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ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC EXTRACT OF *LUFFA ACUTANGULA*

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ABSTRACT

The present study investigates the anti-inflammatory activity of ethanolic extract *Luffa acutangula* (fruit) using carrageenan induced paw edema in wistar albino rats. The medicinal values of the *Luffa acutangula* has been mentioned ancient literature as useful in the treatment of disorders of inflammation. Dried fruits of *Luffa acutangula* were powdered and extracted with ethanol using Soxhlation method. The anti-inflammatory activity was done by carrageenan induced hind paw edema method using Plethysmometer. Indomethacin used as a standard drug. For this activity Control group receive only carrageenan, Standard group receive indomethacin (40mg/kg), induced 0.1 ml carrageenan, test group receive ethanolic Fruit extract of *Luffa acutangula* (500mg/kg). The result showed that ethanolic extract of *Luffa acutangula* fruit exhibited statistically significant ($p<0.05$) inhibition of paw volume at a dose of 500 mg/kg. However, maximum inhibition of paw edema was found to be in Group II 91.70% and although the inhibition of paw edema with the extract was 72.73% which is less than standard group but higher than that of control group.

KEYWORDS: Ethanolic fruit extract, Anti-inflammatory activity, excision wound, Plethysmometer.

INTRODUCTION

Inflammation is a local response of living mammalian tissues to injury due to any agent. It is a body defense reaction to prevent the spread of injurious agent and to remove the necrosed cells and tissues.[1] Inflammation is a normal protective response of body to tissue injury that can be caused by physical trauma, noxious chemicals or microbiologicalagents etc.

Inflammation is the result of concerted involvement of alarge number of vasoactive, chemotactic and proliferative factors at different stages of inflammation and there are many targets for anti inflammatory action. The mechanisms of inflammation involve a series of eventsin which the metabolisms of arachidonic acid plays an important role. Prostaglandins have a important role in the complex process of inflammationand they are responsible for the pain. They can be metabolized by the different pathways like cyclo-oxygenase (COX) pathway to prostaglandins and thromboxaneA2, or by the lipoxygenase (LOX) pathway to hydroperoxyeicosatertraenoicacids (HPETE'S) and leukotrienes (LT's), these areimportant biologically active mediators in a variety of inflammatory events.[2,3]

Inflammatory response is a cascade of biochemical events which propagates and matures, involving the local vascular system, the immune system and various cells within the injured tissue. It is characterized by five cardinal signs: Dolor (pain), Calor (heat), Rubor (redness), Tumor (swelling), Functiolaesa (loss of function).[4]

Specific patterns of acute and chronic inflammation are seen during particular situations that arise in the body called morphologic patterns such as when inflammation occurs or pyogenic bacteria are involved, like Granulomatous inflammation (Characterized by the formation of granulomas), Fibrinous inflammation (in this inflammation a large increase in vascular permeability occurs which allows fibrin to pass through the blood vessels), Purulent inflammation(in this type of inflammation large amount of pus is formed, which consists of neutrophils, dead cells, and fluid), Serous inflammation(it is characterized by the copious effusion of non-viscous serous fluid), Ulcerative inflammation(this type of inflammation occurs near an epithelium can result in the necrotic loss of tissue from the surface, exposing lower layers). In addition to the local changes in an inflammatory area, there are often general systemic manifestations of inflammatory disease. In an inflammatory area, in addition to the local changes there are often general systemic manifestations of inflammatory disease like fever, an increase no. of WBC and the release of acute-phase proteins from liver for ex., C-reactive protein, α2-macroglobulin, fibrinogen, α1-antitrypsin and complement components. C-reactive protein, for example, binds to some microorganisms, which activates complement components.[5,6]

