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Research Article

Preliminary Pharmacological Screening of *Momordica charantia* Linn. Leaves Extracts for Antiulcer Effect on Albino Rats

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Abstract: Ulcer is defined as a rupture in the skin or mucus membrane with loss of surface tissue. It has defects in the epithelium, caused by a lesion with a lost epithelial layer in the stomach or diffused loss of mucosal layer within the oral cavity. The present study aimed to investigate the preliminary screening of *Momordica charantia* Linn. leaves for oral and stomach ulcers. The leaves of *Momordica charantia* Linn. were extracted with different solvents with increasing polarity. Preliminary phytochemical tests were carried out to detect the presence of various chemical constituents in petroleum ether, chloroform, ethyl acetate, ethanol, and aqueous extracts. Initial screening was done for the antiulcer effect on ethanol-induced stomach ulcers and glacial acetic acid oral ulcers. The antiulcer effect was observed by they measured the ulcer index, percentage inhibition of ulcer, gastric pH, total acidity, and gastric volume. In addition, biochemical assessments of proinflammatory mediators (TNF-α, IL-6, and PGE-2) were performed in mucosal tissues. Results of phytochemical screening revealed the presence of steroids, terpenoids, alkaloids, flavonoids, tannins, glycosides, and phenolic compounds. Results of in vivo study showed that the antiulcer effect was observed in a dose-dependent manner. Ethanolic extract of *Momordica charantia* Linn. with 200 and 400mg/kg b.w. Doses have significant antiulcer potential and were comparable to the standard (Omeprazole and ORASORE Gel). A significant ($p<0.05$) reduction in ulcer index and percent inhibition was observed in the stomach and oral cavity ulcers. Furthermore, the practical ($p<0.05$) restoration of inflammatory mediators indicated a supporting mechanism of ulcer healing of ethanolic extract. In conclusion, the antiulcer effect was shown in a dose-dependent manner may be due to the presence of secondary metabolites like phenolic compounds viz., flavonoids, and tannins in the ethanolic extract of *Momordica charantia* Linn.

Keywords: Antiulcer Activity, Antioxidant Activity, *Momordica Charantia* Linn, Flavonoids, Ulcer Index

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1. INTRODUCTION

An ulcer is one of the most common diseases in the mucous membrane or soft tissues, generally by breakdown or rupture in different body organs. Oral ulcer in the oral cavity habitually occurs on the lip, cheeks, and tongue edges with white or yellow color and burning, painful condition. Ulcer in the tissue is inclined to many infections, inflammation, and necrosis in tissue [1]. An oral ulcer may be acute or chronic and could be single or multiple. The ulcer area is covered with a yellowish fibro membrane layer during the healing process starts [2]. A stomach ulcer is a gastric lesion occurring due to an imbalance between aggressive factors like pepsin and helicobacter pylori and defensive factors such as bicarbonate ions, prostaglandin, mucin, and another factor that has been concerned with the pathogenesis of stomach or gastric ulcer is oxidative stress in the gastric mucosa [3]. Previous studies have shown a positive relationship between increased free radicals and the extent of gastric ulceration in experimental animals. Currently, several synthetic drugs are available to treat ulcers, including proton pump inhibitors and H-2 receptor antagonists that reduce gastric acid secretion; sucralfate, colloidal bismuth substrate acting as physical barriers or increasing the bicarbonate secretion, and mucous prostaglandin analogous are available for the treatment of peptic ulcers, but clinical evaluation of these drugs has shown the incidence of relapses and various side effects. It may cause nausea, dryness of mouth, atrophic gastritis, and osteodystrophy [4]. A new approach for managing oral and stomach ulcers is using cytoprotective agents that can also modulate antioxidant defense. Most of the remedies were taken from plants and proved helpful in the indigenous system of medicine. Nature has always been a valuable source of drugs. Plants are a rich source of active principles and antioxidants. So there has been growing interest in identifying and syntonically authenticating agents that have traditionally been used in folk medicine to treat ulcers and other related diseases. The present study aimed to explore new therapeutic effects of *Momordica charantia* leaves for antiulcer potential. *Momordica charantia* Linn. belonging to the family Cucurbitaceae, known as bitter melon, is used not only as a vegetable but also in traditional medicine. The plant is widely grown in tropical and subtropical areas, including Africa, South America, and Asia. The leaves of *Momordica charantia* Linn. pharmacological scrutinizes been revealed to possess a range of anti-inflammatory and hypoglycemic activities [5]. *Momordica charantia* Linn. (bitter melon) has been used traditionally for thousands of years. Every part of the plant exhibits pharmacological action, including the seeds, roots, and leaves which have anti-mutagenic activity, the juice of the leaves was applied externally to cure chronic disorders of the skin as well as in treating burns, boils, and eruptions. In addition, the ingestion of the plant may help to control excessive sugar in the urine and blood [6]. Secondary plant metabolites such as flavonoids, alkaloids, tannins, and saponins are responsible for antiulcer activity [7]. Phytochemical screening of the leaves extract of *Momordica charantia* Linn. revealed the presence of saponins, steroids, tannins, glycosides, alkaloids, and flavonoids [8]. thus, it can reduce mucosal damage. The objective of the present research work was to investigate the therapeutic efficacy of *Momordica charantia* Linn. leaves extracts against oral and stomach ulcers.

2. MATERIAL AND METHODS

2.1 Plant material collection and authentication

The fresh leaves of *Momordica charantia* Linn. were collected in the month of August 2018 from the herbal garden RKDF University, Bhopal, M.P. and identified & authenticated by the Department of Botany APS University, Rewa M.P., India. Herbarium specimens were prepared and deposited with voucher specimen No /B/PAN/484.

2.2 Preparation of plant extracts

The freshly dried leaves of *Momordica charantia* Linn. were shattered and screened with 40 mesh. Shade-dried coarsely powdered leaves (250 g) was loaded in Soxhlet apparatus and were successively extracted using an organic solvent with increasing polarity order i.e., petroleum ether (60-62°C), Chloroform, Ethyl acetate, Ethyl alcohol, and aqueous until the extraction was complete. After completion of extraction, the solvent was removed by distillation. The extracts were dried using a rotator evaporator. The residue was then stored in a desiccator, and the percentage yield was determined [9].

2.3 Phytochemical screening

Phytochemical screening was carried out to get the presence of various phytocompounds viz flavonoids, saponins, glycosides, alkaloids, carbohydrates, tannins, and phenols in different leaves extracts of *Momordica charantia* Linn. using standard methods [10].

