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Protective Role Of Methanolic And Aqueous Extracts Of Cucurbita moschata Linn. Fruits In Inflammation And Drug Induced Gastric Ulcer In Wister Rats.

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Abstract: To validate the science behind the traditional use of *Cucurbita moschata* Linn. fruits in Indian system of traditional medicine to treat inflammation and gastric ulcer, present study was designed to explore the protective effect of methanolic and aqueous extracts of *Cucurbita moschata* fruits against inflammation and drug induced gastric ulcer. Both the extracts exhibits dose dependent (200mg/kg and 400mg/kg) protection against acute inflammation (Carrageenan induced inflammation) and chronic inflammation (cotton pellet granuloma) in experimental animals, these extracts also shows significant activity against hard liquor and drug (Aspirin) induced gastric ulcers in albino rats. To support activity and to find the mechanism of action free radical scavenging activity of the extracts has also been studied. The test extracts shows free radical scavenging activity with IC_{50} value of 39.85 µg for methanolic extract and 12.30 µg for aqueous extract. Moreover both the test extracts has not shown any abnormality and mortality up to 2000mg/kg p.o. in experimented animals, when evaluated for acute toxicity study. The results of the present investigation suggest that *Cucurbita moschata* Linn. fruits having anti-inflammatory and antiulcer activity, and this could be the next generation safe and effective anti-inflammatory drug which helps in protection against drug induced ulcers.

Key Words: *Cucurbita moschata* Linn, anti-oxidant, inflammation, ulcer.

INTRODUCTION

Inflammation is the body's defense reaction to any infection and injury and has been concerned in the pathogeneses of chronic diseases such as arthritis, cancer, atherosclerosis, stroke and epilepsy, as well as neurodegenerative diseases (for example: multiple sclerosis, Alzheimer's and Parkinson's diseases). Inducible COX-2 expressed in immune cells is a key player in initiating the inflammatory response by converting arachidonic acid into notorious proinflammatory prostaglandins and triggering production of other proinflammatory chemokines and cytokines [1].

Gastric ulcer is a lesion of gastric or duodenal mucosa occurring at a site where the mucosal epithelium is exposed to aggressive factors. In spite of the vast amount of research on ulcer, the cause of chronic peptic ulceration is still not clear, it is generally accepted that they result from an imbalance between aggressive factors and the maintenance of mucosal integrity through endogenous defense mechanism[2]. Cyclooxygenase-1 (COX-1) constitutively expressed and thought to mediate 'housekeeping' functions, and thus is responsible for the production of protective prostaglandins in mucosal epithelium layer that are required for achieving normal physiological functions of our gastro-intestinal tract [3].

Now a day growing biomedical system supports the use of modern synthetic medicines which are associated with various side effects and the escalating costs of various synthetic drugs are also the cause for renewed interest in traditional systems of medicine. Chronic use of available anti-inflammatory medicines is a key factor for drug induced ulcers; now it is a challenge for the patients suffers with inflammatory disorders; they may suffer with gastric ulcers. To regain the body's homeostasis, different therapeutic agents including naturally occurring plants and minerals are used. In this background we thought to find out effective, safer and cheaper remedy for inflammatory disorders at a hands stretch which does not cause any harm to the gastro-intestinal tract.

Cucurbita moschata, Linn (Fam. cucurbitaceae) is a cooling astringent fruit which is useful traditional remedy for treating irritable bowel syndrome, purifies blood, increases appetite, and cures leprosy, fatigue and muscle cramping [4]. Fruits and seeds are used in Ayurveda to treat haemorrhage of pulmonary organs, rheumatism and urinary disease [5].

Since pharmacological validation of the ethnomedicinal claims regarding the usefulness of this plant as a drug and since the reports suggests the traditional use of the plant in gastric ulcer and other chronic inflammatory diseases, the present study was designed with an aim to justify the scientific basis of the therapeutic use of *Cucurbita moschata* fruits as an anti-ulcer and anti-inflammatory agent.

MATERIALS AND METHODS

Plant Material

Cucurbita moschata, Linn fruit were collected from market of Neemuch (M.P.) India in the month of August and identified by Dr. H.S. Chatree (Ex. Professor Botany Govt. P.G. College, Mandsaur) and a voucher specimen (T/002/2005) was deposited for future reference in Department of Pharmacognosy, B.R. Nahata College of Pharmacy (BRNCP) Mandsaur (M.P), India.

