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STUDY OF ANTI-INFLAMMATORY ACTIVITY OF AQUEOUS ROOT EXTRACT OF Citrus maxima

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ABSTRACT :

The anti-inflammatory properties of Citrus maxima root extract were assessed utilizing a carrageenan-induced paw oedema model in Wistar rats. Carrageenan-induced paw oedema is a well-known technique for evaluating acute inflammation and the efficiency of anti-inflammatory drugs. In Wistar rats, inflammation was produced by injecting 0.1 ml of 1% carrageenan solution into the sub plantar area of the right hind paw. The rats were placed into five groups: control, standard (Diclofenac sodium, 10 mg/kg), and three test groups treated with Citrus maxima root extract at dosages of 50 mg/kg, 100 mg/kg, and 200 mg/kg. Paw volume was assessed at several periods throughout a 28-day timeframe. The study shows that Citrus maxima root extract has considerable anti-inflammatory capabilities, with a dose-dependent response. The greatest dose (200 mg/kg) had anti-inflammatory effects equivalent to the conventional medication diclofenac sodium. These data imply that Citrus maxima root extract might be a natural treatment for inflammation. More study is needed to understand the bioactive chemicals and methods of action, as well as perform rigorous toxicological tests to ensure long-term safety.

Key Words: Citrus maxima, Diclofenac, Inflammation, Anti-Inflammatory agent.

INTRODUCTION :

Inflammation

Inflammation is the specific reaction of live mammalian apkins to any operator-caused injury. The body's defence reaction is to calm the dangerous operator or stop its spread, after which the necrosed apkins and cells are expelled (2). An early response of the body to a sickness, vexation, or other harm; important side symptoms include pain, swelling, greenishness, and warmth. It is explicitly stated that irritation is a subset of the nonspecific helpless reaction. Carnal apkins respond to damage through an abecedarian mechanism known as aggravation (6). We currently know a great deal about the signalling pathways that connect regular aggravation and vitality collecting (corpulence), but less about the actual typical consequence of the aggravation.

How inflammation capacities

White blood cells release chemicals into the circulatory system or apkins. As a result, the risk of injury or sickness increases due to blood inflow (22). It may have an impact on warmth and greenness. Protuberance is the expansion of a couple of the chemicals that cause fluids to seep into your apkins. This defence mechanism may injure and exasperate nerves (13).

Expanded circumstances of white blood cells and the substances they supply within your joints can ultimately lead to cartilage damage (the bumper fabric between bones) and protuberance of the usual filling.

Causes of inflammation

1. Contagious agents such as bacteria, contagions, and their toxins, as well as fungi, are the primary causes of inflammation.
2. Immunological agents are comparable to antibody-antigen reactions and cell-mediated impunity.
3. Physical agents are analogous to radiation, heat, and cold.
4. Chemical agents, such as venoms, both inorganic and organic.
5. Inadequate opportunities for physical activity.
6. Chronic stress-related illnesses.
7. Disruptions to the circadian rhythm and sleep.

Kinds of inflammation

There are two types of inflammation: acute and chronic, depending on the host's ability to fight itself and the duration of the reaction.

Acute inflammation: It lasts for a short time, is the body's natural reaction, fades away quickly, and is usually followed by healing (1).

• The body uses acute inflammation as a healthy and essential defence medium against bacteria and other external substances.

• Acute inflammation is characterized by the buildup of fluid and tube at the point of injury and the intravascular activation of platelets (e.g., acute bronchitis, dermatitis).

Habitual inflammation (Chronic inflammation):

It is of lengthier duration and occurs either after the causative condition of violent aggravation has endured for a long time, or the fortify is similar in that it includes recurrent vexation beginning in the morning (1).

Table No 1: Overview of the main chemicals released during inflammation.