2.4 Determination of Total Phenolic and Flavonoid Content

The total phenolic content of the ethanol extract of *Momordica charantia* leaves was determined by the standard method using Folin-Ciocalteu reagent [11]. A reaction mixture contained 500µ l of 0.1% diluted extract, 2.5 ml of 0.2M Folin-Ciocaletu reagent, and sodium carbonate solution (2 ml). The sample mixture was placed in the dark under suitable temperature/conditions for 30 min to proceed with the reaction. Measurements of absorbance of the sample mixture were taken at 760nm in a UV–Vis spectrophotometer (Shimadzu, USA). With the help of standard gallic acid, the total phenolic content was expressed as mg of gallic acid equivalents per gram of extract. The full range of flavonoids in the ethanol extract of *Momordica charantia* leaves was calculated using the colorimetric assay method of aluminum chloride. In this method, a small section was taken in a test tube containing 3.4 ml of 30% methanol solvent, 0.15 ml of 0.5M sodium nitrite ($NaNO_2$), and 0.15 ml of 0.3 M $AlCl_3.6H_2O$ were mixed properly trough shaking. After 5 min, one ml of one M NaOH was added with continuous mixing, and absorbance was measured at 510 nm [12]. With the help of pure quercetin, preparing a standard curve and the content were determined in the sample.

2.5 Experimental animals

Whole animal experiments were performed in accordance with the guidelines issued by the Institutional Animal Ethics Committee (IAEC), Vedica College of B. Pharmacy, RKDF University, Bhopal MP. The animals were used with permission number IAEC/VCP/2019/001/7, as per the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Adult albino rats of both sex weighing 150 to 180 g were used for the *in-vivo* antiulcer study. The animals were housed in clean polypropylene cages and maintained in a well-ventilated, temperature-controlled animal house with a

regular 12 hours' light/dark schedule. The animals were fed with a standard rat pellet diet, and clean drinking water was made available ad libitum.

2.6 Toxicity Study

The acute oral toxicity studies were carried out per the guidelines of the Organization for Economic Co-operation and Development (OECD) 423. The acute toxicity of all extracts of *Momordica charantia* Linn. leaves were determined in albino rats. The animals were fasted overnight before the experiment. Animals were divided into 8 groups and the sections were administered orally to various groups of albino rats in doses ranging from 500, 1000, 1500, 2000, 2500, 3000, 3500, and 4000 mg/kg for the acute toxicity study [13]. Observation of all animals for any lethality in any of the groups after 7 days of treatment was noted.

2.7 Experimental protocol

Albino rats of 150-180g of both sexes were used for the study. All the animals were divided into 12 groups, with 6 in each group.
Group I- indicated as Disease Control Saline 5 ml/kg p. o.;
Group II-treated with Omeprazole 20 mg/kg p. o. for the ethanol-induced ulcer model and ORASORE Gel (Wings Biotech, Delhi) for the Glacial acetic acid-induced oral ulcer model
Group III- treated with petroleum ether extract of *Momordica charantia* Linn. leaves (PEEMC) 200 mg/kg p. o.;
Group IV- treated with petroleum ether extract of leaves of *Momordica charantia* Linn. leaves (PEEMC) 400 mg/kg p. o.
Group V- treated with chloroform extract of leaves of *Momordica charantia* Linn. leaves (CEMC) 200 mg/kg p. o.
Group VI- treated with chloroform extract of leaves of *Momordica charantia* Linn. (CEMC) 400 mg/kg p. o.
Group VII- treated with ethyl acetate extract of leaves of *Momordica charantia* Linn. (EAEMC) 200 mg/kg p. o.
Group VIII- treated with ethyl acetate extract of leaves of *Momordica charantia* Linn. leaves 400 mg/kg p. o.
Group IX- treated with ethanol extract of *Momordica charantia* Linn. leaves (EEMC) 200 mg/kg p. o.
Group X- treated with ethanol extract of *Momordica charantia* Linn. leaves (EEMC) 400 mg/kg p. o.
Group XI- treated with aqueous extract of *Momordica charantia* Linn. leaves (AEMC) 200 mg/kg p. o.
Group XII- treated with aqueous extract of *Momordica charantia* Linn. leaves (AEMC) 400 mg/kg p. o.

2.8 Antiulcer activity

Ethanol-induced ulcer

Momordica charantia Linn. leaves extract *of* 200 mg/kg, 400 mg/kg, and Omeprazole 20 mg/kg was administered orally to 48 hours fasted Albino rats. The ulcer was induced by administering 1 ml of 80 % ethanol orally to each animal. [14] After 1 hour, the albino rats were anesthetized using diethyl ether and then euthanized by cervical dislocation. The stomach was removed and opened along the greater curvature. All the stomachs were gently rinsed with water to remove the gastric contents and blood clots. The stomach along the greater curvature is pinned on a soft board for evaluating gastric ulcers. Each stomach was examined for lesions in the four-stomach portion and indexed according to severity. The ulcer scoring was done, and the percentage protection was calculated [14].

Glacial acetic acid-induced oral ulcer model.

The albino rats were anesthetized with 3% sodium pentobarbital at an injection dose of 30mg/kg. Then, 40% glacial acetic acid was administered into the buccal membrane for 60 sec using a glass rod of a diameter of 7 mm and length of 10 cm, then, the cauterization parts were rinsed for 1 min to form a white lesion. Congestion and red swelling were observed in the area of the buccal. Yellow or white pseudo membrane covered the treated parts, indicating that the oral ulcer model was successfully established [15]. All groups were observed macroscopically and ulcer index and percentage inhibition were calculated. After a week of duration, oral mucosa tissue samples were collected from each group, and antioxidants and cytokines assays were analyzed.

2.9 Macroscopic evaluation

The stomach was opened along the wider curvature, rinsed with saline and, extracted stomach contents and blood clots, and inspected for ulcer formation by a 10-lens magnifier [16].
In the case of oral ulcer model, oral mucosal tissue was collected at the junction of the ulcer and normal mucosa from different treatment groups for morphological features of oral ulcer, e.g., hyperemia and the color of the oral mucosa. In addition, the number of ulcers were counted.
Scoring of ulcers was observed as in the following manner:
Standard color …..... (0)
Red coloration…..... (0.5)
Spot ulcer…..... (1)
Hemorrhagic streak… (1.5)
Deep Ulcers…..... (2)
Perforation…..... (3)
Each experimental animal's mean ulcer score is expressed as the ulcer index. The Percentage of protection against ulcers has been given as follows. The Ulcer Index (UI) was calculated using the formula:

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

Where-
UI= Ulcer Index;
UN = Average number of ulcers per animal;
US = Average number of severity scores;
UP = Percentage of animals with ulcers
Percentage inhibition of ulceration

$$\% \, Inhibition \, of \, Ulcer = \frac{Ulcer \, index \, of \, Control \, group - Ulcer \, index \, of \, Test \, group}{Ulcer \, index \, of \, Control \, group} \times 100$$

2.10 Determination of total acidity in stomach ulcer model

The total acidity of the gastric secretion was determined by taking an aliquot of 1 ml of gastric juice diluted with 1 ml of district water and was taken into 50 ml of the conical flask and titrated with 0.01N NaOH and phenolphthalein as indicator until the pink color was observed [17]. The total acidity is expressed as mEquiv/l using the following formula:

$$n \times 0.01 \times 40 \times 1000$$

Where *n* is the volume of NaOH quantified, 40 is the molecular weight of NaOH, 0.01 is the normality of NaOH, and 1000 is the factor represented in liter.