Plant Profile: Plants have been in use for treating various ailments from the pre historic times and still useful to protect against various kind of ailments. The earliest record of herbal treatment can be traced to ancient Chinese and Greek texts. Unani and Ayurvedic systems

also used a large number of plants for the treatment of various ailments.[7] One such plant, *Luffa acutangula*, is a large monoecious, annual climber, found wildand also cultivated throughout the greater parts of India. It contains crystalline bitter principle which is very much similar to cucurbitacin B, luffin and colocynthin.[8] Its seeds show presence of both saturated and unsaturated fatty acid like palmatic, stearic, oleic, linoleic and small amount oflignoceric acid while fruits contain cucurbitacin B, E and oleanalic acid.[9]

Leaves of this plant are orbicular, pale green in colour and fruits are baseball club shaped in structure. The plant possesses laxative, purgative, abortifacient and antifungal property. A survey done in hilly areas of Maharashtra (ethno medico survey) concluded that fruits of *Luffa acutangula*are used in protection from jaundice when taken in the form of very fine powder through nasal route.[10] While the seeds possess emetic, expectorant, and demulcent property.[11,12]

Scientific Classificationof Luffa Acutangula

Kingdom: Plantae

Division: Magnoliophyta.

Class: Magnoliopsida.

Order: Cucurbitales.

Family: Cucurbitacece.

Genus: Luffa.

Species: acutangular.

Vernacular names are turai, satputiya, zinga, turiya, kadawa, gantali, kosataki, ksweda, peerkku.[13]

Growth and Distribution: it is commercially known for its unripe fruits used as a vegetable. Mature fruits can be used to make cleaning sponges. Its fruit shape resembles a cucumber with ridges. It ranges from central Asia and eastern Asia to southeastern Asia. The Luffa acutangulais indigenous to Western, Central and Southern regions of India, and regarded as wild variety ofcultivated species. It is a monoecious, annual, climbing herb, with acutely 5-angled stem. Leaves are alternate, simple, stipules are absent, petiole can be up to 15 cm long, blade broadly ovate to kidney-shaped, 10–25 cm × 10–25 cm, shallowly palmately 5–7-lobed with broadly triangular to broadly rounded lobes, cordate at base, shallowly sinuate-dentate, palegreen, scabrous, palmately veined. Male inflorescence racemose with 15–35 cm long peduncle. Flowers are unisexual, regular, expanded above, 0.5 cm long, lobes triangular,

1–1.5cm long, petals are free, pale yellow in color, male flowers have 3 free stamens, female flowers solitary, on pedicels 2–15 cm long, with inferior, densely pubescent, longitudinally ridged ovary, stigma has 3-lobe. Fruit has a club-shaped, dry and fibrous capsule 15–50 cm × 5–10 cm, acutely 10-ribbed, brownish in color, dehiscent by an apical operculum, many-seeded. Seeds broadly elliptical in outline, compressed, up to 1.5 cm long, smooth, dull black.[14,15,16]

Medicinal Uses: Ayurveda has attributed ridge gourd with a number of health benefits which current clinical research is supporting as well. The ridge gourds are rich in minerals and are very alkaline for the body and hence they have a cooling effect on the body. From Ayurveda point of view, ridge gourd increases vata and kapha, but it cools down and pacifies the dosha pitta in the body. All parts of the ridge gourd plant, fruits, leaves, seeds and even roots are used for their medicinal value.

In spite of their bland taste, ridge gourds have many health benefits

- ✓ It acts as an appetizer
- ✓ It contains a good amount of fiber, vitamins and minerals including Vitamin B2, Vitamin C, carotene, niacin, calcium, phosphorus, iron ad small quantities of iodine and fluorine.
- ✓ Ridge gourd is used as an expectorant and hypoglycemic, bitter tonic, enlargement of spleen and also prevention of premature greying of hair.
- ✓ The roots of are helpful in the removal of kidney stones, swelling of the lymph glands.
- ✓ The leaves useful in the treatment of dysentery conditions, dressing in the diseases such as inflammation of spleen, ringworms, piles and even leprosy.
- ✓ Seeds of ridge gourd are used as a laxative and purgative. Oil is extracted from the seeds of ridge gourd which is used in the treatment of skin diseases.[17]

MATERIALS AND METHODS

The present study was carried out to evaluate the anti-inflammatory activity of *Luffa acutangula*. Qualitative analysis was done by using ethanolic extracts of the fruits. The details of the material used and methods followed are described below.

