International Research Journal of Pharmaceutical and Applied Sciences
Available online at www.irjpas.com
Int. Res J Pharm. App Sci., 2012; 2(4):104-108

Research Article

Evaluation of Anti-Inflammatory Activity of *Citrullus lanatus* Seed Oil by *In-vivo* and *In-vitro* Models

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(Received: 14 August, 2012; Accepted: 27 August, 2012; Published: 29 August, 2012)

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ABSTRACT
The present study aimed for *in-vivo* and *in-vitro* evaluation of *Citrullus lanatus* seed oil for anti-inflammatory activity. *Citrullus lanatus* seed oil is extracted with n-hexane and tested for in-vivo and in-vitro anti-inflammatory activity. The oil was screened for *in-vivo* anti-inflammatory activity by carrageenan-induced paw edema in rat model and *In-vitro* anti-inflammatory activity by human red blood cell membrane stabilization method. The potency of the oil was compared with standard diclofenac (10 mg/kg). The oil showed significant reduction of edema in carrageenan induced rat paw edema model maximum at 3 hr (percentage reduction in paw volume 44.44%, 55.56% and 63.11% for CLSO(50 mg/kg), CLSO(100mg/kg) and diclofenec(10mg/kg) respectably and CLSO at concentration of 100, 250 and 500 mcg/ml showed 42.35%, 68.48% and 78.50% protection of HRBC in hypotonic solution respectably. All the results were compared with standard diclofenac at 50, 100 and 200 mcg/ml which showed 43.74%, 63.93% and 86.73% protection of HRBC in hypotonic solution respectably.

Keywords: *Citrullus lanatus*, anti-inflammatory activity, carrageenan-induced paw edema, human red blood cell membrane.

INTRODUCTION

Inflammation is a complex biological response of vascular tissue to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain. Prolonged inflammation leads to the rheumatoid arthritis, atherosclerosis, hey fever, ischemic heart diseases etc and inflammation is a common manifestation of infectious diseases like leprosy, tuberculosis, syphilis, asthma, inflammatory bowel syndrome, nephritis, vascularitis, celiac diseases, auto-immune diseases etc.

Anti-inflammatory drugs like NSAIDs used to reduce the swelling and pain of inflammation. But these agents carry the risk of gastro-intestinal toxicity, cardiovascular and other toxicity for prolonged use. For these reason, there is a need for ant-inflammatory drugs having less severe side effects to use for chronic inflammatory disease as well. Therefore, in resent time, more interest is shown in alternative and natural drugs for treatment of various diseases, but there is lack of scientific evidence.

Citrullus lanatus of family Cucurbitaceae is commonly known as water melon and in local name Tarmuz (Hindi), Puchakaya (Telugu). The ripe fruits are edible and largely used for making confectionary. Its nutritive values are also useful to the human health. Fruit is used in cooling, strengthening, aphrodisiac, astringent to the bowels, indigestible, expectorant, diuretic, and stomachic, purifies the blood, allays thirst, cures biliousness, good for sore eyes, scabies and itches and as brain tonic to the brain[1]. It also reported having analgesic and anti-inflammatory activity of roots and leaves [2], antimicrobial activity [3], laxative activity of fruit [4] , anti-oxidant and antiulcerative activity [5].

MATERIAL AND METHODS

Collection and extraction of oil

The seeds of *Citrullus lanatus* of family Cucurbitaceae were collected from ripe fruits which were obtained from local fruit market, Kodad, Andhra Pradesh. The seeds collected from fruit and dried and extracted with n-hexane to obtain the oil. The percentage yield was 21.59 % w/w.

Physiochemical studies of oil

The oil obtained from water melon seed were tested for qualitative tests for oraganoleptic characters, solubility, specific gravity, refractive index, saponification value, iodine value and chemical tests for oils.

Drugs and Chemicals

Diclofenac (Symed Pharm. Pvt. Ltd, Hyderabad) used as the standard anti-inflammatory drug, Carrageenan (Type-1, Sigma Chemicals) and all others laboratory reagents were obtained from the institute store and are analytical grade.

