WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 9, 1017-1027 Research Article ISSN 2278 – 4357

COMPARATIVE ANTIULCER ACTIVITY OF PULP & SEED OF ETHANOLIC EXTRACTS OF *CUCUMIS MELON* (LINN) IN RATS

Veer Bala* and Nidhi Tyagi

School of Pharmaceutical Science, Shri Venkateswar University, NH # 24, Delhi Kanpur Highway, Gajraula, Amroha, UP, India.

Article Received on
09 July 2018,

Revised on 29 July 2018,
Accepted on 19 August 2018

DOI: 10.20959/wjpps20189-12293

***Corresponding Author**
Veer Bala
School of Pharmaceutical Science, Shri Venkateswar University, NH # 24, Delhi Kanpur Highway, Gajraula, Amroha, UP, India.

ABSTRACT

The present study was undertaken to investigate antiulcer effect of ethanolic extracts of pulp & seed of *Cucumis melon* L. in Wistar rats. Ethanolic Pulp extract of *Cucumis melon* L. was administered to experimental rats at doses of 200 and 400 mg/kg. & ethanolic seed extract was administered at doses of 200 and 400 mg/kg. Standard drug was Ranitidine (50mg/kg) while normal saline (0.9%) was used as control. The antiulcer effect of the extracts was evaluated measuring different parameters like free acidity, total acidity, ulcer index, ulcer score, gastric secretion and pH of the gastric juice. In addition, total acidity and free acidity were also reduced significantly (**P<0.01, *P<0.05) in dose dependent manner. Further it was observed that ulcer index and ulcer score of extract treated and standard were also reduced significantly (P<0.01) as compared to control group (9.83±1.20, 3.28±0.39). We can conclude that ethanolic extracts of pulp & seed of *Cucumis melon* L. produced notable antiulcer effect which appeared to be comparable with the effect produced by the standard (Ranitidine). The present study provides a quantitative basis for explaining the folkloric use of *Cucumis melon* L. as an Antiulcer agent.

KEYWORDS: Herbal medicine, *Cucumis melon* L., Ethanolic extract, Antiulcer activity.

INTRODUCTION

Thousands of years ago the knowledge of drugs has accumulated by ancient physicians as a result inquisitive nature of men, so today it is said that nature has provided a complete store-house of remedies to cure many disease. Antiulcer agents significantly reduced the volume of

gastric secretion, free acidity total acidity ulcer index ulcer score and increase the pH of the gastric juice. These drugs are also used in the treatment of toxemia oedema, hypertension, diuretics, pulmonary congestion and play vital role in pregnancy/\.[1] Presently synthetic antiulcer is available in market which is having significant side effects. This antiulcer significantly reduced the volume of gastric secretion and increases the pH of the gastric juice.[2] A natural source serves as an additional source for the development of new antiulcer agents as they have significant biological activity. Various plant sources used as antiulcer in different systems of traditional medicine and Ayurveda; *Cucumis melon* L. is most common annual herb and whole plant used to treat various disorders,[3] Review of the literature reveals that no pharmacological and clinical study was carried out to test the diuretic activity of this plant. The main purpose of the present study was to evaluate the antiulcer activity of *Cucumis melon* L. We focused on evaluation of the biochemical parameters like volume of gastric secretion, pH of the gastric juice, free acidity total acidity ulcer index ulcer score.[4]

MATERIALS AND METHODS

Collection and authentication of plant materials: The plant material was collected from Hathras, UP, and authenticated from Birbal Sahni Institute of Palaeobotany, Lucknow, UP.

Extraction process: The pulp & seed of C*ucumis melon* L. were dried in shade. Then extracted in soxhlet assembly with Pet. Ether, ethanol and water successively. Each extract is concentrated by removal of the solvent. Extracted material is dried in hot-air oven below 50_C and pharmacological evaluation was done.[5]

Preliminary phytochemical screening: All the extracts were screened for the presence of various secondary metabolites like glycosides alkaloids, carbohydrates, flavonoids, amino acids, Proteins and tannins [Table 1] using standard Pylorus ligation method.[6]

Experimental Animals: Healthy Wistar rats of either sex (weighing 150-200g) were used in the experiment. All the experimental study was conducted in accordance with CPCSEA and IAEC guidelines.[7]

Toxicity studies: An acute oral toxicity study was performed as per OECD guidelines 423 in female Swiss albino mice weighing 20-25g.[8]

Pylorus ligation method: Gram negative bacteria found in gastric and duodenal mucosa of most persons particularly the elderly. They while in the mucosa, split into ammonia and thus

elevates the local region of the mucosa by high alkalinity. In this way they strongly help the peptic ulcer development.