Material	Prepared by	Trigger for discharge	Primary pro-inflammatory effects
Histamine	Mast cells (in most tissues), basophils; are kept in cytoplasmic granules.	Antibody binding to mast cells and basophils.	Vasodilation, pruritus Vascular permeability Smooth muscle contraction.
(5-HT) Serotonin	Basophils and mast cells	When platelets are activated and when degranulate, mast cells/basophil	Vasoconstriction Vascular permeability
Prostaglandins (PGs)	Mast cells, basophil and platelets. Also, in central nervous system.	Many kinds of stimuli. E.g. toxins, other inflammatory mediators, drugs.	Vasodilation, Vasoconstriction, Diverse.
Heparin	Basophil, liver, mast cells	When cells are degranulate heparin will be release.	Anticoagulant, that will maintain blood supply to injured tissues and wastes and also it washes away microbes.
Bradykinin	Blood and also tissues	When blood clots inside trauma and inflammation.	Vasodilation and Pain.

(NSAIDs) Non-steroidal Anti-inflammatory drugs:

These are referred to as "nonsteroidal" to differentiate them from steroids, which have a similar eicosanoid-depressing, anti-inflammatory impact among a variety of other effects. The unique feature of NSAIDS is that they are not nocturnal. The two most well-known medications in this class are ibuprofen and aspirin (24).

Rofecoxib and Valdecoxib have been taken off the market due to the detection of significant cardiovascular events linked to COX-2 inhibitors (celecoxib is still accessible for usage in patients RA).

Non-steroidal Classification of anti-inflammatory drugs:

1. Salicylates, such as aspirin
2. Derivatives of protonic acid: Ibuprofen, Naproxen, Ketoprofen, Flurbiprofen.
3. Derivatives of anthranilic acid: Mephenamic acid
4. Derivatives of aryl-acetic acid: Aceclofenac and Diclofenac

OBJECTIVES OF THE STUDY :

Using rats as test subjects, the proposed study aims to assess the anti-inflammatory properties of *Citrus maxima* root extracts.

Phase 1:

Preparation of aqueous extracts of root of the *Citrus maxima* by using Soxhlet apparatus.

Phase 2:

Determination of LD50 and selection of 3 doses for the study (i.e. 1/20th low, 1/10th medium, and 1/5th high with respect to the LD50 doses tested with each extract in rats up to maximum dose level of 2000 mg/kg).

Phase 3:

To assess aqueous extracts' ability to reduce inflammation using several experimental animal models, such as.

1. Rats with paw oedema model induced by carrageenan.
2. Rat model of paw oedema produced by histamine.
3. Rat oedema model caused by formalin.

REVIEW OF THE LITERATURE :

Name in botany**:** *Citrus maxima*
Organonomy classification:

- Family: Rutaceae;
- Order: Rosidae;
- Common name: Pomelo

A few aliases for *Citrus maxima*.

Words	Common name
Nepali	Bhogate
English	Shaddock, pumelo, pumelo
Hindu	Madhukar Kati
Italian	Pompelmo
French	Pappémousse

Overview :

The pomelo (scientific name C. grandis or C. maxima) grows mostly in Southeast Asia, with locations including Malaysia, Thailand, southern China, and Fiji. Farmers in other warm, humid climates, including Australia, the Pacific, the Caribbean, and the southern United States, have just lately began to cultivate the fruit (1).
Because of its wonderful aroma, some Asians collect pomelo blooms to make perfume. It is used medicinally in the Philippines and other Southeast Asian countries to treat epileptic seizures and chronic coughing.
When a pomelo is cross-pollinated with another pomelo, it produces a large number of seeds. Cross-pollination with mandarin or sweet oranges will not result in seedy fruit.
Roots: Pomelo plants' roots make up more than 70% of the top meter of soil. Taproots can extend up to two meters below the surface. Fibrous roots can extend much below the canopy. Their extensive root systems allow them to vigorously compete with neighbouring plants. Pomelo plants seldom cause damage to man-made structures since their individual roots are smaller than those of other fruit trees (11). Given how frequently citrus is watered underground across the world, it is unlikely that citrus roots would cause problems with pipelines.