Animals

Rats of either sex weighing 150-200 g were used in experiment. Animals were obtained from Anurag Pharmacy College, Kodad. Animals were kept under standard conditions at 23-25^{0}C 12 hr light/dark cycle and given standard pellet diet and water. Before performing the experiment the ethical clearance was obtained from institutional animal ethics committee (IEAC).

Acute oral toxicity studies

Acute oral toxicity study was carried out for n-hexane extracted *Citrullus lanatus* Seed oil (CLSO*)* using Acute Toxic Class Method as described in OECD (Organization of Economic Co-operation and Development) Guidelines No. 423. The *Citrullus lanatus* seed oil was safe up to a dose of 2,000 mg/kg body weight.

Experimental design

The animals were divided into four groups of six animals each as follows:

Group I: Control: received 1 % aqueous solution of 2% Tween-80, p.o (1 hr before carragenan injection)

Group II: Drug treated: received CLSO 50 mg/kg, p.o (1 hr before carragenan injection)

Group III: Drug treated: received CLSO 100 mg/kg, p.o (1 hr before carragenan injection)

Group IV: Drug treated: Diclofenac 10 mg/kg, p.o (1 hr before carragenan injection)

***In-Vivo* anti-inflammatory activity**

Carrageenan-induced paw edema in rats

The acute hind paw edema in Wister rats (150-200 gm) was produced by Carrageenan-induced paw edema in rats [6]. 0.1 ml of carrageenan (prepared as 1% w/v suspension in saline) locally injected into subplantar region of the left hind paw of rats. *Citrullus lanatus* seed oil (CLSO*)* (50 and 100 mg/kg, p.o. and diclofenac 10 mg/kg, p.o.) were given orally 1 hour prior of carragenan injection. Other group served as control in this experiment which got carragenan and vehicle. The rat paw volume up to the ankle joint was measured at 0 hr (30 min before caragennan injection), 1 hr, 2 hr, 3 hr and 4 hr after the injection of carrageenan using plethysmometer. Increase in the paw edema volume was considered as the difference between initial at 0 hr and of 1 hr, 2 hr, 3 hr or 4 hr. Percent inhibition of paw volume between treated and control groups were calculated as follows:

$$\text{Percent inhibition} = \frac{Vc - Vt}{V_c} \times 100$$

Where V_c and V_t represent the mean increase in paw volume in control and treated groups, respectively.

***In-Vitro* anti-inflammatory activity**

In-vitro anti-inflammatory activity of CLSO was performed by using human red blood cell membrane stabilization method [7, 8]. The blood was collected from healthy human volunteer who was not taken any NSAIDS for 2 weeks prior to the experiment and mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm. The packed cells were washed with isosaline and a 10% suspension was made. Various concentrations of extracts were prepared (100, 250, 500 mcg/ml) using distilled water and to each concentration 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of Human red blood cells (HRBC) suspension were added. It is incubated at 37^{0}C for 30 min and Centrifuged at 3,000 rpm for 20 min. the hemoglobin content of the supernatant solution was estimated spectrophotometrically at 560 nm. Diclofenac (50, 100 and 200 mcg/ml) were used as reference standard and a control was prepared omitting the extracts.

Statistical Analysis

The data were expressed as mean ± standard error mean (SEM). The data were analyzed by using Graph pad software Prismversion5 by one way analysis of variance (ANOVA). The test was followed by Dunnett's 't'-test, p values less than 0.05 were considered as significance.

RESULTS AND DISCUSSION

Preliminary Physicochemical Screening

The CLSO was screened for various Physicochemical test as per the reported methods and found the oil as golden yellow colour, having pungent smell and soluble in organic solvent such as ethanol. It was found of Specific gravity -0.925 at 25^{o}C, refractive index 1.46 at 25^{0}C Saponifiacation value 168.5, iodine value 121.3 and. chemical tests confirms the presence of oil, terpenoids, and phenolic compounds.