Weighing Albino Wistar rats between (150-200 Gms) and either sex are divided into six groups and each group has three animals.

Group I- Control 1% w/v CMC p.o.

Group II- Standard (Renitidine 50 mg/kg p.o. in 1% w/v CMC)

Group III- EECMP 200 mg/kg p.o. in 1% w/v CMC

Group IV- EECMP 400 mg/kg p.o. in 1% w/v CMC

Group V- EECMS 200 mg/kg p.o. in 1% w/v CMC

Group VI - EECMS 400 mg/kg p.o. in 1% w/v CMC

In this method albino Wistar rats were fasted and places individual cages for 24 hours. First of all control vehicle or standard drug or test drug is administered 30 minute prior to pyloric ligation. Under light ether anaesthesia, the abdomen is opened and the pylorus was ligated. The abdomen is then sutured. At the end of 4 hours after ligation the animals are sacrificed with excess of anaesthetic ether, and the stomach is dissected out gastric juice is collected were drained into tubes and were centrifuged at 1000 rpm for 10 minutes and the volume is noted. The pH of gastric juice is recorded by pH meter. Then the contents are subjected to analysis for free and total acidity. The stomachs are then washed with running water to see for ulcers in the glandular portion of the stomach. The numbers of ulcers are noted in the individual stomach and severity of the ulcers score are note down by microscopically with the help of hand lens (10 xs) and scoring was done.[9]

The ulcer scoring done as below

Normal stomach.....0

Red coloration.....0.5

Spot ulcer.....1.0

Hemorrhagic streak.....1.5

Ulcers.....2.0

Perforation.....3.0

Mean ulcer score for each animal will be expressed as ulcer index.

Calculation of ulcer index

$UI = (UN+US+UP) \times 10^{-1}$

UI = Ulcer index

UN = Average of number of ulcer per animal

US = Average of severity score

UP = Percentage of animal with ulcer

Using flowing formula to calculate percentage protection

$$\text{Percentage protection} = 1 - \frac{Ut}{Uc} \times 100$$

Where Ut = Ulcer index of treated group

Uc = Ulcer index of control group.

ESTIMATION OF PARAMETERS

Estimation of total acidity and free acidity : Pipette out 1 mL of gastric juice into 100 mL conical flask and added 2-3 drops of topfer's reagent and treated with 0.01 N sodium hydroxide until all traces of red colour disappears and the colour of the solution turns to yellowish orange. The volume of the alkali added was noted. This volume corresponds to free acidity and titration was continued until a definite red tinge reappears and 2 to 3 drops of phenolphthalein solution was added. Again the total volume of alkali added is noted. Acidity is calculated by using the formula: Acidity = Volume of NaOH × Normality of NaOH × 100 / 0.1 × meq/L/100 gm.

Estimation of Ulcer index (Ui): Ui = mean degree of ulceration × % group of ulceration /100 % inhibition = (ulcer index in control - ulcer index in test) ulcer index in control x 100.

Histological studies: Gastric tissue samples from each group were fixed in 10% formalin for 24 h. The formalin fixed specimens are embedded in paraffin and section (3-5µm) and stained with haematoxylin and eosin dye. The histochemical section was evaluated by light microscopy.

Determination of pH and gastric volume: An aliquot of 1 mL gastric juice was diluted with 1mL of distilled water and pH of the solution was measured by using digital pH meter.

The gastric juice obtained after pyloric ligation was subjected to centrifugation for 1 hr at 3000 rpm and volume was measured by pipette.[10]

Statistical analysis: SPSS (version 13.0) statistical program was used to carry out one way analysis of variance (ANOVA) on the data, followed by Dunnett's *t*-test. Values are expressed as mean ± SEM of six samples. $P < 0.01$ was considered as significant.

