Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(4), 1587-1593 | ISSN:2251-6727

Evaluation of Anti-Ulcer Studies of Extract of Polyherbal Plants Momordica Charantia [Karela] and Phyllanthus Emblica [Amla] in Albino Rats

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(Received: 14 April 2024 Revised: 1 May 2024 Accepted: 18 June 2024)

KEYWORDS
Polyherbal Plants, Momordica Charantia

ABSTRACT:

Objectives: Momordica charantia and Phyllanthus emblica are well known plants having secondary metabolites which are responsible for different pharmacological activity. This study was aimed at evaluating the anti-ulcer effects of a polyherbal extract of Momordica charantia and Phyllanthus emblica in the Wistar albino rat.

Methods: The plant materials were extracted with 100% methanol in soxhlet apparatus, and preliminary phytochemical screenings and TLC analysis of both plant extracts were carried out for the detection of secondary metabolites. Ibuprofen induced ulcer model was selected for study. Omeprazole 20 mg/kg was used as standard drug.

Results: The result obtained from the data revealed that, MCPE extract at dose of 500mg/kg has highest ulcer healing score (84.5%) as compared to Omeprazole 20mg/kg (78.1%) and MCPE 200mg/kg treated animals (69.1%). Histopathological examination shows the maximum ulcers with perforation in disease control group and minimum ulcer development was observed in MCPE extract treatment group.

Conclusions: Present study concluded that, a polyherbal mixture of Momordica charantia and Phyllanthus emblica extract at 500mg/kg dose level could be used for treatment of Peptic ulcers.

Introduction

Plants and animals are the richest source of daily required ailments of our life [1]. Worldwide, interest in herbal remedies has been expanding quickly due to their safe traditional benefits, particularly in treating resistant illnesses, antioxidant qualities, and effectiveness in immune modulation, prophylaxis, and palliation [2].

Peptic ulcer is a prevalent and long-lasting gastrointestinal problem, with a lifetime prevalence of 15% and a point frequency of 5%. The proportion of duodenal to stomach ulcers was 17:1. Stomach and duodenal ulcers were common among male. There are several reasons why gastric ulcer disease development occurs, but the two most prevalent ones are Helicobacter pylori-caused PUD and NSAID usage [3].

Momordica Charantia (Bitter melon) and *Phyllanthus Emblica* (Amla) are the herbal plants of indian native and belonging to *Cucurbitaceae* and *Phyllanthaceae* family [4],[5]. These plants was previously reported to have a number of secondary metabolites which possess anti-inflammatory, anti-oxidant, anti-diabetic, antidepressant activity [6],[7].

Material and Methods

Collection and Authentication of Plant

The plant materials were collected from rural area of Bihar. The Phyllanthus emblica and Momordica

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charantia fruits were dried in sunshine at 42ºC and stored in a paper bag till utilized for study. The plants were authenticated at regional office of Botanical Survey of India, Prayagraj, Uttar Pradesh.

Selection of Animal

Healthy, male wistar albino rats of 120–150 gm were selected for the study. The animals were acclimatized for seven days prior to the experiments. A total of 35 animals were employed for the study. The animals were kept separately in a clean propylene cage with a room temperature of 22±2 ºC, free access to water, and a standard food pallet supply.

Preparation of extract

The dried fruit of P. Emblica and M. charantia was grounded separately into coarse powder using a mechanical grinder. A soxhlet apparatus was used to continually extraction by heating 100g of powdered material of each separately, and 100% methanol was used as solvent for extraction. To make a semisolid mass, after extraction, the both of extract solvent was concentrated in a Lyophilizer after being heated to a temperature of 40 to 45 ºC in a rotary evaporator [8].

Preliminary Phytochemical Screening

Plant species yield wide ranges of bioactive components including as tannins and phenolic compounds, as well as sugars, flavonoids, alkaloids, glycosides, sterols, and lipids. A number of phytochemical tests were carried out for identification of plants secondary metabolites [9],[10].

