Available online on 15.08.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Phytochemical Screening and *In Vivo* Anti-inflammatory Activity of Hydroalcoholic Extract of *Luffa acutangula* (L) Roxb

Shloke Kumar Dwivedi *[1], Sailesh Kumar Ghatuary[1], Satkar Prasad[1], Prabhat Kumar Jain[2], Geeta Parkhe[2]

[1]RKDF School of Pharmaceutical Science, Bhopal (M.P.)

[2]Scan Research Laboratories, Bhopal (M.P.)

ABSTRACT

Natural products are always helpful in the maintenance of life and good health. *Luffa acutangula* (L) Roxb (*L. acutangula* Cucurbitaceae) ranges from central and eastern Asia to south eastern Asia and is commercially grown for its edible unripe fruits, which are cooked and eaten as vegetable in Bangladesh and many parts of India. It is commonly known as ridge gourd, sponge gourd or angled luffa, Karvitura i in *hindi* and dodake in *marathi* .The plant possesses various medicinal properties such as treatment of jaundice, splenic enlargement and laxative. Inflammation is a reaction of a living vascularised tissue to an injury. Conventional or synthetic drugs used in the treatment of inflammatory diseases are inadequate, it sometimes have serious side effects. So number of herbal medicines is recommended for the treatment of inflammation that has no side effects. The present study is aimed to evaluate the anti-inflammatory activity of *L. acutangula* on carrageenan-induced rat paw edema method in rats as for controlling inflammatory disorders. Acute toxicity of the extract (2000 mg/kg) was examined in wistar rats for 14 days. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics and flavonoids were determined by the well-known test protocol available in the literature. Phytochemical analysis revealed the presence of phenols, flavonoids, tannins, saponins, alkaloids ect. The total phenolics content of *L. acutangula* extract was (0.897mg/100mg), followed by flavonoids (0.765mg/100mg) respectively. Hydroalcoholic extract up to 2000 mg/kg did not produce any toxic effects. The hydroalcoholic extract of *L. acutangula* (100 and 200 mg/kg) inhibited the inflammation induced by carrageenan in rats in a dose dependent manner. The hydroalcoholic extract of *L. acutangula* possesses a strong anti-inflammatory activity and may be considered an interesting source of effective anti-inflammatory compounds.

Keywords: *Luffa acutangula* (L) Roxb, Acute toxicity, Anti-inflammatory effect, Phytochemical screening, Flavonoid, paw edema

Article Info: Received 14 June 2019; Review Completed 22 July 2019; Accepted 27 July 2019; Available online 15 August 2019

Cite this article as:

Dwivedi SK, Ghatuary SK, Prasad S, Jain PK, Parkhe G, Phytochemical Screening and *In Vivo* Anti-inflammatory Activity of Hydroalcoholic Extract of *Luffa acutangula* (L) Roxb, Journal of Drug Delivery and Therapeutics. 2019; 9(4-s):390-393
http://dx.doi.org/10.22270/jddt.v9i4-s.3342

***Address for Correspondence:**

Shloke Kumar Dwivedi, RKDF School of Pharmaceutical Science, Bhopal (M.P.)

INTRODUCTION

Since, the main concern of the general public and science is in finding new natural and therapeutically active agents; scientists all over the globe have started screening plants for searching new phytochemicals[1]. Inflammation is one of the most important physiological reactions of a body to stimuli such as irritation, trauma, tissue injury and infection, but excessive or persistent inflammation results in a variety of pathological conditions or organ damage[2]. Usually, inflammation develops through infiltration of leukocytes to the injury sites and production of specific cytokines such as IL-1b and TNF-a. Reactive oxygen species (ROS) also are released during the inflammation process to exert a protective effect against invading pathogens[3,4]. Chronic anti-inflammatory diseases including rheumatoid arthritis are still one of the main health problems of the world's population. At present, although synthetic drugs are dominating the market but element of toxicity that these drugs entail, cannot be ruled out. Their prolonged use may cause severe adverse effects on chronic administration[5], the most common being gastrointestinal bleeding and peptic ulcers[6]. Consequently there is a need to develop a new anti-inflammatory agent with minimum side effects. Search for safe and effective anti-inflammatory agents have been given priority in scientific research in herbal system of medicine.