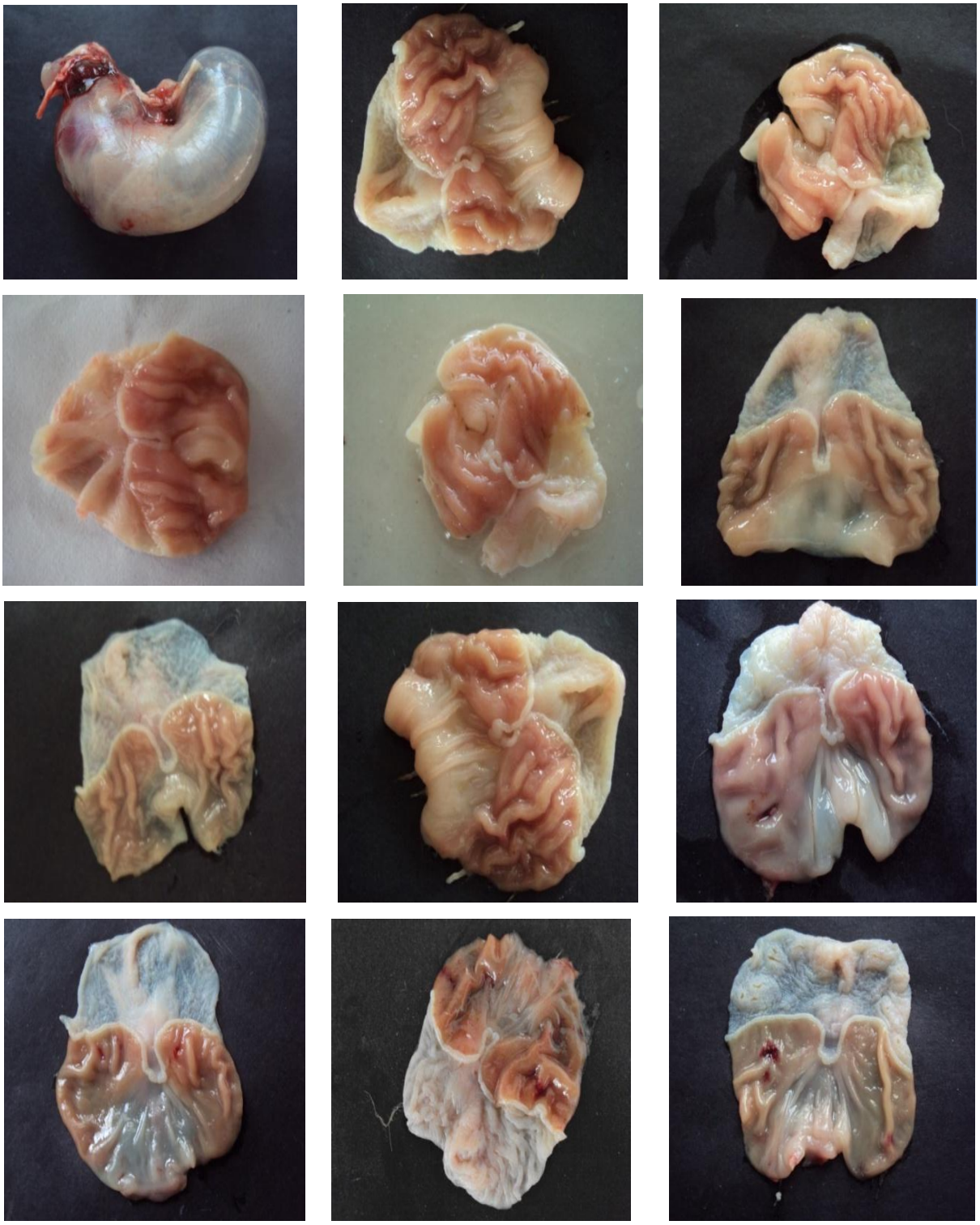


Figure: Ulcer in Stomachs.

RESULTS

The preliminary phytochemical analysis showed the presence of Carbohydrates, Flavanoids, Proteins, Glycosides, Amino acids, Tannins, Saponin, and Alkaloids in all the extracts [Table 1]. All extracts were subjected to pharmacological screening to evaluate acute toxicity studies and antiulcer activity.