Preparation of Extract

Shade-dried fruit was placed in electric herb grinder to collect powder materials. This powder raw material was extracted with the petroleum ether, methanol and water as solvents in 1 **:** 4 (w/v) Raw materials **:** Solvent ratios. Methanol and water extracts obtained were collected, filtered through Whatman filter paper and concentrated in vacuum evaporator (40°C) under reduced pressure and dried in desiccators to get thick brown methanolic extract (MECM) and aqueous extract (AECM), then stored in refrigerator 8 ± 2°C and were used for *in-vitro* and *in-vivo* studies.

Experimental Animals

Male Wistar rats (150-200gms) and male albino mice (20-30gm) were procured from the Institutional Animal House of B.R. Nahata College of Pharmacy, Mandsaur (M.P). Animals were maintained under standard environmental condition: (Room temperature = 27 ± 3°C, Relative humidity = 65 ± 10 % and 12 hours light / dark cycle) and acclimatized for one week before commencing the experiments. The animals were fed with standard diet and water *ad libitum* under strict hygienic conditions. Experiments were performed in accordance with the current guidelines of CPCSEA India. Ethical clearance for handling the animals and experimentation was obtained from the Institutional Animals Ethical Committee (IAEC), B.R. Nahata College of Pharmacy, Mandsaur (M.P) prior to the commencement of the experimental works.

In-vitro antioxidant activity

The assay was based on the capacity of the sample to inhibit blue formazan formation by scavenging the super oxide radicals generated in riboflavin-NBT system. 100 µl riboflavin solution, 200 µl

EDTA solution, 200 µl ethanol and 100 µl Nitro Blue Tetrazolium (NBT) solution was mixed in a test tube and the reaction mixture were diluted up to 3 ml with phosphate buffer (pH. 7.4). The absorbance of solution was measured at 560 nm using phosphate buffer as blank after illumination for 15 min. This was taken as control reading. Different concentration (10 µg, 25 µg, 50 µg and 100 µg) of MECM and AECM and L- Ascorbic acid (Positive control) in 100 µl, was mixed with 100 µl riboflavin, 200 µl EDTA, 200 µl ethanol and 100 µl NBT solution in the test tubes, then the reaction mixtures were diluted up-to 3 ml with phosphate buffer. The absorbance of solution was measured after illumination for 15 min at 560 nm in Thermo Multiscan Spectrum [6, 7, 8]. Percentage inhibition was calculated and this activity was expressed as an inhibition concentration 50 (IC 50). The % inhibition was calculated by using the formula.

% Inhibition = [{Control OD – Test OD}/ Control OD]/ X 100

Determination of acute toxicity (ALD_{50})

The acute toxicity for MECM and AECM were determined in albino mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment. Fixed dose (OCED Guideline no. 423, Annexure – 2d) method of CPCSEA, Govt. of India was adopted for toxicity studies. The tested extracts were administrated orally. The mortality and behavioral abnormalities of all experimented animals was observed in 48hr after administering 2000mg/kg in the all cages [9, 10].

Evaluation of Anti-inflammatory Activity

Carrageenan Induced Paw edema

Paw edema was induced by injecting 0.1ml of 1% w/v carrageenan sodium salt (*Ozone* pharmaceuticals and chemicals, Gujarat, India) subcutaneously into the sub-plantar region of the rat right hind paw to a group of 6 animals each, which were pre-treated either with normal saline 2 ml/kg (Control), diclofenac sodium 10 mg/kg p.o. (Standard) and two different doses (200 mg/kg, & 400 mg/kg, p.o) of test samples (MECM and AECM) to the respective test groups, 30 min before the carrageenan injection. The paw volume was measured plethysmometrically (UGO Basile, Italy) before administering carrageenan and ½, 1, 2 and 3 hours after. Inhibition of Inflammation was calculated as the decreased in volume (ml) of the paw after carrageenan treatment compared to control group [11].

Cotton pellet granuloma method

The effect of test products on chronic or proliferative phase of inflammation was assessed in cotton pellet granuloma rat model as described by Deb et al. (2007). The animals were divided into seven groups of 5 animals each. Autoclaved cotton pellets weighing 50 ±1 mg each were implanted subcutaneously through small incision made along the axilla or flank region of the anesthetized rats. Animal in test groups received the test samples MECM and AECM at two different doses of 200 mg/kg, & 400mg/kg p.o., once daily for fourteen consecutive days. Control group rats (n = 5) received vehicle (dist. water; 1 ml/Animal, p.o.) and the standard group received Diclofenac sodium (10 mg/kg) orally for fourteen days.