Thin Layer Chromatography

A TLC glass plate having 10 × 1.5 cm diameter with silica gel-G F254 was heated to 100 °C for 10 minutes for activation, and allowed to cool at room temperature. One edge of the plates was marked with 1cm pencil lines. Thin capillary pipettes were used to spot extract samples onto the pencil line. In a development chamber containing a trial solvent of ethyl acetate: methanol: water (4:2:1, v/v/v), and chloroform: ethyl acetate: methanol (6:3:1) the plates were put. The solvent front was permitted to move up to one centimeter from the top end. After removing the TLC plates, a soft pencil was used to mark the solvent front. After being air dried, observed at 365nm and 254nm under a UV light source. Retention factor (RF) was calculated and noted [11].

Acute oral toxicity study

Using the methodology outlined in OECD guideline, the acute oral toxicity of polyherbal fruit extract of Momordica charantia and Phyllanthus emblica was conducted on five wistar albino rats. Rats were administered a limit test dosage of 200 and 500 mg/kg of the test sample orally by oral gavage following a three-hour fast. After receiving the extract, all of the mice were watched for general behavioral changes, signs of toxicity, and death for the first 4 hours (critically), then every day for the next 10 days [12].

Grouping of Animals

All the animals were divided into 5 groups (n=7). Each group having six animals and divided as normal control, disease control, standard control and two tests group (low and high dose). Animals were kept separately in propylene cage at 22ºC and (12:12 hours) light and dark cycles. All these animals were acclimatized for seven days prior to the experiments. The animals having continuous standard food and water supply. The food was taken out 24 hours before to the experiment, but water was still available.

Dosing of Animals

Throughout the study, the animals in the normal control only received normal saline and disease control group were received distilled water and Ibuprofen 400 mg/kg for 7 days. Omeprazole 20 mg/kg was given to the standard group, Group III and IV was administered 200 mg/kg and 500 mg/kg of the polyherbal extract of MCPE respectively for 14 days. The study protocols were approved by Institutional Animal Ethics Committee (IAEC).

Ulcer Induction

Ibuprofen at dose of 400mg/kg dissolved in 15 ml distilled water and administered orally twice daily for seven days. After that, all treatment group animals were received respective drug treatment for 14 days as per protocol. At day 15th, all animals were sacrificed under light chloroform anesthesia and stomach was dissected out with the help of scalpel and surgical scissor for histopathological observations [13].

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Evaluation Parameters

The stomach was opened by a gentle cut along greater curvature, and number of ulcer was counted macroscopically with the help of simple lens [14].

Ulcer Score

The ulcer score was calculated by following systems:

- 0 = No ulcer
- 1 = Superficial ulcer
- 2 = Deep ulcer
- 3 = Perforation

Ulcer Index

Ulcer index (UI) was calculated by following formula:

$$\text{Ulcer Index (UI)} = (\text{UN+US+UP})/10$$

Where,

UN= Average number of ulcer per animal

US= Average severity score

UP= % of ulcerated animals.

Percentage Ulcer Inhibition

Percentage of ulcer protection of all group of animals were calculated by following formula:

$$\%\ \text{Ulcer inhibition} = \text{UI (control)} - \text{UI (test group)}/\ \text{UI (control)} \times 100$$

Histopathological Examination

Histological examination of stomach tissue was done after experimental procedures. Sections of tissue with a thickness of 5 µm were cut, float in a water bath, and be selected using albuminized frosted end slides to improve section adhesion. In addition, the slices were stained using the hematoxylin and eosin staining technique to illustrate the tissue anatomy by microscope with suitable magnifications and observed for hemorrhagic lesion, ulceration and inflammation [15].

Statistical Analysis

The data collected from the study were expressed as Mean ± Standard error of mean (SEM). Statistical analysis of the data was performed by one way analysis of variance (ANOVA) single factor and Tukey's tests was used to determine the difference between groups. $P<0.05$ was considered as level of significance.

Results

Physicochemical Analysis

Powdered crude drug material of Momordica charantia seeds and Phyllanthus emblica fruits were subjected for determination of different preliminary physicochemical analysis such as ash value, acid insoluble ash etc. were carried out and the result obtained were mentioned in table-1.