Collection of plant materials: Fruit pulp of *Luffa acutangula*were collected in the month of sep, 2017 from local areas of Indore (Madhya Pradesh). Fruit pulp of Fresh fruits of *Luffa acutangula*were crushed and used for the study.

Chemicals and Reagents: Ethanol, Fehling's reagent, Hydrochloric acid, sulphuric acid, Ferric chloride, acetic anhydride, chloroform, Mayer's reagent, glacial acetic acid, ammonia, magnesium, Anthrone reagent, Bradford reagent.

Extraction: The fruits were dried under shade and then powdered and 25gm of powder sample was extracted with 125ml of ethanol (1:5) by using soxhlet apparatus. The whole apparatus was kept over a heating mantle and was heated continuously for 4 hours at boiling point of solvent. The extract was concentrated to dryness and the residues were transferred to a preweighed sample bottle and were stored in desiccators for further studies.

Qualitative analysis: Different biochemical parameters like reducing sugar, Flavonoid, Terpenoid, Tannin, Saponin, Anthraquinone, glycosides, alkaloids etc. were tested.

Test for Flavonoids: The extract and add a few magnesium turnings, followed by the addition of con. HCl drop by drop. Pink colour indicates the presence of flavonoids.

Test for Steroids and Terpenoids: Extract, dry and dissolve in chloroform. Add a few drops of acetic anhydride and conc.H2SO4 and keep undisturbed for few minutes. Formation of green colour indicates the presence of steroids, while pink colour indicates the presence of terpenoids.

Test for Saponin: To extract was shaken with 5ml of distilled water and was heated to the boiling point. Frothing indicates the presence of saponin.

Test for Tannins: To extract, add 2 drops of 5% FeCl3. Presence of dirty green precipitate indicates the presence of tannin.

Test for Reducing Sugars: The aqueous extract was added to boiling Fehling solution in a test tube, a brick red colour indicates the presence of reducing sugars.

Test for Alkaloids: 5 ml of extract evaporated to dryness. Residue heated on a boiling water bath with 2% HCl. Then filtered, treated Mayer's reagent. Yellow precipitate indicating the presence of alkaloid.

Test for Anthraquinones: To powdered material add 10 ml of 1% HCl and boiled for 5 minutes. Filter the sample and allowed to cool. Partition the cool filtrate against equal volume of chloroform. Carefully transfer the chloroform layer into clean test tubes. Shake with equal

volume of 10% ammonia solution and allow the layer to separate. Presence of delicate rose pink colour indicates the presence of combined anthraquinones.

Test for Glycosides: To 0.5 gm of extract diluted to 5ml with distilled water and add 2ml of glacial acetic acid and containing one drop of ferric chloride solution. This was underplayed with 1ml of conc. H2SO4. Brown rings at the interface the presence of glycosides.[18,22]

Safety Profile: The ethanolic extract of the leaves of *Luffa acutangula was* found to be safe in Wistar rats upon single exposureup to dose of 2000mg /kg orally. Acute toxicity study of ethanolic extract *Luffa acutangula* fruits hasshown that, it is safe up to 2000mg/kg in Wistar rats.

Experimental Animal: Male Albino rats weighing 200-250 gm were for animal studies. The animals were grouped in polyacrylic cages andmaintained under standard laboratory conditions. They were allowed free access to standard dry pellet diet and water ad libitum. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) constituted under CPCSEA. The rats were acclimatized to laboratory condition for 14 days beforecommencement of experiment.[23,28]

Chemicals: Carrageenan, Standard drug (Indomethacin).

Experimental Design: Acute inflammation is provided by injection of 0.1 ml of carrageenan into the sub plantar region of rat hind paw.