On 14th day, the cotton pellets covered by the granulomatous tissue were removed from all animals under ether anesthesia and dried in hot air oven at 55 ± 5°C till a constant weight was achieved. Granuloma weight was obtained by subtracting the weight of cotton pellet on 0 day (before start of experiment) from the weight of the cotton pellet on 14th day [11].

Evaluation of Anti ulcer activity

Gastric ulceration was induced in overnight fasted rats by the administration of necrotizing agent like hard liquor (42.8% ethanol at 10ml/kg. p.o.) and ulcerogenic drugs like Aspirin (200mg/kg, p.o.) to group of 6 animals in respective experimental groups. Each animal were pre-treated either with distilled water (2ml/kg p.o.) for control group, Lansoprazole 8mg/kg p.o. for standard group and with test drugs MECM and AECM (200mg and 400mg/kg, p.o.) for test groups, one hour before administering the ulcerogenic agents. The animals were sacrificed 1 hr. after the administration of necrotizing agent or 6 hr. after administration of ulcerogenic agents by anaesthetizing with an over dose of ether. The stomachs were removed, cut and opened along with the greater curvature, the ulcer index were evaluated according to severity and scored with the help of hand lens (10X) as follows: 0 = Normal coloured stomach, 0.5 = Red coloration, 1 = Spot ulcers, 1.5 = Haemorrhaegic streaks, 2 = Ulcer > 3mm but < 5mm, 3= Ulcers > 5mm [12, 13, 14].

Statistical analysis

Data were expressed as mean ± Standard Error Mean (SEM) of six observations. Differences were considered significant at ***P<0.001, or **P < 0.01 or * P<0.05 when compared test groups V/S control group. For numerical results, one-way analysis of variance (ANOVA) followed by post

hoc Dunnett Multiple Comparisons Test were performed using GraphPad InStat Version 3 (GraphPad Software).

RESULTS

In the present study of NBT- superoxide anion system, it is observed that the test extracts demonstrated dose dependent increase in super oxide scavenging activity and IC_{50} values of test extracts reveals that AECM (12.30µg) shows highest activity than MECM (39.85 µg) but their activity was less than standard ascorbic acid (7.60 µg). Details of the results were illustrated in Table 1.

All the test samples MECM and AECM employed in acute toxicity study does not show any sign of abnormality and mortality at the dose of 2000mg/kg in experimental animals for a period of 48 hrs initially and then after for a week observation. Therefore 2000mg/kg dose was considered as ALD_{50} cut off the dose (a safe dose), so $1/10^{th}$ and $1/5^{th}$ of that were selected (200, 400mg/kg dose) for all *in-vivo* experiments as sub-maximal and maximal dose.

Both the test samples MECM and AECM were evaluated for *in-vivo* acute anti-inflammatory activity by using carrageenan-induced rat paw edema model. Both extracts were shown a significant (*p<0.05 & **p< 0.01) anti-inflammatory activity in dose dependent manner when compared with the normal control group (Table 2 & Figure 1).

The effect of samples MECM and AECM of were evaluated on chronic inflammatory model. The test samples were found to cause significant (***P<0.001) inhibition of granuloma formation in dose dependent manner (200 mg/kg and 400mg/kg. p.o.) (Table 3 and Figure 2).

Pre treatment with test samples MECM and AECM (200, 400mg/kg, p.o.) produced significant and dose - dependent decrease in the intensity of gastric mucosal damages induced by both ulcerogenic drug (Aspirin) and necrotizing agent (hard liquor). Details of the results were illustrated in Table 4.

Table 1: Effect of methanolic and aqueous extract of *Cucurbita moschata* Linn. fruit in superoxide anion scavenging activity.

Sl. No.	Treatment	IC50 value (µg) of Super oxide anion scavenging activity
1	Ascorbic Acid	7.60 ± 0.0026
2	Methanolic Extract of *Cucurbita moschata* (MECM)	39.85 ± 0.0047
3	Aqueous Extract of *Cucurbita moschata* (AECM)	12.30 ± 0.0032

Data were expressed as mean ± Standard Error Mean (SEM) (n=3)

Table 2: Effect of methanolic and aqueous extracts of *Cucurbita moschata* Linn. fruit on carrageenan induced paw edema in rats.