2.11 Determination of pH in stomach ulcer model

An aliquot of 1 ml gastric juice was diluted with 1 ml of distilled water, and the solution's pH was measured using a pH meter.

2.12 Measurement of gastric content in stomach ulcer

The stomachs were removed and the volume of the gastric juice was collected and measured.

2.13 Assessment of antioxidant parameters

Assessment of stomach and oral mucosal tissues were collected from all experimental animal groups for antioxidant assay. Catalase was estimated following the breakdown of hydrogen peroxide according to method [17]. The beers and Seizer method was used to determine the activity of the enzyme CAT. The activity was measured by reading absorbance at 240 nm at 30-second intervals for 3 min using a UV-visible spectrophotometer. Superoxide dismutase (SOD) was assayed [18] based on the enzyme's inhibition of epinephrine autoxidation. The SOD activity was measured as a degree of inhibition of auto-oxidation of epinephrine at an alkaline pH by the method.0.1 ml of tissue homogenate was added to the tubes containing 0.75 ml ethanol and 0.15 ml chloroform (chilled in ice) and centrifuged. Absorbance at 480 nm was measured by using Shimadzu UV visible spectrophotometer. Reduced glutathione (GSH) content was determined in tissue by an earlier reported method [19]. Tissue homogenates were immediately precipitated with 0.1 ml of 25% TCA, and the residue was removed after centrifugation, and the absorbance was read at 412 nm using a UV visible spectrophotometer.

2.14 Cytokines assays

Cytokines parameters such as tumor necrosis factor-α (TNF-α), Prostaglandin E2 (PGE2), and Interleukin-6 (IL-6) were assayed in the tissue samples by ELISA Reader [20]. (Erba Lisa Scan EM, Mumbai). The assays were performed according to the protocol recommended by the manufacturer (TRANSASIA, Mumbai, India)

3. STATISTICAL ANALYSIS

Statistical analysis of the results was done by one-way analysis of variance (ANOVA) using GraphPad Prism 5 software, followed by the Bonferroni comparison test for significance. Significance has been set at ($p<0.05$) and compared to the inducer. Results were presented as Mean± S.D.

4. RESULTS AND DISCUSSION

4.1 Phytochemical Screening

The plant material *Momordica charantia* Linn. leaves were collected and identified. Then, the powdered materials plants were successively extracted with petroleum ether, chloroform, ethyl acetate, ethyl alcohol, and water. Finally, the percent yields of each extract were calculated. The percent yields of extracts of *Momordica charantia* Linn. leaves were 12.8 % w/w in petroleum ether, 11.2 % w/w in chloroform, 9.8 % w/w in ethyl acetate, 23.6 % w/w in ethanol, and 10.3 % w/w in aqueous (Table 1).

Table 1: Percentage yield of various extracts of *Momordica charantia* Linn leaves

Extracts	% Yield (w/w)
Pet. Ether	12.8
Chloroform	11.2
Ethyl acetate	9.8
Ethanol	23.6
Aqueous	10.3

The phytochemical analyses of all extracts were performed qualitatively for different phytoconstituents. The *Momordica charantia* Linn. leaves give positive tests of steroids, terpenoids, and fatty acids in petroleum ether, alkaloids, and terpenoid compounds in chloroform extract.

Table 2: Qualitative tests of various extracts of leaves of *Momordica charantia* Linn leaves

Phytoconstituents Tests	Extracts				
	Petroleum Ether	Chloroform	Ethyl Acetate	Ethanol	Aqueous
Carbohydrates					
Molisch's test	–	–	–	–	+
Fehling's test	–	–	–	–	+
Tollen's test	–	–	–	–	+

Barfoed reagent test	_	_	_	_	+
Protein and amino acid					
Millon's test	_	_	_	_	_
Xanthoproteic test	_	_	_	_	_
Ninhydrin test	_	_	_	_	+
Steroids and Terpenoids					
Liebermann-Burchard test	+	+	_	+	_
Salkowski test	+	+	_	+	_
Antimony trichloride test	+	+	_	+	_
Trichloro acetic acid test	+	+	_	+	_
Glycosides					
Keller–Killiani test	_	_	_	+	+
Legal's test	_	_	_	+	+
Bontrager's test	_	_	_	+	+
Alkaloids					
Mayer's test	_	+	_	_	_
Dragendorff's test	_	+	_	_	_
Wagner's test	_	+	_	_	_
Hagger's test	_	+	_	_	_
Tannins & Phenolic compounds					
Ferric chloride test	_	_	+	+	+
Lead acetate test	_	_	+	+	+
Potassium chromatic test	_	_	+	+	+
Flavonoids					
Shinoda test	_	_	+	+	+
Ferric chloride test	_	_	+	+	+
Saponins					
Foam test	_	_	_	_	+
Test for fatty acid					
Spot Test	+	_	_	_	_
Saponification Test	+	_	_	_	_

- Negative, + Positive

The ethyl acetate and ethanol extract contain flavonoids, tannins, Phenolic compounds, and glycosides. The aqueous extract was found the presence of saponins, glycosides, Tannins and Phenolic compounds, flavonoids and carbohydrates (Table 2).

4.2 Total Phenolic and Flavonoid Content

The results showed that the total phenolic content in the ethanol extract of *Momordica charantia* leaves was 64.71±2.08 mg gallic acid equivalent/g. The flavonoid content in ethanol extract of *Momordica charantia* leaves was found as 97.42±4.83 mg (quercetin equivalent) per 100 gm of ethanol extract (Table 3).

Table 3. Total Phenolic and Flavonoid Content determination in ethanol extract of *Momordica charantia* leaves

Quantitative test	Methods	Observation of extract
Total Phenolic content	Folin-Ciocaletu method	64.71±2.08 mg gallic acid equivalent/g
Total Flavonoid Content	Aluminum chloride method	97.42±4.83 mg (quercetin equivalent) per 100 gm

4.3 *Acute toxicity study*

All extracts of *Momordica charantia* Linn. leaves were found safe in the dose used and there was no mortality up to an amount of 4000 mg/kg body weight. In the first to fourth step of the acute toxicity studies, no great signs of toxicity were observed at 2000 and 4000 mg/kg dosage. Therefore, there is a zero mortality 0/3 and total mortality 0/3, i.e., between 500 and 4000 mg/kg. Based on the above study, the dose 200 and 400 mg/kg p.o. was taken for the antiulcer activity.