The plant *L. acutangula* (Cucurbitaceae), commonly known Ridge Gourd in English and Kadudodaka in Marathi, is fairly large climber found in Western, Central and Southern India and regarded as the wild form of cultivated species. In Ayurveda fruits and seeds of *L. acutangula* used to treat jaundice, biliousness, bronchitis and asthma[7]. *L. acutangula* has been shown to possess CNS depressant activity[8], in vitro

antioxidant activity[9], and larvicidal activity[10]. Phytochemical studies and documented report have indicated that *L. acutangula* contains β-carotenes[11], flavonoids[12], acutosides A-G, oleanane type triterpene saponins[13], acutosides H-I, oleanolic acid saponins[14]. Since hydroalcoholic extract of *L. acutangula* was shown to possess protective potential against the free radicals activity, as evidenced by in vitro antioxidant effect[10], Therefore, the present study was designed to investigate anti-inflammatory activities of hydroalcoholic extract of leaves of *L. acutangula* by using carrageenan-induced rat paw edema model.

MATERIALS AND METHODS

Plant material

Fresh leaves of *L. acutangula* were collected from area adjoining forests of Bhopal in the month of October, 2018.

Chemical reagents

Diclofenac sodium (Themis Pharmaceuticals, Mumbai), carrageenan (Sigma Chemical Co, St Louis, MO, USA) were used in present study. All other chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Extraction of plant material

Leaves of *L. acutangula* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place. 65 gm of dried powdered leaves of *L. acutangula* has been extracted with 80% methanol using maceration process for 48 hrs. The extraction procedure was ensured by pouring a few drops of extract from thimble left no residue on evaporation. After complete extraction the solvent was evaporated and concentrated to dry residue and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts[15].

Phytochemical screening

Phytochemical screening to detect the presence of bioactive agents was performed by standard procedures[16, 17]. After the addition of specific reagents to the solution, the tests were detected by visual observation of color change or by precipitate formation.

Total phenolic contents

The total phenolic content was determined using the method of *Olufunmiso et al*[18]. A volume of 2 ml of *L. acutangula* leaves extracts or standard was mixed with 1ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (75g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The blue colour developed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/g).

Total flavonoid contents

The total flavonoid content was determined using the method of *Olufunmiso et al*[18]. 1 ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 60 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/g).

Animals

In the present investigation the Wistar rats (150-200 gm) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

Acute oral toxicity

Preliminary experiments were carried out on rats (n=6). Hydroalcoholic extract leaves of *L. acutangula* were administered orally in different doses to find out the range of doses which cause zero and 100 % mortality of animals. Acute oral toxicity was conducted according to the method of Organisation for Economic Co-operation and Development (OECD) [19]. Animals were kept fasting providing only water, extract were given p.o. in doses of 500, 1000 and 2000 mg/kg/p.o. administered orally for 4 days of different groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-inflammatory effect.

Carrageenan induced hind paw oedema

Experimental designs

Group –1: Control

Group –2: Indomethacin (10 mg/kg, bw, Standard)

Group –3: Hydroalcoholic extract leaves of *Luffa acutangula* (100mg/kg, p.o.)

Group –4: Hydroalcoholic extract leaves of *Luffa acutangula* (200mg/kg, p.o.)

Anti-inflammatory activity was measured using carrageenan induced rat paw oedema assay. The rats were divided into 4 groups of 6 animals each (plant extract was dissolved and administered per oral at different dose levels). Group 1 was treated as control (0.1 ml of 1% (w/v) of was treated with carrageenan (1%w/v) in saline in the sub planter region of the right hind paw), Group 2 was administered Indomethacin (10 mg/kg, bw) and considered as standard. Group 3 were treated with hydroalcoholic extract leaves of *L. acutangula* (100mg/kg, p.o.). Group 4 were treated with Hydroalcoholic extract leaves of *L. acutangula* (200mg/kg, p.o.). Oedema was induced by injecting 0.1 ml. of a 1% solution of carrageenan in saline into the sub plantar region of the right hind paw of the rats. The volumes of oedema of the injected and the contralateral paws were measured after the induction of inflammation using a plethysomgraph to calculate the percentage of paw oedema inhibition[20].

$$\text{Percentage Inhibition} = \frac{Vc-Vt}{Vc} \times 100$$

Where, Vc- Edema volume of control group, Vt- Edema volume of test group

Statistical analysis

All analysis was performed using graph pad prism for Windows. All statistical analysis is expressed as mean ± standard error of the mean (SEM). Data were analyzed by one way ANOVA, where applicable $p<0.05$ was considered statistically significant, compared with vehicle followed by Dunnett's test.