Treatment	Dose (mg/kg)	Mean paw volume (ml)				
		0 hrs	½ hrs	1 hrs	2 hrs	3 hrs
Control (2ml/kg water p.o)	-	0.955±0.053	1.23±0.041	1.43±0.084	1.77± 1.293	1.91± 0.105
Diclofenac Sodium	10	0.686±0.055	0.81± 0.066	1.273±0.068	0.93± 0.078	0.50± 0.076**
MECM	200	1.06±0.070	1.23 ± 0.017	1.43±0.027	1.85± 0.021	1.63± 0.016**
MECM	400	1.116±0.065	1.23± 0.048	1.54±0.024	1.72± 0.032	1.57± 0.032**
AECM	200	1.030±0.124	1.24 ± 0.011	1.33±0.014	1.82± 0.008	1.66± 0.015*
AECM	400	0.96± 0.147	1.18 ± 0.009	1.74±0.020	1.62± 0.021	1.59± 0.018**

Data were expressed as mean ± Standard Error Mean (SEM) n=6. Differences were considered significant at ***P<0.001, or **P < 0.01 or * P<0.05 when compared test groups V/S control group.

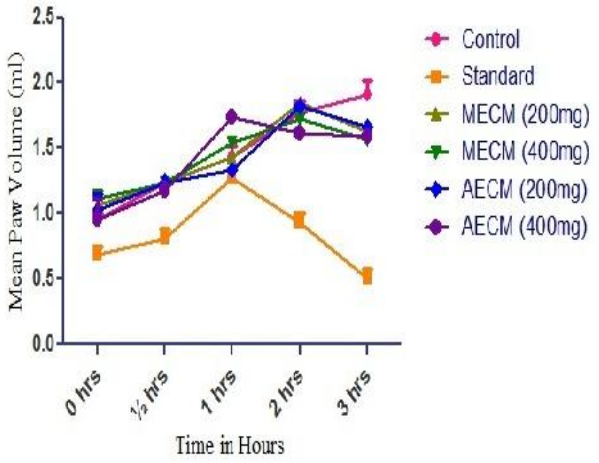

Fig. 1: Effect of methanolic and aqueous extracts of *Cucurbita moschata* Linn. fruit on carrageenan induced paw edema in rats

Table 3: Effect of methanolic and aqueous extracts of *Cucurbita moschata* Linn. fruit on cotton pellet induced granuloma in rats.

Treatment	Dose mg/kg/day	Mean weight of the granulation (mg) ± S.E.	Anti-inflammatory effect (%)
Control (2ml/kg water p.o)	-	85±2.221	
Diclofenac Sodium	10	58.5±1.176**	31.17
MECM	200	73.66±3.902**	13.34
MECM	400	69.86 ± 1.615**	17.81
AECM	200	74.58 ± 1.802**	12.25
AECM	400	67.66±1.116**	20.40

Data were expressed as mean ± Standard Error Mean (SEM) n=6. Differences were considered significant at ***P<0.001, or **P < 0.01 or * P<0.05 when compared test groups V/S control group.

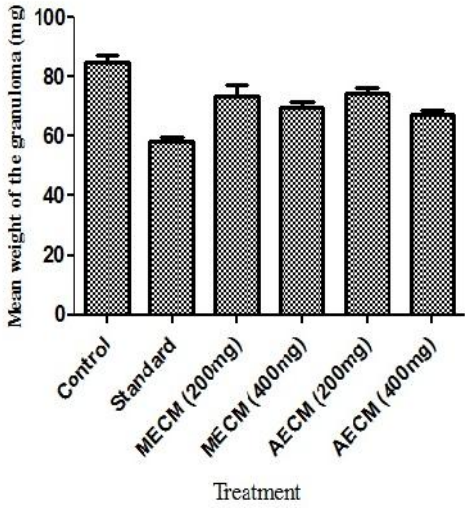

Fig. 2: Effect of methanolic and aqueous extracts of Cucurbita moschata Linn. fruit on cotton pellet induced granuloma in rats

Table 4: Effect of methanolic and aqueous extracts of *Cucurbita moschata* Linn. fruit on gastric ulcers induced by ulcerogenic drugs and necrotizing agents in rats.