4.4 *Determination of ulcer index*

The *Momordica charantia* Linn. leaves extracts were investigated for two different models i.e., Ethanol-induced stomach ulcer and Glacial acetic acid-induced oral ulcer model. Ethanol administration resulted in the production of gastric mucosal damage. The ulcer index of animals in ethanol induced ulcer model, in case of a disease control group of animals, was 41.85±2.88. Ethanol extract of *Momordica charantia* Linn. leaves at the dose 200 mg/kg body weight and 400 mg/kg body weight treated animals showed 14.62±1.25 and 11.87±1.56, respectively that is significantly reduced the ulcer index at level ($p<0.05$) as compared to control group (Table 4 and figure 1). However, the reduction in ulcer index by other extracts was not found to be significant.

Table 4: Effect of different extracts of *Momordica charantia* Linn. leaves on Ulcer Index and percent inhibition in ethanol-induced ulcer model in albino rats

Animal groups	Ulcer Index	% Inhibition
Disease Control (Saline 5 ml/kg)	41.85±2.88	-
Omeprazole (20mg/kg)	11.54±1.25*	72.42*
PEEMC (200 mg/kg)	38.20±2.36	08.72
PEEMC (400 mg/kg)	35.28±2.21	15.69
CEMC (200 mg/kg)	29.55±1.72	29.39
CEMC (400 mg/kg)	27.31±1.96	34.74
EMC (200 mg/kg)	34.24±2.24	18.18
EMC (400 mg/kg)	31.66±2.65	24.34
EEMC (200 mg/kg)	14.62±1.25*	65.06*
EEMC (400 mg/kg)	11.87±1.56*	71.63*
AEMC (200 mg/kg)	28.30±2.27	32.37
AEMC (400 mg/kg)	26.71±1.90	36.17

n = 6, value represents Mean ± S.D. *P< 0.05

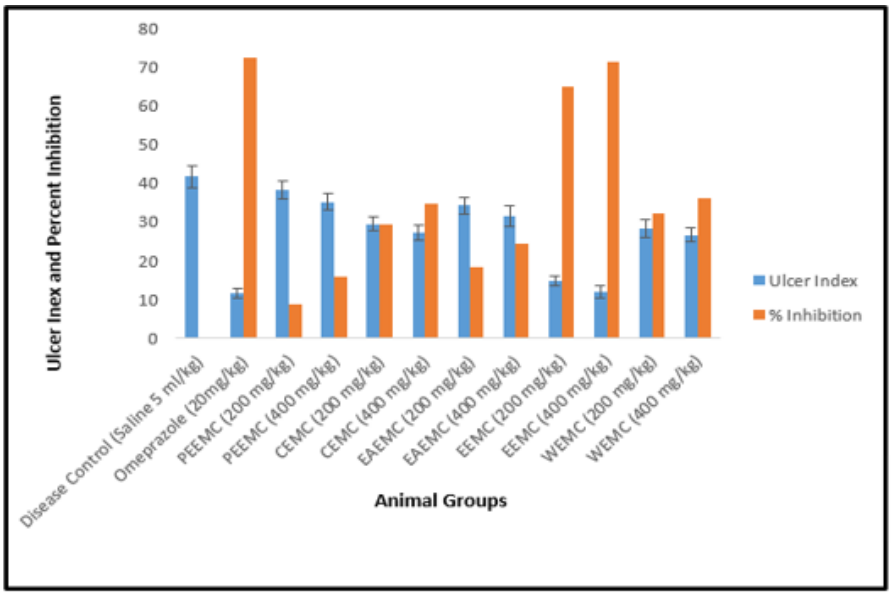

Fig 1: Effect of different extracts of *Momordica charantia* leaves on Ulcer Index and percent inhibition in ethanol-induced ulcer model

Glacial acetic acid-induced oral ulcer shows the ulcer index of animals in the oral ulcer model, in the control group of animals, was 28.74±2.71. Ethanol extract of *Momordica charantia* Linn. leaves 200 and 400 mg/kg treated animals showed 11.75±0.84 and 10.32±0.73, respectively and the ulcer index was significantly reduced ($p<0.05$) as compared to the control group (Table 5 and Figure 2). However, the reduction in ulcer index by other extracts was not found significantly.

Table 5: Effect of different extracts of *Momordica charantia* Linn. leaves on ulcer index and percent inhibition in Glacial acetic acid-induced oral ulcer model in albino rats

Animal groups	Ulcer Index	% Inhibition
Disease Control (Saline 5 ml/kg)	28.74±2.71	-
ORASORE Gel (Once a day)	09.62±0.89*	66.52*
PEEMC (200 mg/kg)	25.34±1.56	11.83
PEEMC (400 mg/kg)	22.64±1.82	21.22
CEMC (200 mg/kg)	24.69±1.87	14.09
CEMC (400 mg/kg)	21.45±2.32	25.36
EMC (200 mg/kg)	20.96±1.65	27.07
EMC (400 mg/kg)	19.64±1.28	31.66
EEMC (200 mg/kg)	11.75±0.84*	59.11*
EEMC (400 mg/kg)	10.32±0.73*	64.09*
AEMC (200 mg/kg)	19.44±1.21	32.35
AEMC (400 mg/kg)	18.55±1.62	35.45

n = 6, value represents Mean ± S.D. *P< 0.05

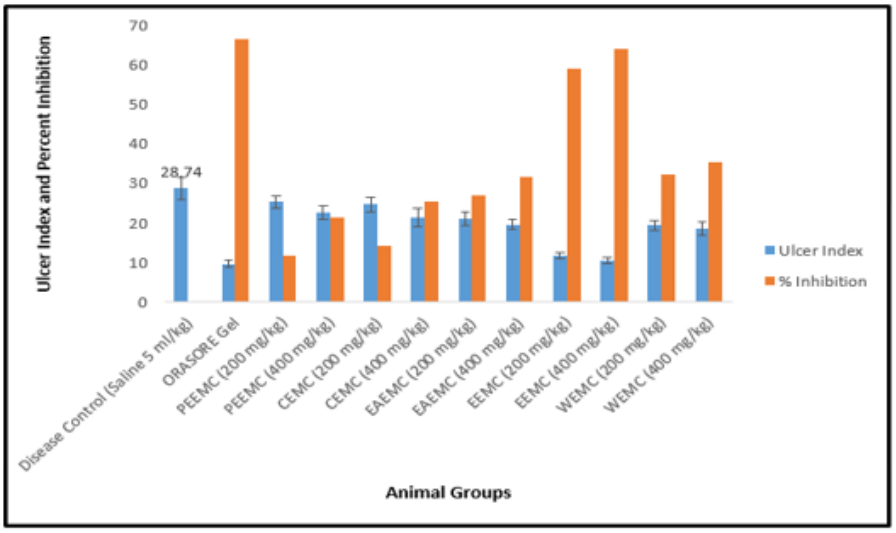

Fig 2: Effect of different extracts of *Momordica charantia* leaves on Ulcer Index and percent inhibition in Glacial acetic acid-induced oral ulcer model

4.5 Gastric volume

From the results, in control group the average gastric volume was found to be 5.08 ml, whereas the ethanolic leaf extract of *Momordica charantia* Linn. with dose of 200 mg/kg and 400 mg/kg showed significantly reduced gastric volume of 2.48 and 2.43, respectively as compared to control (Table 6 and Figure 3). However, the gastric book shown by Omeprazole, a standard drug, was 2.72 ml, which is statistically significant ($p<0.05$) (Table 6 and figure 3).