Table 1: Preliminary phytochemical test of the ethanolic extract of pulp & seed of *cucumis melon* Linn.

Sr. No.	Phytochemical test	Ethanolic extract of *Cucumis melon* seed	Ethanolic extract of *Cucumis melon* pulp
1.	Carbohydrates	+	+
2.	Flavanoids	+	+
3.	Proteins	+	+
4.	Glycosides	+	+
5.	Amino acids	+	+
6.	Tannins	+	+
7.	Alkaloids	+	+
8.	Amino Acid	_	_
9.	Reducing sugar	+	+
10.	Mucilage	_	_
11.	Saponin	_	_

From preliminary toxicity studies, it was observed that animals were found to be safe up to a maximum dose of 1000 mg/kg b. w. However, there were few changes in the behavioral response.

Table 2: Effect of Pulp and seed extract of *Cucumis melon* Linn on general behavioral profile.

Sr. No.	Treatment	EECMP 40mg/kg	EECMP 200mg/ Kg	EECMP 400mg/ Kg	EECMS 40mg/ Kg	EECMS 200mg/ Kg	EECMS 400mg/ Kg	Chlorpromazine	Vehicle 5ml/kg
1.	**Activity**	4.0±0.0	3.9±0.0	3.8±0.2	4.0±0.0	3.7±0.3	3.5±0.34	7.7±0.2***	4.0±0.0
2.	**Alertness**	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	3.0±0.52	7.7±0.2***	4.0±0.0
3.	**Awareness**	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	8.0±0.0***	4.0±0.0
4.	**Sound response**	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	8.0±0.0***	4.0±0.0
5.	**Touch response**	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	7.8±0.2***	4.0±0.0
6.	**Pain response**	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	4.0±0.0	8.0±0.0***	4.0±0.0

N=6. All values are Mean ± SEM. The scores indicate the peak effect. ***P<0.001 compared to the base line score (Kruskal -Wallis test followed by Dunn‘s multiple comparison test)

Table 3: Effect of *Pulp and seed* of *Cucumis melon* Linn ethanolic extract on exploratory behavior (Y-maze test) in rats.

Sr. No.	Treatment	Dose	Number of eateries in open arm after treatment (min.)			
1.	Vehicle	5ml/kg	11.3±0.33	12.0±1.15	11.3±0.67	13.0±0.58
2.	Diazepam	25mg/kg	3.0±0.58**	2.6±1.20**	3.0±0.58**	4.0±1.00**
3.	EECMP	200mg/kg	11.0±0.58	10.0±0.58	10.7±0.33	11.0±0.58
4.		400mg/kg	10.7±0.33	11.0±0.58	10.0±0.58	11.0±0.00
5.	EECMS	200mg/kg	10.3±0.88	11.0±0.00	9.7±0.88	11.3±0.33
6.		400mg/kg	11.7±0.33	10.0±1.00	9.0±0.58	11.0±0.58

N=3, All values are Mean±SEM. Statistical comparison was performed by Graph pad software using Dunnett's test followed by ANOVA, **P<0.01 when all compared with the vehicle group.

Table 4: Effect of *Pulp and seed* of *Cucumis melon* Linn ethanolic extract on exploratory behavior (Plus maze test) in rats.

Sr. No.	Treatment	Dose	Number of entary in open arm	
			Before treatment	After treatment
1.	Vehicle	5ml/kg	8.0±0.58	8.7±0.67
2.	Diazepam	25mg/kg	8.0±0.58	8.3±0.88
3.	EECMP	200mg/kg	8.7±0.33	8.3±1.20
4.		400mg/kg	8.0±0.58	8.7±0.67
5.	EECMS	200mg/kg	7.3±0.33	9.0±0.58
6.		400mg/kg	8.7±0.88	12.3±1.45**

N=3, All values are Mean±SEM. Statistical comparison was performed by Graph pad software using Dunnett's test followed by ANOVA, **P<0.01 when all compared with the vehicle group

Table 5: Locomotor activity of *Pulp and seed* of *Cucumis melon* Linn ethanolic extract by using actophotometer