Parameter	Result (%w/w)	
	P. emblica	M. charantia
Total Ash	8.09±0.2	4.9±0.4
Water soluble Ash	3.42±0.4	4.12±0.4
Acid insoluble Ash	1.12±0.1	2.60±0.2
Moisture content	6.54±0.2	14.50±0.2
Extractive Value	14.00	12.60
Foreign Particles	1.29	3.06

Table-1: Preliminary physicochemical analysis of M. charantia and P. emblica fruit powder.

Preliminary Phytochemical Analysis

The result suggests that, M. charantia containing secondary metabolites such as alkaloids, Tannins, sterols, flavonoids, phenolics while glycosides, carbohydrates, and sterols are absent. Extract of P. emblica fruits gives positive results for presence of alkaloids, Tannins, flavonoids, saponins, carbohydrates and polyphenols while sterols and glycosides are absent.

Thin Layer chromatography

TLC plates with M. charantia and P. emblica sample develops six spots and four spots respectively (Figure-1) and Rf values was found through Thin layer chromatography was listed in Table-2. The observed Rf values was compared to the standard literature and suggested that plant contains flavonoids, alkaloids, phenolic compounds and Tannins in M. charantia seed extract and P. emblica may contains alkaloids, Flavonoids and saponins which are responsible for pharmacological effects.

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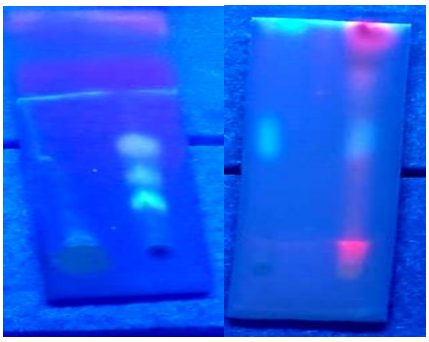

(A) **(B)**

Figure-1: TLC analysis of fruit and seed extract of P. emblica **(A)** and M. charantia **(B)**.

S.No.	Rf Values	
	M. charantia extract	**P. emblica extract**
1	0.18	0.18
2	0.22	0.32
3	0.31	0.42
4	0.56	0.52
5	0.64	--
6	0.98	--

Table-2: Rf Values of the TLC analysis of M. charantia and P. emblica extract.

Acute Oral Toxicity Study

Acute oral toxicity (LD50) study of extract was performed according to CPCSEA guideline. There are no mortality was found for both groups. During first 48 hours animals were observed for abnormal physical and behavioral symptoms but there are no any such abnormal symptoms were found. On the basis of safety data of both plant extract at equal portions of 200mg/kg and 500mg/kg was found safe dose for administration by oral route.

Anti-ulcer Activity

Anti-ulcer activity of methanolic fruit extract of Phyllanthus emblica and Momordica charantia had been carried out according to CPCSEA guidelines. Data obtained from experiment was listed below **(Table-11)** suggested that no ulcer was seen in normal control group animals, disease control (group II) which received normal saline having large number of ulcer developed, while standard group consist of less ulcer number. Test group-II also having lesser number of ulcer development as compared to test group-I, standard and control group.

Group	Number of Ulcer (UN)	Severity Score (US)	Ulcer Index (UI)	Ulcer Protection (%) (UP)
Normal control (MCPE 200mg/kg)	0.3	0.5	0.8	0
Disease Control (Normal saline)	5.6±0.8	11.0±1.17	16.7±3.07	0
Standard (Omeprazole 20mg/kg)	2.5±0.28	2.16±0.69	3.6±1.80	69.1
Test 1 (MCPE 200mg/kg)	3.3±0.54	2.8±0.65	5.1±2.38	69.15
Test 2 (MCPE 500mg/kg)	1.3±0.54	1.25±0.46	2.5±1.63	84.57

Data was expressed as Mean ± SEM (P <0.05), n=6.

Table-11: Observation data for Anti-ulcer activity of MCPE extract against Ibuprofen induced gastric ulcer.

The result from above table suggested that, after Ibuprofen 400mg/kg administration the development of gastric ulcers with ulcer index of 16.7±3.07 which was highest in disease control group. While standard group having 3.6±1.80, test group-I with ulcer index of 5.1±2.38 and test group-II with 2.5±1.63 (P<0.001)

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which was lesser than other groups. Test group-I having less ulcer index observed as compared to standard group.