Table 6: Effect of different extracts of *Momordica charantia* Linn. leaves on various ulcer parameters in ethanol induced ulcer model in albino rats

Animal groups	Gastric volume (ml)	pH	Total acidity (mEq/l)
Disease Control (Saline 5 ml/kg)	5.08±0.06	2.4±0.06	605.45±35.25
Omeprazole 20mg/kg	2.72±0.03*	4.6±0.02*	320.14±28.32*
PEEMC (200mg/kg)	4.86±0.05	2.8±0.07	534.25±30.53
PEEMC (400mg/kg)	4.35±0.02	3.0±0.04	526.99±28.42
CEMC (200 mg/kg)	4.99±0.04	3.0±0.02	504.64±22.12
CEMC (400 mg/kg)	4.10±0.02	3.2±0.04	490.25±17.34
EMC (200mg/kg)	4.28±0.04	2.8±0.03	486.22±21.58
EMC (400mg/kg)	4.03±0.02	2.9±0.05	475.20±16.52
EEMC (200 mg/kg)	2.48±0.05*	4.2±0.02*	351.88±13.53*
EEMC (400 mg/kg)	2.43±0.02*	4.8±0.06*	318.11±17.83*
AEMC (200 mg/kg)	4.77±0.03	3.4±0.03	475.36±16.80
AEMC (400 mg/kg)	4.52±0.02	3.6±0.02	410.28±19.85

n = 6, value represents Mean ± S.D. *P< 0.05

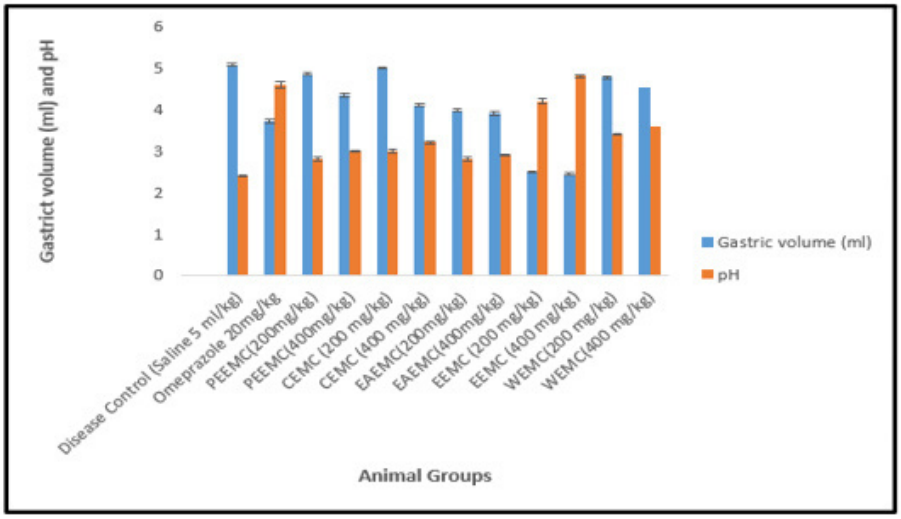

Fig 3: Effect of different extracts of *Momordica charantia* leaves on various ulcer parameters in ethanol induced ulcer model

4.6 *the pH of gastric contents*

In control animals, without any drug treatment, the average pH was 2.4, the ethanol extract of *Momordica charantia* Linn. leaves 200 mg/kg and 400 mg/kg have shown a significant increase in pH 4.2 and 4.8, respectively as compared to control (Table 6 and Figure 3). On the other hand, the rise in pH shown by Omeprazole, a standard drug was 4.6, which is statistically significant ($p<0.05$). This has revealed that the extract i.e., EEMC at a dose 400 mg/kg body weight is more potent than Omeprazole.

4.7 *Total acidity of gastric contents*

The disease control group showed gastric total acidity 605.45±35.25 mEq/litre. After treatment with 200 and 400 mg/kg of ethanolic leaf extract of *Momordica charantia* Linn. showed significant decrease in total acidity 351.88±13.53 and 318.11±17.83 mEq/litre, respectively when compared with control group of animals. Other extracts, i.e., petroleum ether, chloroform, ethyl acetate and aqueous extracts did not show significant reduction in the total acidity (Table 6 and Figure 4). This indicates that the ethanol extracts of *Momordica charantia* Linn. leaves showed a significant effect in various ulcer parameters when compared with standard animal groups. They also exhibit better antisecretory activity as evidenced by a reduction in the mean volume of gastric secretion, rise in pH and reduction in total acidity[7].

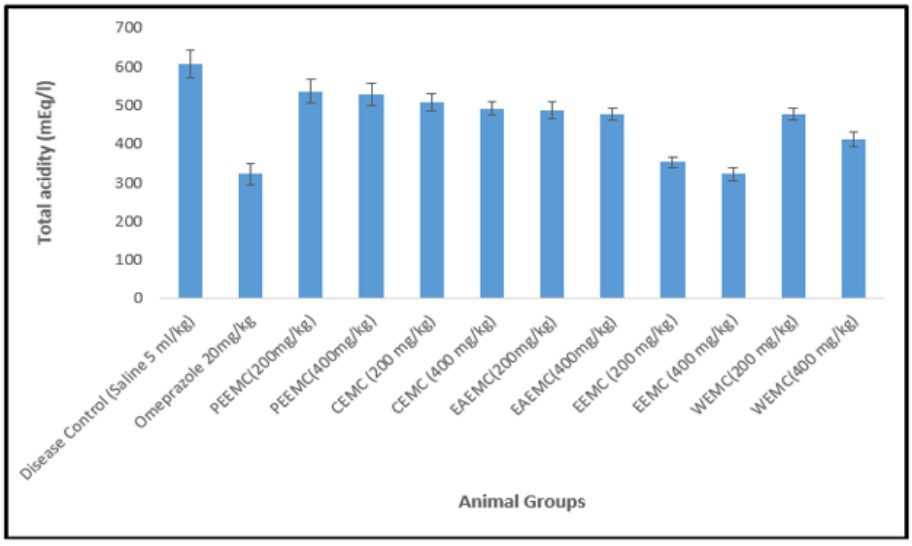

Fig 4: Effect of different extracts of *Momordica charantia* leaves on total acidity in ethanol induced ulcer model