Sr. No	Treatment	Dose	Before treatment	Locomotor activity after treatment (min.)		
				30	60	120
1.	Vehicle	5ml/kg	160.7±0.7	156.6±1.7	153.3±4.4	146.7±6.7
2.	Diazepam	25mg/kg	154.0±7.0	87.7±3.4**	72.0±2.1**	51.0±1.0**
3.	EECMP	200mg/kg	158.3±1.7	148.6±5.8	138.3±1.6	157.3±1.5
4.		400mg/kg	161.7±1.7	156.7±1.7	143.3±8.3	145.0±7.6
5.	EECMS	200mg/kg	155.3±5.5	148.3±3.3	131.7±6.0	151.7±6.3
6.		400mg/kg	168.0±4.2	162.7±1.5	153.3±6.7	143.7±9.8

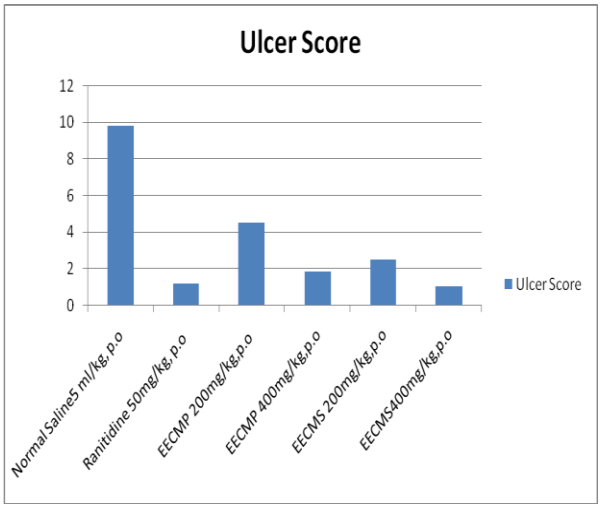
N=3, All values are Mean±SEM. Statistical comparison was performed by Graph pad software using Dunnett's test followed by ANOVA, **P<0.01 when all compared with the vehicle group.

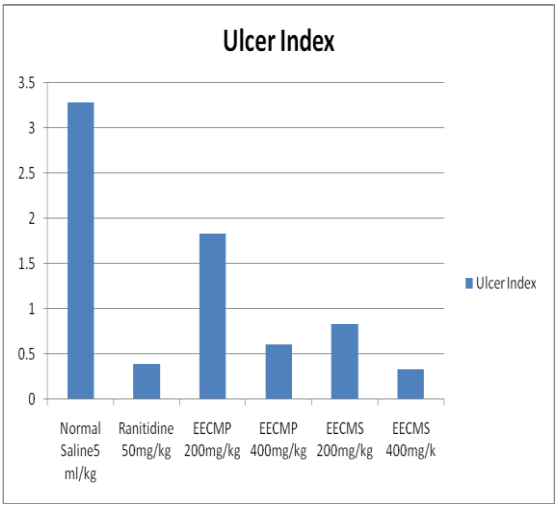
Effect of antiulcer activity of ethanolic extract of pulp & seed of *Cucumis melon* Linn by pylorus ligation induced ulcer model

The results obtained when ulceration of the gastric mucosa was provoked by pylorus ligation are shown in [Table 6]. Pyloric ligation of rats in the control group produced lesions or raised inflammations. The pyloric ligation has caused the accumulation of gastric secretions of 1.47±0.15 ml with pH 2.17±0.50 in a control group. The free acidity and total acidity of the gastric secretion were found to be 92.33±7.26 and 169.33±17.47mEq/L respectively. Pretreatment with the ethanolic extract of pulp & seed of *Cucumis melon* Linn at dose of 200 and 400mg/kg, significantly (P<0.05) reduced the volume of gastric secretion and increase the pH of the gastric juice. In addition, total acidity and free acidity were also reduced significantly (**P<0.01, *P<0.05) in dose dependent manner. Further it was observed that ulcer index and ulcer score of extract treated and standard were also reduced significantly (P<0.01) as compared to control group (9.83±1.20, 3.28±0.39) as shown in [Table 6].

Table 6: Evaluation of antiulcer activity of ethanolic extract of pulp & seed of *Cucumis melon* Linn by pylorus ligation.