RESULTS AND DISCUSSION

The crude extracts so obtained after maceration extraction process was concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extract. The yield of extracts obtained from different samples using Pet. ether, hydroalcoholic as solvents are depicted in the Table 1. The results of qualitative phytochemical analysis of the crude powder of leaves of *L. acutangula* are shown in Table 2. The determination of the total phenolic content, expressed as mg gallic acid equivalents and per 100 mg dry weight of sample. TPC of hydroalcoholic extracts of leaves of *L. acutangula* showed the content values of 0.897. The total flavonoids content of the extracts was expressed as percentage of quercetin equivalent per 100 mg dry weight of sample. The total flavonoids estimation of hydroalcoholic extracts of leaves of *L. acutangula* showed the content values of 0.765 Table 3 and Fig. 1&2. No adverse changes and mortality were observed in animals, which orally received hydroalcoholic extract (2000 mg/kg) of *L. acutangula*. This indicates that 2000 mg/kg is maximum safe dose. So 1/20th and 1/10th *i.e.* 100 and 200 mg/kg of body weight of the maximum safe dose were selected for studying *in vivo* anti-inflammatory effects. Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. It is characterized by redness, swollen joints, joint pain, its stiffness and loss of joint function. Inflammation is currently treated by NSAIDs. Unfortunately these drugs cause increased risk of blood clot resulting in heart attacks and strokes. Therefore, the developments of potent anti-inflammatory drugs from the natural products are now under considerations. Carrageenan induced acute inflammation is one of the most suitable test procedure to screen anti-inflammatory drugs. The time course of edema development in carrageenan induced edema is represented by a biphasic curve. The first phase of inflammation occurs within an hour of injection of carrageenan which occurs partly due to trauma of injection and partly due to serotonin and histamine component. Carrageenan induced paw edema is sensitive to cycloxygenase inhibitors and are used to evaluate the effect of non steroidal anti-inflammatory agents, which primarily inhibit the cycloxygenase involved in prostaglandin synthesis[21]. As shown there is a significant percentage inhibition 54% and 60% ($p < 0.05$) of paw edema at the different doses i.e. Hydroalcoholic leaves extract of *L. acutangula* 100 and 200 mg/kg p.o. The percentage inhibition of standard anti-inflammatory drug indomethacin was (74%; 10 mg/kg, bw) Table 4 and Fig. 3. Therefore it can be inferred that the possible inhibitory effect of hydroalcoholic leaves extract of *L. acutangula* in carrageenan induced inflammation may be due to inhibition of cycloxygenase leading to inhibition of prostaglandin synthesis.

Table 1 % Yield of plant material

S. No.	Solvents	*Luffa acutangula*
1	Pet. ether	1.8%
2.	Hydroalcoholic	2.4%

Table 2 Phytochemical screening of extract of *Luffa acutangula*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Mayer's Test	-ve
	Wagner's Test	-ve
	Dragendroff's test	-ve
	Hager's test	-ve
2.	**Glycosides**	
	Modified Borntrager's Test	-ve
	Legal's test	-ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	+ve
4.	**Phenolics**	
	Ferric Chloride Test	+ve
5.	**Proteins and Amino acids**	
	Xanthoproteic test	-ve
	Ninhydrin Test	-ve
6.	**Carbohydrates**	
	Molisch's Test	-ve
	Benedict's Test	-ve
	Fehling's test	+ve
7.	**Saponins**	
	Froth Test	+ve
	Foam test	+ve
8.	**Diterpins**	
	Copper acetate test	+ve