4.8 *Effect of different extracts on oxidative status in ethanol induced ulcer model and Glacial acetic acid-induced oral ulcer model in albino rats*

Momordica charantia Linn. leaves the decreasing level of SOD 48.35±2.74, CAT 32.56±1.24 and GSH 45.27±2.84 in Ethanol-induced stomach ulcer model was slightly improved in treatment group with ethanol extract at 200 mg/kg dose. But a significant improvement in level of SOD 51.56±3.25, CAT 34.43±1.59 and GSH 48.89±2.16 were found in treatment group of 400mg/kg dose. as well as standard drug treated group when compared to control group (Table 7 and Figure

5). In Glacial acetic acid induced oral ulcer model significant improvement in level of SOD 60.25±1.14, CAT 37.47±1.51 and GSH 50.43±2.34 were found in treatment group of 400mg/kg dose by producing natural antioxidants (SOD, CAT, GSH), the cell reacts to increased concentrations of free radicals [21]. which can reduce or remove free-radical harm to cellular structures (Table 8 and Figure 6). In particular, glutathione peroxidase catalyzes the conversion of hydroxide ions to water. SOD able to converts ions from superoxide to hydrogen peroxide and is then converted by catalase to oxygen and water [22]. Superoxide dismutase occurs in a variety of different isoforms, each specializing in particular areas of the cell [23]. The cell increases the expression of antioxidant enzymes when subjected to rising ionizing radiation levels. However, if these cellular defenses are overcome by the amount of ROS, the cell will experience damage (dose-dependent) that can lead to carcinogenesis, teratogenesis, necrosis or apoptosis. Anti-inflammatory and antioxidant compounds can control free radical generation, eliminate free radicals and induce natural development of antioxidant (such as SOD, GSH, and CAT). They improve DNA repair, suppresses many inflammatory reactions, or stay cell division, allowing cells to undergo apoptosis process longer[7].

Table 7: Effect of different extracts of *Momordica charantia* Linn. leaves on antioxidants level of stomach tissues of albino rats in ethanol induced ulcer model in rats.

Animal groups	Antioxidants level		
	SOD (µ g/50 mg tissue)	CAT (µ mol/50 mg tissue)	GSH (µ mol/50 mg tissue)
Disease Control (Saline 5 ml/kg)	16.24±1.73	14.62±2.14	25.43±2.15
Omeprazole 20mg/kg	56.43±4.35*	34.24±2.35*	47.37±3.16*
PEEMC (200mg/kg)	22.25±1.26	16.84±1.23	26.52±1.15
PEEMC (400mg/kg)	23.82±1.12	18.58±1.54	27.46±1.43
CEMC (200 mg/kg)	19.65±1.84	19.45±2.14	27.41±1.46
CEMC (400 mg/kg)	21.26±1.57	21.34±2.15	28.72±1.43
EMC (200mg/kg)	23.56±1.45	23.41±1.54	26.56±1.15
EMC (400mg/kg)	24.62±1.26	24.23±1.16	24.87±1.14
EEMC (200 mg/kg)	48.35±2.74*	32.56±1.24*	45.27±2.84*
EEMC (400 mg/kg)	51.56±3.25*	34.43±1.59*	48.89±2.16*
AEMC (200 mg/kg)	24.64±1.16	15.81±1.43	19.34±1.85
AEMC (400 mg/kg)	24.82±1.74	16.52±1.16	20.75±1.16

n = 6, value represents Mean ± S.D. *P< 0.05

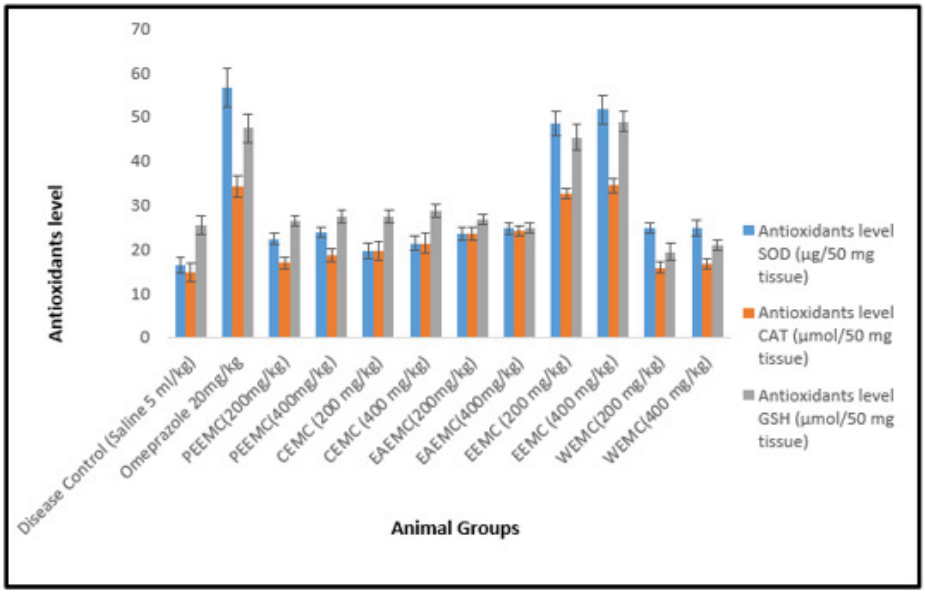

Fig 5: Effect of different extracts of *Momordica charantia* leaves on antioxidants level of stomach tissues of rats in ethanol induced ulcer model

Table 8: Effect of different extracts of *Momordica charantia* Linn. leaves on antioxidants level of oral mucosal tissue of albino rats in Glacial acetic acid-induced oral ulcer model

Animal groups	Antioxidants level		
	SOD (µ g/50 mg tissue)	CAT (µ mol/50 mg tissue)	GSH (µ mol/50 mg tissue)
Disease Control (Saline 5 ml/kg)	16.24±1.14	18.85±1.25	28.74±2.51
ORASORE Gel (Once a day)	61.23±2.24*	39.82±2.14*	51.32±3.12*
PEEMC (200mg/kg)	23.65±1.15	19.80±1.16	31.46±1.96
PEEMC (400mg/kg)	24.45±1.16	21.82±1.31	32.66±1.42
CEMC (200 mg/kg)	18.59±1.44	22.25±1.45	30.46±2.41
CEMC (400 mg/kg)	19.51±1.15	23.46±1.95	31.56±1.64
EMC (200mg/kg)	20.53±1.61	20.87±1.48	29.53±1.31
EMC (400mg/kg)	21.72±1.56	20.53±1.12	30.48±1.52
EEMC (200 mg/kg)	59.56±1.12*	33.65±1.56*	48.72±2.56*
EEMC (400 mg/kg)	60.25±1.14*	37.47±1.51*	50.43±2.34*
AEMC (200 mg/kg)	23.93±1.43	19.73±1.34	20.32±1.41
AEMC (400 mg/kg)	24.72±1.41	20.51±1.36	23.41±1.44