Group I: Received saline solution orally served as Control and 0.1 ml of carrageenan in left paw.
Group II: Received indomethacin 10mg/kg orally served as Standard and 0.1 ml of carrageenan in left paw.
Group III: Received Ethanolic extract of *Luffa acutangula* 500mg/kgorally served as 0.1 ml of carrageenan in left paw.

RESULTS AND OBSERVATION

Extraction

The phytochemicals present in the plant material was extracted by the use of soxhlet apparatus. The solvent, ethanol was used for the separation of chemical component.

Phytochemical analysis

Standard phytochemical screening for flavonoid, glycosides, alkaloids, tannin, saponins were done. The qualitative phytochemical investigations of *Luffa acutangula(fruit)* ethanolic extract showed the presence of steroids, flavonoids, saponins, tannin, alkaloids and anthaquinones reducing sugars in the ethanol extracts.

Table. 1: Phytochemicals of *Luffa acutangula* (Fruit).

Test	Test Result
Flavonoids	+
Steroid	+
Saponin	+
Tannin	+
Reducing sugar	+
Alkaloids	+
Anthaquinones	+
Glycosides	-

Anti- inflammatory activity: The anti-inflammatory activity of ethanolic extract was determined according to the method given in Vogel & Vogel 1997 all the suspensions were administred 30min before the induction of edema by administer in 0.1% carrageenan in saline. The degree of paw edema of all the groups were measured by using plethysmometer at 5 hr of carrageenan administration to each group.

% Inhibition was calculated by formula % inhibition (treated) = $(V_5 – V_0 / V_0) * 100$

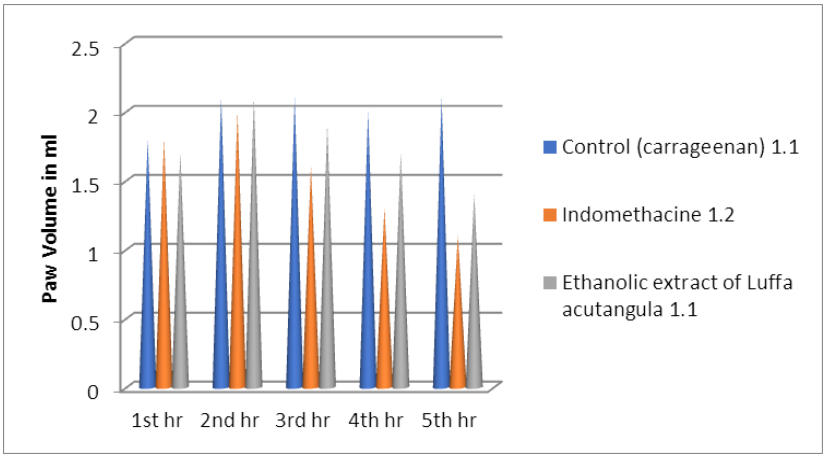
V_5= Right paw volume at 5^{th} hr after carrageenan administration

V_0=Right paw volume before hr carrageenan administration

Table. 2: Anti-inflammatory activity of *Luffa acutangula* fruit ethanolic extract in experimental rats.

Group	Initial paw volume (ml)	Paw volume after induction (ml)				
		1st hr	2nd hr	3rd hr	4th hr	5th hr
Control (carrageenan)	1.1±0.05	1.8±0.10	2.1±0.13	2.1±1.1	2.0±0.11	2.1±0.13
Indomethacine	1.2±0.89	1.8 ±0.11	2.0± 0.84	1.6 ± 0.94	1.3± 1.41	1.1 ± 0.71
Ethanolic extract of *Luffa acutangula*	1.1± 0.07	1.7 ± 0.89	2.1± 0.12	1.9 ± 0.81	1.7 ±0.12	1.4 ±0.89

N = 5, no of rats in each group.



Graph. 1: Anti inflammatory response on paw volume.