Chemical Components that are present in *Citrus maxima*
Phytochemicals are classified into various chemical groups, including phenols, alkaloids, saponins, sugars, and flavonoids. There include carotenoids, terpenoids, amino acids, glycosides, and anthraquinones. Alkaloids (24). The bulk of the plant's components, including the stem, blossom, peel, root, and bark, were discovered to contain alkaloids. Some of the isolated acridone alkaloids are Citpressine-I and II, 5-hydroxynoracronycine, bunta-nine, citracridone-I, II, and III, citrus nine-I, gran-disine I and II, glycocitrine-I, Natsu Citrine-II, and prenylcitpressine (14). Examples of alkaloids include geibalansine, pumiline, p-synephrine, baiyumine-A and -B, caffeine, citbismine-A, -B, and -C, and citropten and -B.

METHODOLOGY :

Table No.2: Materials:

1.	Metabolic cages
2.	Vernier calipers
3.	Soxhlet Apparatus
4.	Analytical Balance
5.	Microscope
6.	China disc
7.	Beaker
8.	Gavage needle

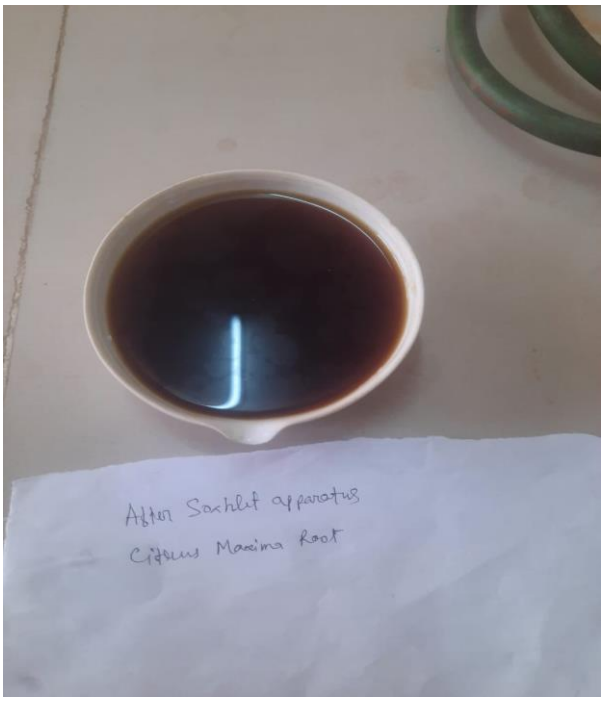
Methods:

1. Preparation of Plant Material: The roots of *Citrus maxima* were cleaned and dried and grinded into a fine powder.

2. Weighing: 20 grams of the powdered roots are weighed and placed into a filter paper.

3. Setup: The filter paper is placed containing the plant material into the Soxhlet extractor. Water is added into the round-bottom flask.

4. Extraction: The solvent is heated to its boiling point. The solvent vapor will rise through the condenser, condense, and drip onto the plant material, extracting the bioactive compounds.
5. Cycling: The solvent will be siphoned back into the flask and reheated, creating a continuous cycle until the extraction is complete (typically 6-12 hours).
6. Post-Extraction: Once the extraction is complete, the filter paper is removed and the solvent is evaporated from the extract to obtain the concentrated extract.



7. Storage: The extract is stored in a suitable container for further analysis or use.

Phytochemical screenings:

Phytochemical Constituents	**Constituents in *Citrus maxima* root extract**
Polyphenols	Present
Flavonoids	Present
Alkaloids	Present
Proteins	Absent
Terpenoids	Present
Sterols	Present
Simple sugars	Absent
Carotenoids	Present
Lignin	Absent
Coumarins	Present
Glycosides	Present

Study Protocol:

1. Induction of Inflammation
2. Administration of Extract
3. Measurement of Inflammation
4. Data Collection and Analysis

Screening method for anti-inflammatory activity

Carrageenan-Induced Paw Oedema model in Rats

Concentration: 1% carrageenan in saline
Volume: 0.1 ml injected into the sub plantar region of the rat's paw

Standard Anti-Inflammatory Drugs:

1. Diclofenac Sodium: 10 mg/kg, orally
2. Indomethacin: 2.72 mg/kg, orally
3. Aspirin: 200 mg/kg, orally

Citrus maxima Extract:

1. Extract Preparation: Derived using Soxhlet extraction with aqueous solvent.
2. Administration:

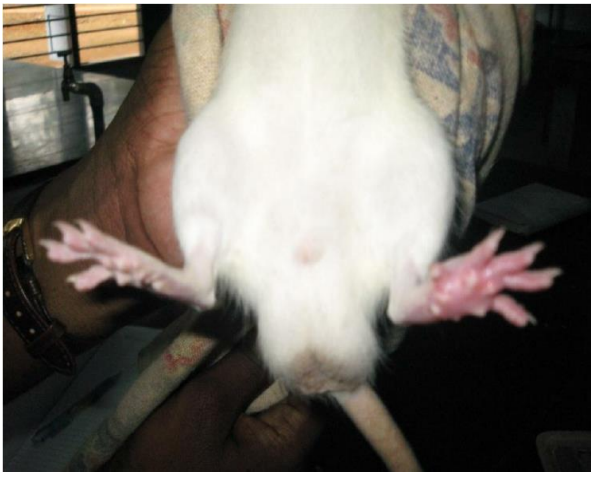
Dose: Start with a preliminary dose range (e.g., 50 mg/kg, 100 mg/kg, and 200 mg/kg)
Route: Orally (PO) or intraperitoneally (IP).

Table No 3: The following study is done by selecting 30 healthy Wistar rats and divided into 5 groups as follows:

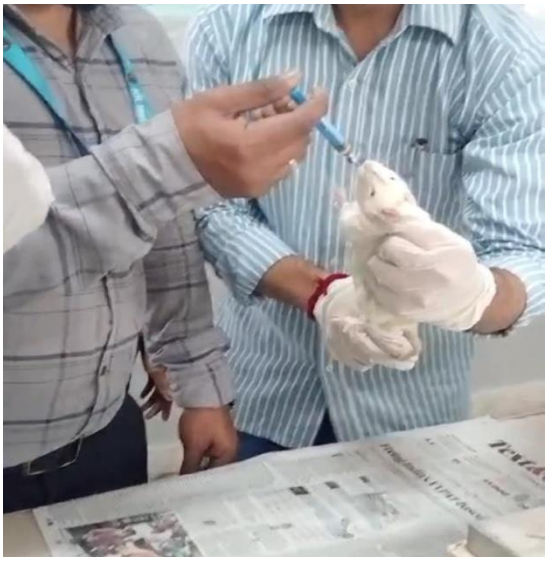
	Group 1	**Group 2**	**Group 3**	**Group 4**	**Group 5**
Group	Control Group	Carrageenan + Diclofenac Sodium (Standard)	Carrageenan + *Citrus maxima* Extract (Low Dose)	Carrageenan + *Citrus maxima* Extract (Medium Dose)	Carrageenan + *Citrus maxima* Extract (High Dose)
Treatment	Saline solution	Diclofenac sodium, (10 mg/kg), orally	*Citrus maxima* extract, (50 mg/kg), orally	*Citrus maxima* extract, (100 mg/kg), orally	*Citrus maxima* extract, (200 mg/kg), orally
No. of rats	6	6	6	6	6

Procedure:

1. Induce inflammation by injecting 0.1 ml of 1% carrageenan into the rat's hind paw.



2. -Control Group: The saline is administered orally.



-Standard Drug Group: Diclofenac sodium (10 mg/kg) is administered intraperitoneally.

-Test Groups: Citrus maxima extract at doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg are administered.