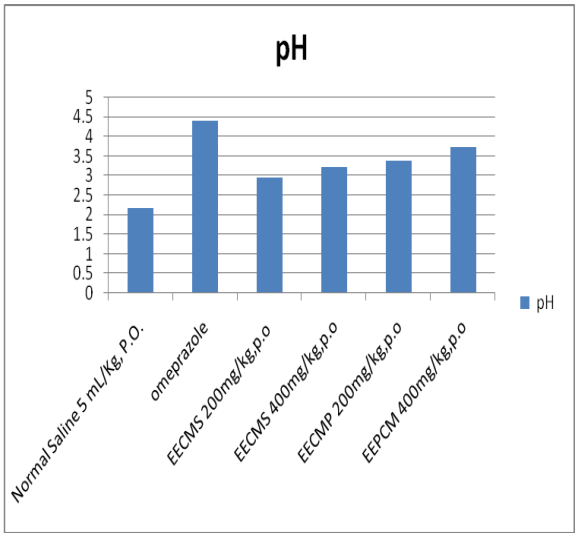
Sr. No.	Treatment	Dose (mg/kg)	Ulcer Score	Ulcer Index	% Protection
1.	Control(1%w/v CMC)	1ml/animal	9.83±1.20	3.28±0.39	-
2.	Ranitidine	50	1.16±0.60**	0.39±0.20**	88.11
3.	EECMP	200	4.50±0.08	1.83±0.69	54.22
4.		400	1.83±0.33**	0.61±0.11*	81.40
5.	EECMS	200	2.50±0.58*	0.83±0.19*	74.69
6.		400	1.00±0.50**	0.33±0.17**	89.94

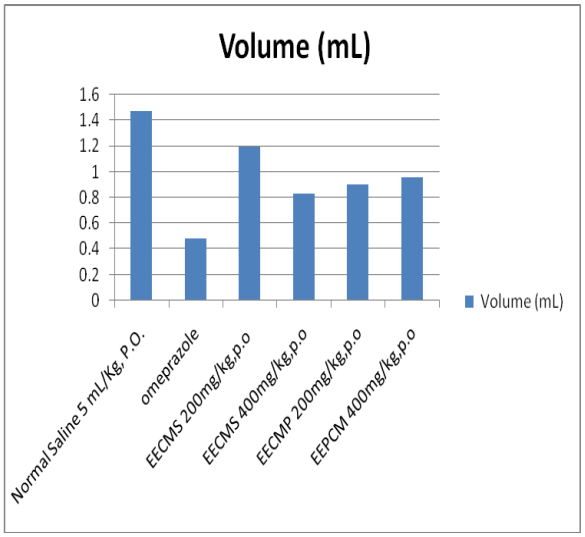




All values are Mean±SEM for 3 rats, Statistical comparison was performed by Graph pad software using Dunnett's test followed by ANOVA, **P<0.01, when all compared with the control group.

Sr. No.	Treatment	Dose (mg/kg)	pH	Volume (ml)	Free Acidity (mEq/L)	Total acidity (mEq/L)
1.	Control	1ml	2.17±0.50	1.47±0.15	92.33±7.26	169.33±17.47
2.	Renitidine	50	4.41±0.29**	0.48±0.07**	58.00±7.81**	90.33±1.67**
3.	EECMP	200	2.97±0.49	1.20±0.17	93.00±1.00	128.67±2.19*
4.		400	3.23±0.26	0.83±0.20	48.33±2.40**	95.00±6.35**
5.	EECMS	200	3.40±0.29*	0.90±0.12	82.67±4.70	130.33±11.79
6.		400	3.73±0.17*	0.96±0.17	64.67±7.26*	96.33±9.82**





All values are Mean±SEM for 3 rats, Statistical comparison was performed by Graph pad software using Dunnett's test followed by ANOVA, **P<0.01, *P<0.05 when all compared with the control group.

DISCUSSION

In the present investigation different doses of ethanolic extract of pulp & seed of *Cucumis melon* L. in Wistar rats. Ethanolic extract of pulp & seed of *Cucumis melon* L seeds were screened for gastroprotective effects by employing various experimentally induced ulcer models in rats. Gastrointestinal ulcer is a common disease in clinic. The imbalance between aggressive (gastric juice, pepsin) and protective factors (mucosal blood flow, bicarbonate secretion, the secretion of mucosa integrality of cellular membrane, cell regeneration, prostaglandin and other hormones), is considered as the major mechanism for ulcer. The general anti-ulcer drugs, inhibit the acid secretion, protect the mucosa, and inhibit the *Helicobacter pylori*.