Table 3 Estimation of total phenolics and total flavonoids content

S. No.	Extract	Total Phenol (mg/100mg)	Total flavonoid (mg/100mg)
1.	Hydroalcoholic	0.897	0.765

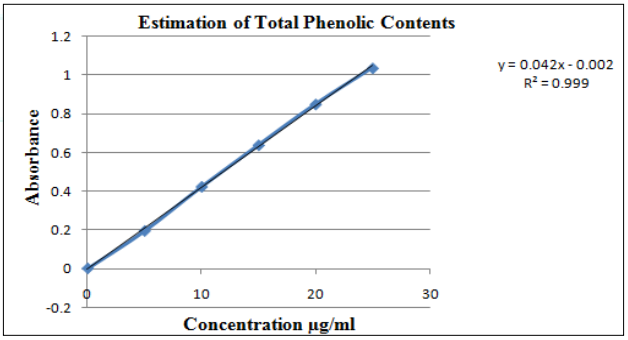

Fig 1 Graph of estimation of total phenolic content

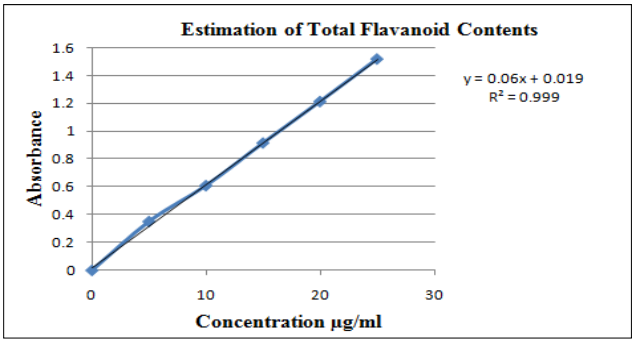

Fig 2 Graph of estimation of total flavonoids content

Table 4 Effect of hydroalcoholic extract of leaves of *L. acutangula* on paw oedema induced by carrageenan in rats

Group	Treatment	Dose (mg/kg)	Mean differences in Paw Volume (ml)	Percentage of Inhibition (%)
Group I	Control	0.1 ml of 1% (w/v) treated with carrageenan (1%w/v) in saline	3.99 ± 0.2	--
Group II	Indomethacin	10	1.2 ± 0.6***	74.00
Group III	Hydroalcoholic extract of *L. acutangula*	100	2.0 ± 0.5**	54.00
Group IV	Hydroalcoholic extract of *L. acutangula*	200	1.8 ± 0.1***	60.00

Values are expressed as mean ± SD. *P < 0.05-significant compared to carragenan treated group.

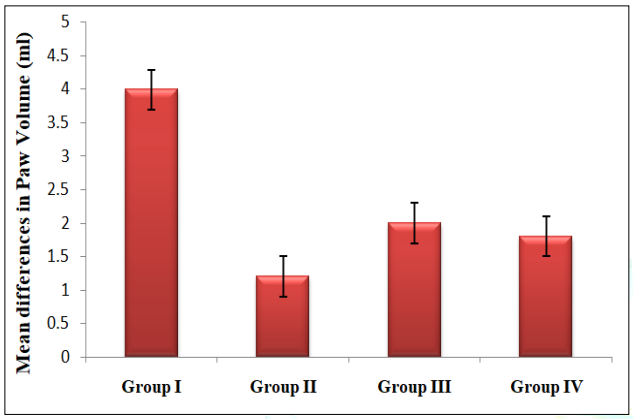

Fig 3 Effect of hydroalcoholic leaves extract of *L. acutangula* on paw edema induced by carrageenan in rats

CONCLUSION

Altogether, the present study results confirmed that hydroalcoholic extract of leaves of *L. acutangula* possess significant anti-inflammatory activity, which may be devoted to major secondary active metabolite present in it. In conclusion we suggest that the future studies on *L. acutangula* could be useful for the management of inflammatory diseases and oxidative stress.

