.12 | 4.12 ± 0.1 |
| Stem 50 mg/kg | 5.48 ± 0.07 | 4.96 ± 0.14 | 4.64 ± 0.10 | 4.34 ± 0.07 |
| Stem 100 mg/kg | 5.62 ± 0.08 | 4.61 ± 0.05 | 4.34 ± 0.03 | 4.06 ± 0.05 |
| Stem 200 mg/kg | 5.43 ± 0.05 | 4.66 ± 0.05 | 4.36 ± 0.06 | 3.97 ± 0.09 |
| Leaf 50 mg/kg | 5.09 ± 0.11* | 4.49 ± 0.14* | 4.14 ± 0.09* | 3.67 ± 0.03* |
| Leaf 100 mg/kg | 5.02 ± 0.28* | 4.29 ± 0.1*2 | 3.94 ± 0.10* | 3.67 ± 0.05* |
| Leaf 200 mg/kg | 5.35 ± 0.19* | 4.70 ± 0.06* | 4.17 ± 0.11* | 3.51 ± 0.07* |

TABLE 3: % INHIBITION TABLE

| Groups | 0 h | 1 h | 2 h | 3 h |
|----------------|--------|--------|--------|--------|
| Standard | 0.12 | 4.04 | 7.62 | 10.23 |
| stem 50 mg/kg | 5.10 | 5.92 | 7.46 | 20.04 |
| stem 100 mg/kg | 7.05 | 12.52 | 13.4 | 11.55 |
| stem 200 mg/kg | 5.98 | 11.57 | 7.02 | 13.51 |
| Leaf 50 mg/kg | 11.76* | 14.65* | 17.40* | 21.57* |
| Leaf 100 mg/kg | 13.03* | 18.44* | 21.89* | 20.26* |
| Leaf 200 mg/kg | 7.80* | 10.30* | 16.80* | 23.37* |

Values are the mean ± S.E.M. (n=6), p* < 0.05) P* < 0.05 compared to Standard one way ANOVA followed by Newman keules multiple comparisons Test

CONCLUSION:

1. For determination of dose acute toxicity study was carried out in which no mortality was found up to the dose of 5000 mg/kg body weight, and hence the doses of 50, 100, 200 mg /kg body weight were selected approximately for pharmacological studies Stem juice and leaf juice were obtained and the average % practical yields were 3.22 and 4.57 respectively
2. The juices, when subjected to phytochemical tests, were found to contain carbohydrates, tannins, steroids, and flavonoids. In the anti-inflammatory studies leaf juice at the doses of 50, 100 and 200 mg/kg body weight was effective to reduce inflammation. The activity of leaf juice was more than that of stem juice but both can be claimed to have analgesic and anti-inflammatory activity. The activity may be due to the presence of tannins, steroids, and flavonoids.

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CONFLICT OF INTEREST: Nil

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