The values obtained from each group were expressed as Mean ± Standard deviation. Dunnet's test was done to compare the statistical significant changes between controls, (Carrageenan induced paw edema) and indomethacin treatment rats and with *Luffa acutangula* fruit ethanolic extract treatment. The significant levels between the groups was Compared using row wise comparison between Initial with different hours.

Table. 3: Percentage of Inhibition.

Group	Initial paw volume	Paw volume After 5th hr	Difference in paw volume (ml)	Inhibition percentage
Control (carrageenan)	1.1±0.52	2.1±0.13	1.0	09.10
Indomethacine	1.2±0.89	1.1±0.71	0.1	91.70
Ethanolic extract of *Luffa acutangula*	1.1±0.07	1.4±0.89	0.3	72.73

The effect of ethanolic extract of *Luffa acutangula* was studied inwistar albino rats by seeing its anti inflammatoryactivity where inflammation induced by Carrageenan. The experiment results showed that (Table2) the ethanolic extract exhibited statistically significant at doses of 500 mg/kg within 5th hr of administration of *Luffa acutangula.* The effect of ethanolic extract of *Luffa acutangula*on carrageenan-induced rat paw edema at different hours of study wascompared to that of control for the evaluation of anti inflammatory activity on the basis of percent inhibition of paw edema volume.

The Group I is carrageenan induced group in which results showed an elevated level of paw volume in each hour upto 2nd hr. At the end of the 5th hr the paw volume is higher than the Initial Paw Volume. In Group II the standard Indomethacin is intraperitoneally received which gives low paw volume in each hr from 2st to 5th hr. Finally at the end of 5th hr paw

volume shows least value. The Group III the Carrageenan is subcutaneously induced along with the oral administration of *Luffa acutangula* fruit ethanolic extract of 500mg/kg/i.p. Here the 1st and 2nd hr shows elevated values of Paw Volume. After that the values were lowered in 3rd, 4th, 5th hrs respectively.

The experiment results showed (Table 3) that the ethanolic extract of *Luffa acutangula* fruit exhibited statistically significant ($p<0.05$) inhibition of paw volume at a dose of 500 mg/kg. However, maximum inhibition of paw edema was found to be in Group II 91.70% and although theinhibition of paw edema with the extract was 72.73% which is less than standard group but higher than that of control group.

DISCUSSION

In spite of excellent development in the field of synthetic drugs during recent era, they are found to have major or minor side effects, whereas plants still hold their own unique place, by the way that they have no side effects. Therefore, a systematic approach should be made to find out the efficacy of plants against inflammation so as to exploit them as herbal anti-inflammatory agents with no side effects with high efficacy. The potential effect of the ethanolic extract of *Luffa acutanguta* fruit was investigated. Recent studies suggest that the inflammatory tissue damages are due to the reactive oxygen species which are generated during phagocytosis when phagocytes invading the inflammation sites.

In present study we carried out several tests to evaluate the anti-inflammatory activity of *Luffa acutangula* fruit. Qualitative and Quantitative phytochemical analysis were done. From the results we found that it contains many effective compounds like flavonoids, alkaloids, tannin, anthroquinone etc. Flavonoids have been shown to possess various biological properties related to antioxidant, antinociceptive, and anti-inflammatory mechanisms by targeting reactive oxygen species and prostaglandins which are involved in the late phase of acute inflammation and pain perception. Therefore, it may be said that flavonoids may play importantrole in anti-inflammatory effect of the extract besides other compounds.

CONCLUSION

The overview of *Luffa acutangula* revealed that it is a source of many therapeutically important nutrients and chemical constituents such as luffangulin, cucurbitacin, oleanolic acid, myristic acid, amino acids, oligosaccharides etc. Studies have showed its use in diabetes, Immunomodulation, Tumor suppression, Parkinsonism, Antimicrobial, Ulcer and

Hepatoprotection. From the results of the present study it can be concluded that the ethanolic extract of the *Luffa acutangula* fruits have a significant anti-inflammatory effect on paw edema induced by carrageenan in wistar albino rats.

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