n = 6, value represents Mean ± S.D *P< 0.05

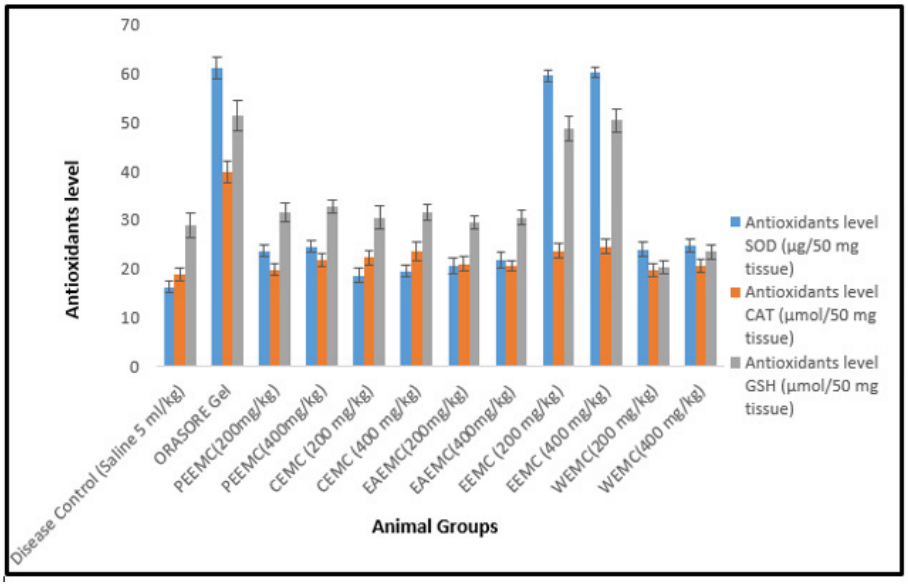

Fig 6: Effect of different extracts of *Momordica charantia* leaves on antioxidants level of oral mucosal tissue of rats in Glacial acetic acid-induced oral ulcer model

The GSH detoxification system is important in cellular defense against a large group of injurious agents [24]. GSH can protect against free radicals and cell death after various inflammation reactions[25]. The endogenous protection mechanism, like the GSH and antioxidant enzymes, defends against oxidative damage under normal conditions. GSH is a flexible protector and performs its protective function by free radical scavenging, restoring the damaged molecules by contributing hydrogen, reducing peroxides and retaining protein thiols in a reduced state [26].

4.9 Effect of different extracts on proinflammatory mediators in ethanol induced ulcer model and Glacial acetic acid-induced oral ulcer model in albino rats

Inhibitory effect of ethanol extract on the tissue level of proinflammatory mediators (TNF-α , IL-6, and PGE2) were measured in the stomach tissue of albino rats by ELISA (Table 9 and 10). Results were confirmed that *Momordica charantia* Linn. leaves extracts treatment groups were showed significant decrease in the tissue level of proinflammatory mediators i.e., TNF-α , IL-6, and PGE2 when compared to the control group of animals. Ethanolic extract of *Momordica charantia* Linn. leaves at both doses 200 and 400 mg/kg showed significant reduction of tissue cytokines level such as TNF-α 18.25±0.56 and 15.23±0.91, respectively, IL-6 16.23±0.95 and 13.54±0.82, respectively and prostaglandin E2 24.52±1.22 and 22.09±1.54 pg/L. This significant reduction was comparable to standard group of animals also. All other extracts were not showed significant reduction in the cytokines level of treated animals (Table 9 and Figure 7).

Table 9: Effect of different extracts of *Momordica charantia* Linn. leaves on proinflammatory mediators in stomach tissue of albino rats in ethanol induced ulcer model in albino rats

Animal groups	TNF-α (pg/L)	IL-6 (pg/L)	PGE2 (pg/L)
Disease Control (Saline 5 ml/kg)	39.23±1.78	29.23±1.06	51.23±2.32
Omeprazole 20mg/kg	17.65±0.59*	12.52±0.56*	22.32±1.06*
PEEMC (200mg/kg)	37.23±1.27	23.54±1.24	42.23±2.12
PEEMC (400mg/kg)	32.56±2.30	22.32±1.05	38.54±2.14
CEMC (200 mg/kg)	27.53±1.78	25.63±1.33	36.47±2.32
CEMC (400 mg/kg)	24.32±1.65	23.54±1.54	33.79±1.56
EMC (200mg/kg)	28.56±1.23	20.62±1.88	36.25±1.28
EMC (400mg/kg)	27.62±1.89	20.85±1.72	32.46±1.45
EEMC (200 mg/kg)	18.25±0.56*	16.23±0.95*	24.52±1.22*
EEMC (400 mg/kg)	15.23±0.91*	13.54±0.82*	22.09±1.54*
AEMC (200 mg/kg)	27.32±1.23	27.23±1.05	31.22±2.12
AEMC (400 mg/kg)	26.53±1.37	24.85±1.91	30.54±1.98

n = 6, value represents Mean ± S.D. *P< 0.05

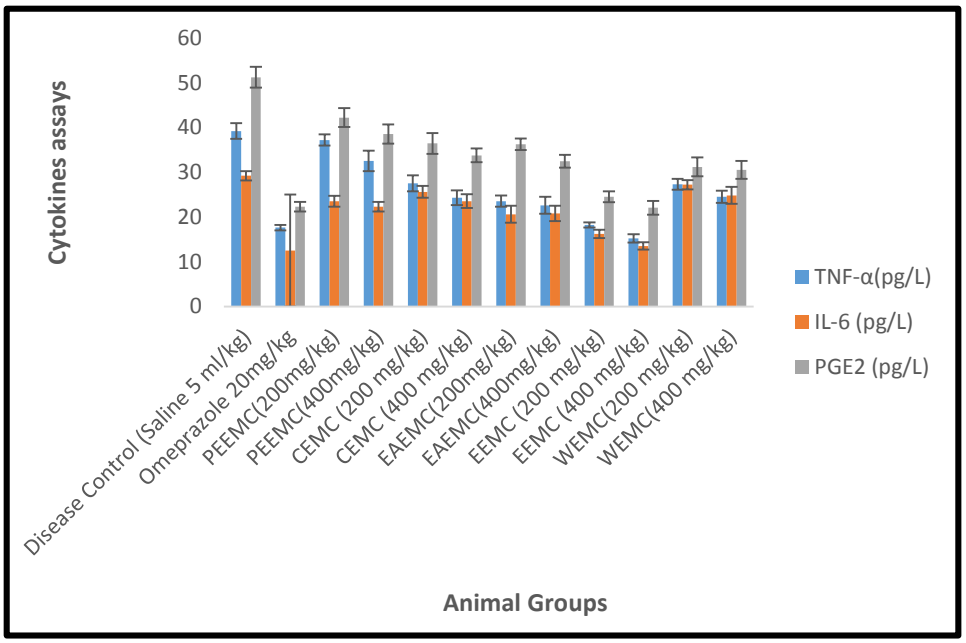

Fig 7: Effect of different extracts of *Momordica charantia* leaves on proinflammatory mediators in stomach tissue of rats in ethanol induced ulcer model