***In-vivo* anti-inflammatory activity of *Citrullus lanatus* seed oil (CLSO)**

The results of *in-vivo* anti-inflammatory activity of *Citrullus lanatus* seed oil (CLSO) on carragenan induced paw edema in rats were given in **Table 1.** Anti-inflammatory effect of *citrullus lanatus* seed oil was evaluated after sub plantar injection of carragenan in rats. Sub plantar injection of carragenan results in significant increased in paw edema after one hour in control rats as compared to normal rats which was subsequently increased up to 3 hrs. Rat treated with CLSO (50mg/kg and 100mg/kg) showed significant decrease in paw edema in paw edema on 1 hr, 2 hr, 3 hr and 4hr and was comparable with standard drug diclofenac (10 mg/kg). The difference in paw volume of treated group was most significant ($p<0.001$) at 3 hr.

***In-vitro* Anti-Inflammatory of *Citrullus lanatus* seed oil (CLSO)**

The results of *in-vitro* anti-inflammatory activity of *Citrullus lanatus* seed oil (CLSO) on carragenan induced paw edema in rats were given in **Table 2.** *In-vitro* anti-inflammatory activity of CLSO was performed by using human red blood cell membrane stabilization method. CLSO showed significant anti-inflammatory activity in a concentration dependent manner. CLSO at concentration of 100, 250 and 500 mcg/ml showed 42.35%, 68.48% and 78.50% protection of HRBC in hypotonic solution respectably. All the results were compared with standard diclofenac at 50, 100 and 200 mcg/ml which showed 43.74%, 63.93% and 86.73% protection of HRBC in hypotonic solution respectably.

Table1: Effect of *Citrullus lanatus* Seed oil (CLSO) on carragenan induced paw edema in rats

Treated groups	Difference in paw edema volume (ml)							
	1 hr		**2 h**		**3h**		**4h**	
	PV	% RPV	PV	% RPV	PV	% RPV	PV	% RPV
Control	0.300±0.02	-	0.366±0.03	-	0.450±0.02	-	0.333±0.04	-
CLSO 50mg	0.201 ±0.02 *	33.34	0.231±0.02**	36.88	0.250±0.02***	44.44	0.216±0.01*	36.03
CLSO 100 mg	0.21±0.01*	30.00	0.232±0.02**	36.61	0.200±0.25***	55.56	0.183±0.01**	45.04
Diclofenac 10 mg	0.166±0.02**	44.67	0.18±0.01***	50.81	0.166±0.03***	63.11	0.116±0.01***	65.16

Values are in Mean ± S.E.M (n=6); ^{ns} -Non Significant, *p<0.05, **p<0.01, ***p<0.001 when compared with Control using One way ANOVA followed by Dunnet's "t" test, PV= Paw Volume, %RPV= Percentage Reduction in Paw Volume

Table 2: *In-Vitro* anti-inflammatory activity of *Citrullus lanatus* Seed oil (CLSO) by HRBC method

Treatment	**Conc. mcg/ml**	**Absorbance 560 nm**	**Percentage inhibition**
Control	---	2.526	-
CLSO	100	1.456	42.35%
CLSO	250	0.796	68.48%
CLSO	500	0.543	78.50%
Diclofenec	50	1.421	43.74%
Diclofenec	100	0.911	63.93%
Diclofenec	200	0.335	86.73%

The edema and inflammation induced by Carrageenan is shown to be mediated by histamine and serotonin during first 1 h. After which increased vascular permeability is maintained by the release of kinins upto 2.30 h, followed by the release of kinins and finally through the release of bradykinin, prostaglandin and lysosomes from 2.30 to 6 h. The later phase is reported to be sensitive to most of the clinically effective anti-inflammatory agents. The mediators appear to be prostaglandins, the release of which is closely associated with migration of leucocytes into the inflamed site [9]. The Carrageenan induced paw edema model in rats is known to be sensitive to cyclo-oxygenase (COX) inhibitors and has been used to evaluate the effect of non-steroidal anti-inflammatory agents [10, 11]. Though CLSO (50 and 100 mg/kg, p.o.) significantly reduced the paw edema in rats but the effect was of less intensity, when compared with diclofenec (10 mg/kg, p.o).

The *Citrullus lanatus* seed oil exhibited membrane stabilization effect by inhibiting hypotonicity induced lyses of erythrocyte membrane. The erythrocyte membrane is analogous to the lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membranes[12] . Stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release[13].

