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ANALGESIC AND ANTI INFLAMMATORY ACTIVITIES OF METHANOLIC EXTRACT OF FRUIT RIND OF *CITRULLUS LANATUS* (THUNB)

Md. Abdul Jalil, Nushrat Jahan, Md. Mohaiminul Islam, A.B. Ripon Khalipha, Chand Sultana Khatun, Md. Mofizur Rahman, Md. A.K. Azad*

Dept. of Pharmacy, Bangladesh University, Mohammadpur, Dhaka-1207, Bangladesh

***Corresponding author e-mail:** ak_azad5271@yahoo.com

ABSTRACT

Almost all diseases are associated with the inflammation and pain. The Present study was designed to evaluate analgesic and anti inflammatory potentialities of methanolic extract of Cirtrullus lanatus rind which belongs to Cucurbitaceae family. Potent analgesic activity was observed ($p<0.05$) in extract 400mg/kg by 66.45% inhibition of writhing reflex and mild (50.63% inhibition) activity was observed in 200mg/kg compare to inhibition of 82.91% of standard Diclofenac 10 mg/kg. On the other hand, anti-inflammatory effect was remarkable ($p<0.05$) with 400mg crude compare with Standard Diclofenac 5 mg/kg body weight. Dunet test represents 46% reduction of paw edema compare to standard of 56.71%. Preliminary phytochemical screening of the plant extract showed the presence of Glycosides, saponins, flavonoids, steroids and protein were present in the rind of Citrullus lanatus one of which has analgesic, anti inflammatory properties. These provide scientific evidence to support the isolation and development of biologically active components as analgesic and anti-inflammatory agents.

Key words: Citrullus lanatus, Analgesic activity, anti inflammatory activity, phytochemical screening, Carrageenan

INTRODUCTION

Medicine is the wonder of the world and blessings for mankind. From the very ancient time men used various plant parts as their wound healing. It is estimated that 70-80% of people worldwide rely chiefly on traditional, largely herbal medicine to meet their primary healthcare needs**.[1]**The global demand for herbal medicine is not only large, but growing**.[2]** The market for Ayurvedic medicines is estimated to be expanding at 20 annually in India**[3],**while the quantity of medicinal plants obtained from just one province of China (Yunnan) has grown by 10 times in the last 10 years**.[4]** Medicinal herbs have been used as a firm of therapy for the relief of pain throughout the history**.[5]** In generally narcotic and non steroidal drugs are used against pain and inflammation. As their adverse effect and to limit it the present study was designed to obtain a new source of analgesic and anti inflammatory drug. Citrullus lanatus Thunb (Cucurbitaceae) is a reptant flowering plant commonly known as watermelon. Watermelon is indigenous to tropical Africa, growing profusely in the Kalahari Desert Namibia and Botswana **[6].** In this study we examined the analgesic and anti inflammatory effects of methanolic extract of Citrullus lanatus fresh fruit rind in experimental animal models.

MATERIALS AND METHODS

Collection and Preparation of rind extract: Citrullus lanatus (CL) fruits were collected from local market during the month of May 2011 and authenticity was confirmed from the Bangladesh National Herbarium, Mirpur, Dhaka. The rinds of Citrullus lanatus were shade dried for fifteen days at room temperature to ensure the active constituents free from decomposition. The dried rinds were powdered in an electrical grinder after overnight drying in an oven below 50°C. The powder was extracted with 96% methanol at room temperature. The bottle was kept at room temperature and allowed to stand for 10 days with occasional shaking. When

the solvent become concentrated, the liquid alcohol contents were filtered through cotton and then through filter paper (Whatman Fitter Paper No. 1). Finally, a highly concentrated methanolic crude extract were obtained. The extract was dried under reduced pressure using a rotary vacuum evaporator and the extract was kept in refrigerator for further use.

Phytochemical screening: Phytochemical studied of methanolic extract of Citrullus lanatus fruit was carried out for preliminary chemical investigation for the direction of practical pharmacognosy text book **[7]**

Drugs and chemicals: Carrageenan was purchased from Otto chemicals, India. The standard drug Diclofenac-Na was purchased from Square Pharmaceuticals Limited of Bangladesh. Acetic acid, methanol and other chemicals supplied from laboratory of Bangladesh University were analytical grade.

Experimental animals: Eight week-old Swice albino mice (27-30g) purchased from Jahangirnagar University, Dhaka, Bangladesh and were housed in animals cages under standard environmental conditions (22-25°C, humidity 60-70%, 12 hr light: 12 hr dark cycle). The mice were feed with standard pellet diet taken from, Jahangirnagar University Dhaka. The animals used in this study were cared in accordance with the guidelines on animal experimentation of our institute.