Treatment	Dose mg/kg/p.o	Mean Ulcer index	% inhibition
Hard liquor induced ulcer			
Normal control (water 2ml/kg water p.o)	-	0.333 ± 0.247	-
Ulcer control (5ml/kg hard liquor p.o)	-	18.66 ± 1.838	-
Standard (Lansoprazole) + (5ml/kg hard liquor p.o)	8	3.116 ± 1.121**	83.30
MECM + (5ml/kg hard liquor p.o)	200	4.25 ± 0.901 **	77.22
MECM + (5ml/kg hard liquor p.o)	400	1.583 ± 0.611**	91.51
AECM + (5ml/kg hard liquor p.o)	200	3.73 ± 0.72**	80.01
AECM + (5ml/kg hard liquor p.o)	400	1.83 ± 0.27**	90.19
Aspirin induced ulcer			
Normal control (water 2ml/kg water p.o)	-	0.33 ± 0.247	
Ulcer control (200mg/kg Aspirin p.o)	-	17.16 ± 1.459	
Standard (Lansoprazole) + (200mg/kg Aspirin p.o)	8	3.416 ± 1.121**	80.12
MECM + (200mg/kg Aspirin p.o)	200	4.83 ± 0.988**	71.85
MECM + (200mg/kg Aspirin p.o)	400	2.50 ± 0.527**	85.43
AECM + (200mg/kg Aspirin p.o)	200	4.16 ± 0.21**	75.75
AECM + (200mg/kg Aspirin p.o)	400	2.00 ± 0.22**	88.34

Data were expressed as mean ± Standard Error Mean (SEM) n=6. Differences were considered significant at ***P<0.001, or **P < 0.01 or * P<0.05 when compared test groups V/S control group.

DISCUSSION

In present study MECM and AECM demonstrated dose dependent superoxide radical scavenging activity. It was reported that oxidative stress occurs when free radical formation exceeds the body's capacity to protect itself and contributes different biological chronic conditions such as arteriosclerosis, arthritis, cancer, gastric ulcer, diabetes and various neurodegenarations. The primary antioxidants that react with free radicals, which may limit free radical damage occurring in the human body [6]. The observation of present study supported ability of MECM and AECM in protection against oxidative damage.

MECM and AECM shows significant anti-inflammatory activity, this activity may be due to inhibition of the mediators of inflammation such as histamine and cytokines. In chronic inflammation model (cotton pellet granuloma method) the results indicate that MECM and AECM has anti-transudative, anti- exudative and anti-proliferative activity, and this may be due to the inhibition of COX-2 production and decrease in release of inflammatory prostaglandins at the site of inflammation 14.

Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the developments of the haemorrhage and necrotic aspects of tissue injury. Even, there are reports that alcohol increases the secretion of protein into the gastric juice; decreases the GSH level and increases the production of oxygen derived free radicals. Decrease in mucosal resistance is considered to be most important etiological reason in alcohol induced gastric ulcers [15, 16]. The result shown that pretreatment with MECM and AECM has reduced the ulcer index significantly; this may be due to decrease in the production of reactive oxygen radicals and enhancement of mucosal resistance in gastric mucosal layer.

NSAIDS are the most popular class of pain killers and are possessing side effects like gastric irritation and gastric ulceration. This is because of inhibition COX-1 and resulting in the inhibition of synthesis of prostaglandins consequently, there is enhanced 5-lipoxygenase pathway liberating

leukotrienes and these leukotrienes are reported to have role in ulcerogenesis [17]. In addition there is some evidence that aspirin induces gastric ulcer by causing back diffusion of H^+ ions into the mucosal cells [16, 18]. Since the result of present study demonstrated the gastro protective activity of MECM and AECM against Aspirin induced ulcer**,** it may be suggested that this gastro protection occurs by inhibiting the back diffusion of H^+ ions in the gastric mucosal cells and also by enhancing the expression of COX-1 production.

Overall in all the models of ulcers and inflammation studied, it was observed that there was a great imbalance between the aggressive physiological factors that contributes to build protective mechanisms. Treatment with MECM and AECM has reduced the ulcer progression in both the models and this may be because of enhancement of COX-1 expression and increase in production of mucosal layer; on the contrary, protective action against inflammation may be because of inhibition of COX- 2 production and inhibition of release of proinflammatory mediators at the site of inflammation.

CONCLUSION

Present investigation revealed that the protective effect of *Cucurbita moschata,* Linn fruit against gastric ulcer and inflammation is thought to its anti oxidant mechanisms. This study confirms the scientific basis of therapeutic use of *Cucurbita moschata,* Linn and it could be a next generation drug candidate for treating patients suffering with chronic inflammatory disorders and drug induced gastric ulcers. Further research is required to find out the bioactive phytoconstituent(s) present in *Cucurbita moschata,* Linn fruit, actual mechanism of action and its toxicological profile.

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