Table 10: Effect of different extracts of *Momordica charantia* leaves Linn. on proinflammatory mediators in oral mucosal tissue of glacial acetic acid induce oral ulcer model in albino rats

Animal groups	TNF-α (pg/L)	IL-6 (pg/L)	PGE2 (pg/L)
Disease Control (Saline 5 ml/kg)	47.53±1.24	36.12±1.08	58.65±3.30
ORASORE Gel (Once a day)	17.53±0.89*	15.23±0.94*	25.32±1.25*
PEEMC (200mg/kg)	42.65±1.54	27.65±1.20	47.53±2.41
PEEMC (400mg/kg)	39.34±2.69	24.82±1.09	45.22±1.89
CEMC (200 mg/kg)	27.53±1.82	25.73±1.23	41.25±1.65
CEMC (400 mg/kg)	25.65±1.26	24.65±1.08	38.52±1.82
EMC (200mg/kg)	28.65±1.24	23.54±1.32	34.56±2.04
EMC (400mg/kg)	26.35±1.98	21.56±1.04*	32.54±1.45*
EEMC (200 mg/kg)	18.56±0.54*	17.32±0.98*	23.54±1.84*
EEMC (400 mg/kg)	16.49±0.87*	14.65±0.62*	21.36± 1.93*
AEMC (200 mg/kg)	32.56±2.41	21.54±1.08	37.23±2.02
AEMC (400 mg/kg)	30.12±1.48	20.45±1.90	32.23±1.97

n = 6, value represents Mean ± S.D. *P< 0.05

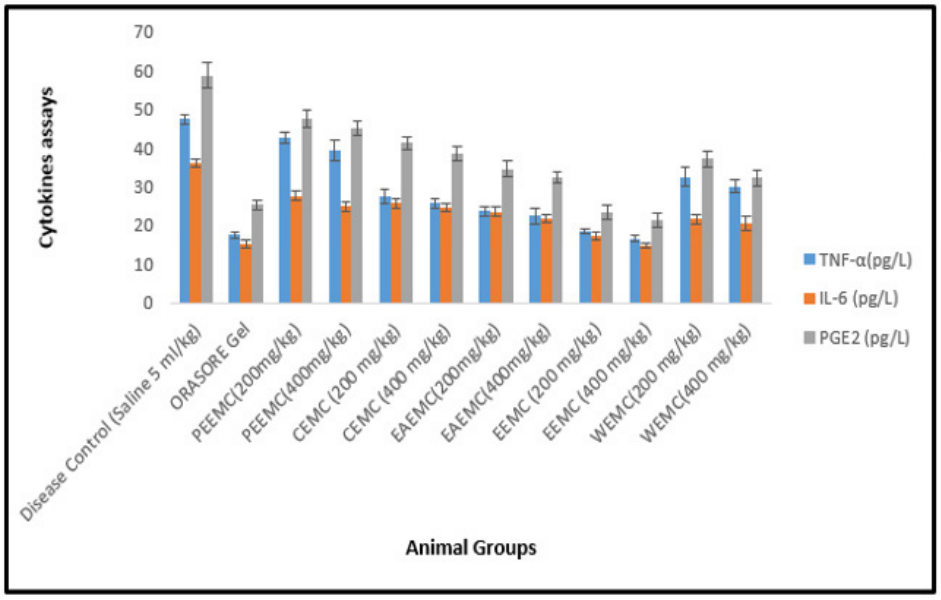

Fig 8: Effect of different extracts of *Momordica charantia* leaves on proinflammatory mediators in oral mucosal tissue of glacial acetic acid induce oral ulcer model in rats

Glacial acetic acid-induced oral ulcer model also characterized by the marked expression of some proinflammatory cytokines. Inhibitory effect of ethanol extract on the tissue level of proinflammatory mediators (TNF-α , IL-6, and PGE2) were measured in oral mucosa of albino rats. Ethanolic extract of *Momordica charantia* Linn. leaves at both doses 200 and 400 mg/kg showed significant reduction of tissue cytokines level such as TNF-α 18.56±0.54 and 16.49±0.87, respectively, IL-6 17.32±0.98 and 14.65±0.62, respectively and prostaglandin E2 23.54±1.84 and 21.36±ss 1.93 pg/L. This significant reduction was comparable to standard group of animals also. Other extracts of *Momordica charantia* were not showed significant reduction in the cytokines level (Table 10 and Figure 8) in the Glacial acetic acid-induced oral ulcer model. The results were demonstrated that ethanol extract of *Momordica charantia* Linn. leaves inhibit the increased levels of cytokines. Therefore, the inhibitory effects on the production of inflammatory cytokines account for its anti-oral ulcer activity [27]. Flavonoids are believed to act as health-promoting substances as they have antioxidant and anti-inflammatory properties [28]. In the inflammation phase, macrophages and neutrophils are attracted to the injured tissues that release inflammatory mediators, such as tumor necrosis factor-alpha (TNF-a) and interleukin-1 (IL-1) [29]. Neutrophils contain high levels of destructive proteases and oxygen free radicals that are released into the wound area when cells die. This can cause extensive tissue damage and prolong the inflammatory phase. These free radicals are produced during oxidative stress, which causes lipid peroxidation, DNA breakage, and scavenging enzymes inactivation. In case of hemorrhoid, one of the major causes of delayed healing is the persistence of inflammation or an inadequate angiogenic response [30, 31]. It has been postulated that an anti-inflammatory response after cutaneous wound induction is a prerequisite for healing [32, 33]. Potent antioxidant, anti-inflammatory agents such as flavonoids can play an important role in restoring physiological conditions, allowing a major improvement in ulcer healing [34].

5. CONCLUSION

Present study was concluded that ethanolic extract of *Momordica charantia* Linn. leaves showed significant antiulcer effect in both type of ulcer i.e., oral and stomach ulcer through possible antioxidant mechanism. The antiulcer effect may be due to the presence of reported flavonoids. Study confirms the traditional claim of *Momordica charantia* Linn. for management of ulcer proven.

6. ACKNOWLEDGEMENT

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7. AUTHORS CONTRIBUTION STATEMENT

Mr. Punit Singh has compiled the whole data and arranged as per journal guidelines. All data was compiled, and results of each observation were discussed by both authors. Dr Mohan Lal Kori was read whole manuscript and checked for calculation and grammatical corrections and given necessary input towards design the final editing of manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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