Analgesic activity: For analgesic test all mice were divided into four groups. Each group comprises 4 mice. Control group (received 0.5% methyl cellulose, per oral), Standard Group (received Diclofenac 10mg/kg intraperitoneally), and CL extract Group (received 300mg/kg CL extract per oral). The analgesic activity of the samples was studied using acetic acid-induced writhing model in mice. Test samples and vehicle were administered orally 30mins before intraperitoneal administration 10ml/kg of .7% acetic acid but Diclofenac-Na was administered intraperitoneally 15 minutes before the acetic acid injection, the mice were observed for specific contraction of body referred to as "writhing" for the next 10minutes**.[8].** Percentage protection of acetic acid induced writhing was calculated by the formula. Percentage protection = (Wc-Wt)/Wc x100.

Where, wc is the mean values of control group and Wt is the mean values of treated group.

Anti-inflammatory activity: Inflammation (paw edema) was induced by injecting 0.1ml of 1% Carrageenan in physiological saline into the subplantar tissues of the left hind paw of each mouse.**[9]**. The methanolic extract of Citrullus lanatus fruit rind 400 mg/kg were administered orally 30 min prior to Carrageenan administration. The paw *edema size* was measured at 0, 1, 2, 3 & 4 hours by using dial caliper **[10].** The percentage inhibition of paw *edema* in drug treated group was compared with the control group. Diclofenac sodium (5 mg/kg p.o.) was used as reference standard. 0 hour reading was considered as an initial normal paw size .Data was collected from the paw thickness and percentage inhibition of paw edema of the treated animals. Percentage inhibition of paw edema was calculated by using the formula:

Anti inflammatory activity (%) = (1-T/C) x100
Where T is the change of paw diameter in treated group and C is the change of paw diameter in control group.

Data Analysis: All values were expressed as mean ± Standard error of mean (SEM). Statistical comparison were performed by One-way analysis of variance (ANOVA), followed by using Tukeys test. Results were considered as significant of the differences between the test and control group data when p values less than 0.05 ($p<0.05$).

RESULTS

Phytochemical screening: Preliminary phytochemical analysis of the plant extract showed the presence of Glycosides, saponins, flavonoids, steroids and protein were present in the rind of Citrullus lanatus.

Analgesic activity: Acetic acid writhing: The analgesic effects of methanolic extract of Citrullus lanatus rind was investigated on acetic acid induced writhing were demonstrated in table no 1 and percentages of writhing inhibition indicated in figure no 1. Oral administration of CL in 200mg and 400mg per kg body weight showed significant ($p<0.05$) and dose dependant protection indicated by 50.63 and 66.45 % of writhing inhibition respectively compare with 82.91 % inhibition of standard reference Diclofenac 10mg/kg i.p.

Table no. 1. Effects of Citrullus lanatus on acetic acid induced writhing in mice.

Animal Group	Writhing Counting (Mean ±SEM)	% of Inhibition of Writhing
Control Group	39.5±0.72	_
Standard Group	6.75±0.33*	82.91
200mg CL Group	19.5±0.35*	50.63
400mg CL Group	13.25±0.38*	66.45

Data were expressed as mean ± S.E.M. (n = 4 mice in each group) significantly different from the control at *P<0.05

Anti-inflammatory activity: Table-2 shows the effects of the extract on Carrageenan induced paw edema in mice. The methanolic extract of Citrullus lanatus rind significantly attenuated Carrageenan induced paw edema in mice. However, the maximum reduction of paw edema size was observed at higher dose (400mg/kg, p.o.) and 200mg/kg also remarkably reduces paw edema which were comparable to the effect of Diclofenac (5mg/kg, p.o.). Citrullus lanatus rind significantly (P<0.05) reduces 46.0% of paw edema, compare to Standard inhibition of 56.71%

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Table no. 2 Effects of the methanolic extract of rind of Citrullus lanatus on Carrageenan induced paw edema size in mice

Time → / Animal Group ↓	1hrs	2hrs	3hrs	4hrs	% of inhibition of paw edema
	(Paw edema in mm)				
Carrageenan 1%	0.28±0.01	0.33±0.004	0.30±0.002	0.30±0.002	0.00
Diclofenac Group	0.16±0.003	0.13±0.005	0.12±0.005	0.11±0.005*	56.7
200mg CL Group	0.22±0.002	0.21±0.002	0.19±0.003	0.18±0.004*	34.0
400mg CL Group	0.19±0.003	0.17±0.002	0.15±0.002	0.14±0.001*	46.0

Data were expressed as mean ± S.E.M. (n = 4 mice in each group) significantly different from the control at *P<0.05