CONCLUSION

Citrullus lanatus seed oil possesses potent anti-inflammatory activity both *in-vivo* and *in-vitro* studies which is comparable to standard diclofenec. Since, serotonin, histamine and prostaglandins are the major mediators of inflammation, anti inflammatory effect of *Citrullus lanatus* seed oil either due to inhibition of their synthesis or inhibition of prostaglandin synthesis at third stage of inflammation. Based on the present study, it can be concluded that *Citrullus lanatus* seed oil have potent anti-inflammatory activity and by further studies it can be possible to formulate natural anti-inflammatory drug of *Citrullus lanatus.*

Acknowledgements

This study was supported by giving permission to use instruments and ethical clearance from institute. Our special thanks to Dr. M. Chinna Eswaraiah, Principal, Anurag Pharmacy College, Kodad, Andhra Pradesh, for his valuable suggestion and help.

REFERENCES

1. Rahman A.H.M.M., Anisuzzaman M., Ferdous Ahmed, Rafiul Islam A.K.M. and Naderuzzaman A.T.M.. Study of Nutritive Value and Medicinal Uses of Cultivated Cucurbits, *Journal of Applied Sciences Research***, 2008**; 4(5): 555-558.
2. Deng Jia-Gang, Wang Shuo, Guo Li-Cheng, Fan Li-Li,. Anti-inflammatory and Analgesic Effects of Extract from Roots and Leaves of Citrullus lanatus, *Chinese Herbal Medicines*, **2010**; 2(3): 231-235.
3. Loiy Elsir Ahmed Hassan, Hasnah Mohd Sirat, Sakina M. Ahemd Yagi, Waleed S. Koko and Siddig Ibrahim Abdelwahab. In vitro Antimicrobial activities of chloroformic, hexane and ethanolic extracts of Citrullus lanatus var. citroides (Wild melon), *Journal of Medicinal Plants Research*, **2011**; 5(8):1338-1344.
4. Swapnil Sharma, Sarvesh paliwal, Jaya Dwivedi Amita Tilak. First report on laxative activity of Citrullus lanatus, *Pharmacologyonline* , **2011**; 2: 790-797.
5. Naresh Singh Gill, S. Sood, A. Mutharaman, M. Bali.. Evaluation of antioxidant and anti-ulcerative potential of citrullus lanatus seed extracts in rats. *Latin american journal of pharmacy*, **2011**; 30(3):429-434.
6. Winter C.A., Risley E.A., Nuss C.W.,. Carrageenan-induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. Proceedings of the Society for Experimental Biology and Medicine, **1962**; 111: 544–547.
7. Gandhisan R, Thamaraichelvan A, Baburaj,. Antiinflammatory action of Lannea coromandelica HRBC membrane stabilization. *Fitotherapia,* 1991; 62: 82-83.
8. Rajendran Vadivu and K.S. Lakshmi,. In vitro and In vivo anti-inflammatory activity of leaves of Symplocos cochinchnensis (Lour) Moore ssp laurina, *Bangladesh J Pharmacol*, **2008**; 3: 121-124.
9. Di-Rosa M, Giroud JP, Willoughby DA,. Studies on the mediators of acute inflammatory response induced in rats in different sites of carrageenin and turpentine*, J Pathol.* **1971**;104:15-29.
10. Rao ChV, Kartik R, Ojha SK, Rao AGMM.,. Anti-inflammatory and antinociceptive activity of stem juice powder of Tinospora cordifolia Miers. in experimental animals. *Hamdard Medicus.* **2005**; 48:102-106.

11. Ghule BV, Ghante MH, Upaganlawar AB, Yeole PG,. Analgesic and Anti-Inflammatory activities of Lagenaria siceraria Stand. fruit juice extract in rats and mice. *Phcog Mag*. **2006**; 2(8): 232-238.
12. Chou CT,. The anti-inflammatory effect of Tripterygium wilfordii Hook F on adjuvant-induced paw edema in rats and inflammatory mediators release. *Phytother Res*. **1997**; 11: 152-54.
13. Murugasan N, Vember S, Damodharan C. Studies on erythrocyte membrane IV: In vitro hemolytic activity of oleander extract. *Toxicol Lett*, **1981**; 8: 33-38.