It has been indicated that, interference of gastric blood circulation and digestive effect of accumulated gastric juice are responsible for induction of ulceration in pylorus ligation. In pylorus ligation model antiulcer activity of EECMP & EECMS was evident from its significant reduction in gastric volume, ulcer index, free and total acidities, and increase in pH. Therefore it is suggested that both the extracts can suppress gastric damage induced by aggressive factors. Preventive anti-oxidants, such as catalase (CAT) enzymes and reduced glutathione (GSH) are the first line of defense against reactive oxygen species. Reduced glutathione (GSH) is a major low molecular weight scavenger of free radicals in the cytoplasm and an important inhibitor of free radical mediated lipid per oxidation.

The results concluded that CPEE and CPCE had significant effect when compared with the standard ranitidine and can be predicted that these extracts have potencies to antagonize H_2 receptor and thus are good antiulcer agents. In addition preliminary phytochemical investigation on both the extracts revealed the presence of tannins and flavonoids, substance known to affect the integrity of mucus membrane mentioned. Forming an impervious protective pellicle over the lining to prevent absorption of toxic substances. Tannins, being an astringent, may precipitate microproteins on the site of ulcer and proteolytic enzymes are resisting the attack. Flavonoids have also been reported to offer some protection in ulcer development by increasing capillary resistance and improving microcirculation.

CONCLUSION

The dried seeds were subjected to phytochemical work. The preliminary phytochemical investigation showed the presence of glycosides, flavonoids, carbohydrate, tannins in the ethanolic extract and glycosides, sterols, triterpines and tannins in the chloroform extract.

The ethanolic extracts of pulp & seed of *Cucumis melon* L were employed for the pharmacological screening, these extracts showed significant anti-ulcer activity. The results led to the conclusion that the EECMS exhibited better antiulcer activity then the EECMP. For assessing anti-ulcer activity pylorus ligation induced ulceration models were used and various parameters like gastric pH, gastric volume, free acidity, total acidity, carbohydrates, hexoses, hexosamine, fucose, anti-oxidant study, protein estimation, mucin estimation and ulcer index observations were determined.

Both ethanolic extracts of pulp & seed of *Cucumis melon* L raised the gastric pH, and increased the anti-oxidanst level, they lowered the free acidity, total acidity and ulcer index as compared to the control group.

REFERENCES

1. Thamotharan, G., Seka,r G., Ganesh, T., Saikat, S., Chakrborthy, R. and Kumar, S.N., 2010, Anti-ulcerogenic effects of *Lanata camara* Linn leaves on In-vivo test models in rats, Asian J Pharma Clin Res, 3(3): 57-60.
2. http://en.wikipedia.org/wiki/peptic_ulcer (accessed on 22/06/11).
3. Agharkar SP. Medicinal plants of Bombay presidency, PBI, Scientific Publishers, Jodhpur (India), 1991; 230.
4. Ramaswamy, S., Sengottuvelu, S., Sherief, S.H., Jaikumar, S., Saravanan, R., Prasadkumar, C., Sivakumar, T., 2010, Gastroprotective activity of ethanolic extract of *Trachyspermum ammi* fruit, Int J Pharma Bio Sci, 1(1): 1-15.
5. Trease G.E., Evans W.C., Pharmacognosy, 13th ed, ELBS Publication, Delhi, 1989, 171.
6. Kokate CK. Practical Pharmacognosy.4th ed. Delhi: VallabhPrakashan, 1994.
7. Patel R, Mahobia N k,Gendle R,et al. Diuretic activity of leaves of *Plectranthus amboinicus* (Lour) Spreng in male albino rats, 2010; 2: 86-88.
8. Organization for Economic Co-operation and Development. Guidance Document on Acute Oral Toxicity Testin. Environment Directorate, Peris: OECD, 2001; 1- 24.
9. Kulkarni, S.K., 1999, Hand book of experimental pharmacology, Vallabh Prakashan, New Delhi, 148-50.
10. Turner, R.A., 1965, screening methods in Pharmacology, 3rd edition, Academic press Nic., London, 23-41.