DISCUSSIONS

Our present study was made an attempt to find out new analgesic and anti-inflammatory drug from Citrullus lanatus. Present study demonstrated the analgesic and anti-inflammatory activity of methanolic extract of Citrullus lanatus according to acetic acid induced writhing inhibition and Carrageenan induced paw edema size reduction. Acetic acid increases the peripheral fluid levels of prostaglandins (PG-E_2 & PG-F) and releases other analgesic and anti-inflammatory mediators such as bradikinin, histamine and 5-hydroxytryptamine **[11, 12]** Carrageenan injection causes local inflammatory reaction edema which is suitable criteria for the anti-inflammatory evaluation **[9].** Carrageenan induced inflammatory paw edema development is a biphasic reaction. In first phase is developed by releasing histamine and serotonin and in second edema fulfilled by releasing bradikinin, protease, prostaglandins and lysosome. **[13, 14, 15]** Inflammation is also caused the increasing activity of free radicals. **[16]** Many researchers have paid attention to the anti-inflammatory and analgesic potentials of members of Cucurbitaceae, a plant family widely known to synthesize cucurbitacins predominantly **[17, 18, 19]** Most of the herbal plants attribute their activity for the combination of different active constituents. Preliminary phytochemical analysis of the plant extract showed the presence of Glycosides, saponins, steroids and protein were present in the rind of Citrullus lanatus. Analgesic and anti-inflammatory of many plants have been attributed for the presence of flavonoids**[20]**, It is therefore present study may be attributed for the presence of flavonoids.

CONCLUSION

To summarize the study of methanolic extract of rind of Citrullus lanatus on Carrageenan induced paw edema can provide the scientific basis as a traditional

medicinal source for analgesic and anti-inflammatory activities. But further study needed to understand the mechanism of action for analgesic and anti-inflammatory activities.

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REFERENCES

1. Farnsworth, N. R. & Soejarto, D. D. Global importance of medicinal plants. In the conservation of medicinal plants (ed. O. Akerele, V. Heywood and H. Synge), Cambridge University Press, Cambridge, UK. 1991, pp. 25-51.
2. Srivastava, R.Studying the information needs of medicinal plant stakeholders in Europe. TRAFFIC Dispatches .2000
3. Subrat, N. Ayurvedic and herbal products industry: an overview. Kathmandu, Nepal, Program in Asia (MAPPA), IDRC, Canada, and the WWF-UNESCO People and Plants Initiative. 15-20 December 2002.
4. Pei Shengji. Ethnobotany and modernisation of Traditional Chinese Medicine. Kathmandu, Nepal, Medicinal and Aromatic Plants Program in Asia (MAPPA), IDRC, Canada, 2002, 2002b,.
5. Almeida, R.N., D.S. Narvarro and J.M. Barbosa-Filho,Plants with central analgesic activity. Phytomedicine, 2001, 8: 310-322
6. North Carolina State University: Watermelon biogeography.
7. Kokate, C.K.,. Practical Pharmacognosy. New Delhi: Vallabh Prakashan, 1994, pp: 107-107
8. Ahmed F, Selim MST, Das A.K. and Choudhuri MSK. Anti-inflammatory and antinociceptive activities of Lippa nodiflora Linn. Pharmazie, 2004, 59: 329-333.
9. Winter, C. A., Risley, E. A., and Nuss, G. W. Carrageenan induced oedema in hind paw of the rats as an assay for anti-inflammatory drugs. Proc. Soc. Exp. Bio Med, 1962, 111: 544-547
10. Al-Haboubi, H.A and I.J. Zeitlin,. Re-appraisal of the role of histamine in carageenan-induced oedema. European. J. Pharmacol., 1983, 88: 160-176.
11. Whittle, B.A., The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesics. Br.J. Pharmacil., 1964, 22: 246-253
12. Deraedt, R., S. Joughney, F. Delevakee and M. Falhour,. Release of prostaglandin E and H in an algogenic reaction and its inhibition. Eur. J. Pharmacol., 1980, 51: 17-24
13. Vinegar, R., W. Schreiber and R. Hugo,. Biphasic development of Carrageenan oedema in rats. J. Pharmacol. Exp. Ther., 1969, 166: 96-103
14. Crunkhorn, P. and S.C. Meacock, Mediators of the inflammation induced in the rat paw by Carrageenan. British J. Pharmacol., 1971, 42: 392-402
15. Di Rosa, M. and D.A. Willoughby,. Screens for anti-inflammatory drugs. J. Pharm. Pharmacol., 1971, 23:297-298
16. Mantle, D., F. Eddeb and A.T. Pickering,. Comparison of relative antioxidant activities of British medicinal plant species in vitro. J. Ethnopharmacol., 2000, 72: 497-510
17. Tannin-Spitz T, Bergman M, Grossman S Cucurbitacin glucosides: Antioxidant and free-radical scavenging activities. Biochem. Biophys. Res. Commun., 2007, 364(1): 181-86.
18. Haritunians T, Gueller S, Zhang L, Badr R, Yin D, Xing H, Fung MC, Koeffler HP Cucurbitacin B induces differentiation, cell cycle arrest, and actin cytoskeletal alterations in myeloid leukemia cells. Leuk. Res., 2008, 32(9): 1366-1373.
19. Wakimoto N, Yin D, O'Kelly J, Haritunians T, Karlan B, Said J, Xing H, Koeffler HP Cucurbitacin B has a potent antiproliferative effect on breast cancer cells in vitro and in vivo. Cancer Sci., 2008, 99 (9): 1793-1797.
20. Pathak, D., K. Pathak and A.K. Sigla,. Flavonoids as medicinal agents: recent advances. Fitoterapia, 1991, 62: 371-388.