3. Measurement: The paw volume can be measured by using a Plethysmometer or Vernier Callipers. Also, it can be measured by inserting the paw into a tube containing fluid and analysing the rise in the level of fluid and specific gravity of that fluid.

RESULTS :

The detailed study of Carrageenan-Induced Paw Oedema in Rats. The initial paw volume was 0.5 ml and the changes in paw volume are observed through the tabular column given below:

Table No 4: 0.1 ml of 1% carrageenan solution is injected into the paw of Wistar rats and the changes in the volume of the paw was observed.

Group	**1 hr**	**2 hr**	**3 hr**	**4 hr**	**5 hr**
Control	1.33 ml	1.21 ml	1.11 ml	1.03 ml	0.96 ml
Diclofenac (10 mg/kg)	0.80 ml	0.72 ml	0.71 ml	0.65 ml	0.54 ml
Citrus maxima (50 mg/kg)	0.86 ml	0.83 ml	0.80 ml	0.71 ml	0.60 ml
Citrus maxima (100 mg/kg)	0.83 ml	0.80 ml	0.75 ml	0.65 ml	0.59 ml
Citrus maxima (200 mg/kg)	0.81 ml	0.76 ml	0.70 ml	0.67 ml	0.58 ml

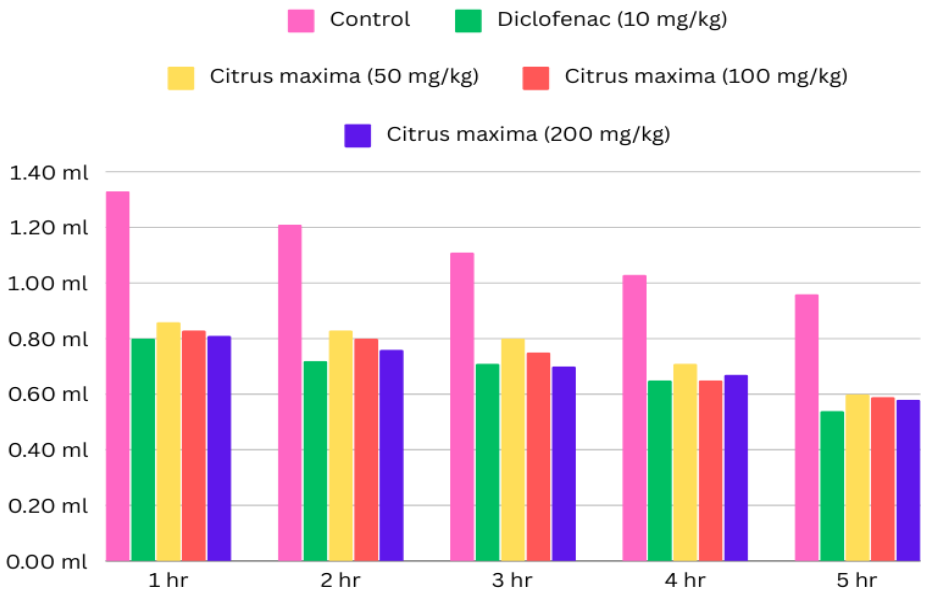


Figure 1: Graphical representation anti-inflammatory activity in rats

CONCLUSION :

The anti-inflammatory properties of *Citrus maxima* root extract were examined utilizing a carrageenan-induced paw oedema model in Wistar rats. The findings revealed that *Citrus maxima* extract had strong anti-inflammatory action in a dose-dependent manner (12).

Citrus maxima extract shows effects comparable to those of the common anti-inflammatory drug Diclofenac sodium (10 mg/kg) at its maximal dose of 200 mg/kg. Paw volume was significantly reduced by both treatments, demonstrating strong anti-inflammatory effects. The observed dose-dependent response suggests that the root extract of Citrus maxima contains potent bioactive compounds with anti-inflammatory properties (2). These results point to the potential use of Citrus maxima as a supplement or natural substitute for traditional anti-inflammatory medications.

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