REFERENCES

1. Abdel-Massiha RM, Faresa R, Bazzia S, El-Chamib N, Baydounb E. The apoptotic and anti-proliferative activity of *Origanum majorana* extracts on human leukemic cell line. Leukemia Research. 2010; 34:1052–1056.
2. Dinarello CA. (1997). Proinflammatory and anti-inflammatory cytokines as mediators in the pathogenesis of septic shock. Chest 112:321S-29S.
3. Frode TS, Buss ZS, dos Reis GO, Medeiros YS. (2009). Evidence of anti-inflammatory effects of pioglitazone in the murine pleurisy model induced by carrageenan. Int Immunopharmacol 9:1394-400.
4. Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. (2010). Oxidative stress, inflammation and cancer: How are they linked? Free Radic Biol Med 49:1603-16.
5. Yesilada E, Ustun O, sezik E, Takaishi Y, Ono Y, Honda G. Inhibitory effect of turkish folk remedies on inflammatory cytokines: Interleukins-1-alpha, interleukins-1-beta and tumor necrosis factor-alpha. J Ethnopharmacol. 1997; 58:59-73.
6. Corley DA, Kerlikowske K, Verma R, Buffler P. Protective association of aspirin/NSAIDS and esophageal cancer: A systemic review and meta analysis. Gastroenterology. 2003; 124:47-56.
7. Kirtikar K R, Basu B D, Luffa acutangula var amara Clarke, in Indian Medicinal Plants, (International Book Distributors, Book Sellers and Publishers, Uttaranchal). 2006, 1123.
8. Misar A V, Upadhye A S & Mujumdar A M, CNS depressant activity of Luffa acutangula var. amara C. B. Clarke fruits in mice, Indian J Pharm Sci , 66 (2004) 463.
9. Ansari N M, Houlihan L, Hussain H & Pieroni A, Antioxidant activity of five vegetables traditionally consumed by South-Asian Migrants in Bradford, Yorkshire, UK, Phytother Res, 19 (2005) 907.
10. Prabhakar K & Jebanesan A, Larvicidal efficacy of some cucurbitacious plant leaf extracts against Culex quinquefasciatus (Say), Biores Tech, 95 (2004) 113.
11. Kandlakunta B, Rajendran A & Thingnaganing L, Carotene content of some common (cereals, pulses, vegetables, spices and condiments) and unconventional sources of plant origin, Food chem, 106 (2008) 85.
12. Schilling E E & Heiser C B, Flavonoids and the systematics of Luffa, Bioch systematics and ecol, 9 (1981) 263.
13. Nagao T, Tanaka R, Iwase Y, Hanazono H & Okabe H, Studies on the constituents of Luffa acutangula Roxb. I. Structure of acutosides A-G, oleanane type triterpene saponins isolated from the herb, Chem Pharm Bull, 39 (1991) 599.
14. Nagao T, Tanaka R, Iwase Y, Hanazono H & Okabe H, Studies on the constituents of Luffa acutangula Roxb. II. Structure of acutosides H-I, oleanolic acid saponins isolated form the seeds, Chem Pharm Bull, 39 (1991) 889.
15. Mukherjee, P. K., (2007). "Quality Control of Herbal Drugs", 2nd Edition, Business Horizons, 2007, 2-14.
16. Khandelwal KR. Practical pharmacognosy technique and experiments. 23rd Ed. Nirali Prakashan; 2005.
17. Kokate CK. Practical pharmacognosy. 4th Ed. Vallabh Prakashan; 1994.
18. Olufunmiso OO, Afolayan AJ, Phenolic content and antioxidant property of the bark extract of Ziziphus mucronata willd. Subsp. mucronata willd, BMC Complement Alternative Medicine, 2011; 11:130.
19. Guideline Document on Acute oral Toxicity Testing, Series on Testing and Assessment No. 423. Paris: Organization for Economic Co-Operation and Development, OECD Environment, Health and Safety Publications; 1996. Available from: http//www.oecd.org/ehs.
20. Winter C.A., Risley E.A., Nuss G.W. Carrageenan-induced oedemain the hind paw of rat as an assay for anti-inflammatory activity. Proc. Soc. Exp. Biol. Ther. 1962; 111:544–547.
21. Seibert K., Masferrer J.L. Role of inducible cycloxygenase (COX 2) in inflammation. Receptor. 1994;4(1):17–23.