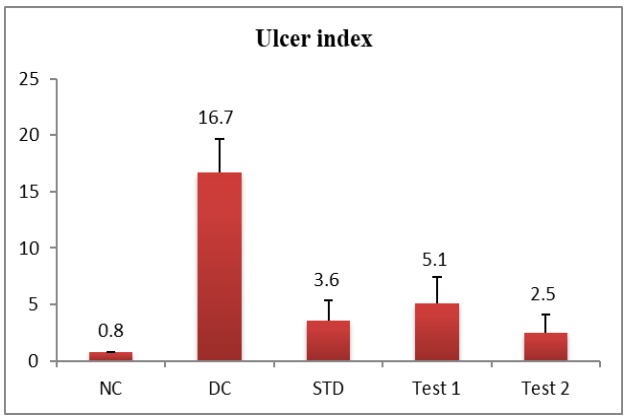

Figure 2: Ulcer index of animals after receiving different treatment.

Observation of peptic ulcers after treatment shows the highest ulcer number (5.6±0.8) of ulcer was developed in disease control group. Standard group was recognized for less ulcer number than disease control and test group-I **(Figure-2)**. Test group-II having minimum ulcer number (1.3±0.54) observed as compared to all experimental groups.

Ulcer index among different groups, observations and data revealed that disease control group having no protection of ulcer observed (Figure-18**)**. Standard group which received standard treatment of Omeprazole was found ulcer protection of 78.1%. Test group-I received treatment of MCPE extract at dose of 200mg/kg having ulcer protection of 69.1% which was less protection as compared to standard group. Test group-II received treatment of MCPE extract at 500mg/kg was found 84.5% (P<0.001), which was significant ulcer protection as compared to all other groups.

Severity score of ulcers of different group of animals was described by Figure-3. The above figure suggested that the disease control group have maximum severity score (62%) of ulcers.

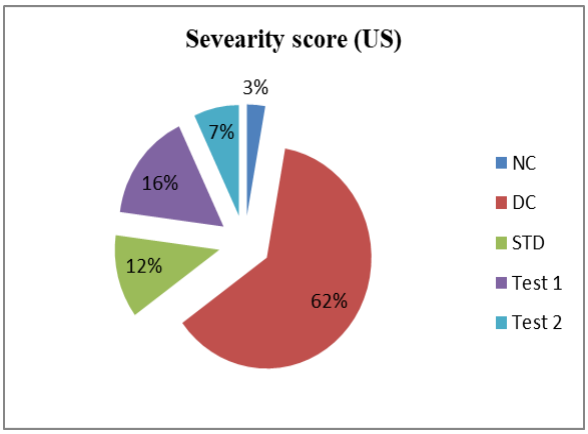

Figure-3: Severity score of different group of animals with ulcer induced by Ibuprofen in wistar albino rats.

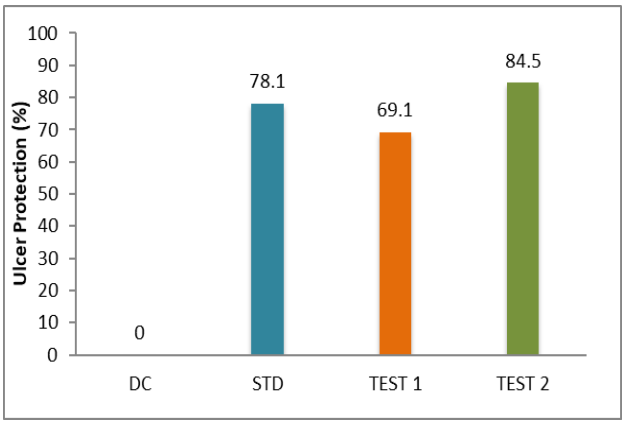

Figure-4: Percentage of ulcer protection of different group of animals after treatment.

After analyzing data obtained from study, it was revealed that, disease control group having higher ulcer index with less protection of gastric mucosa while, standard group having less ulcer index 3.6±1.80with 69.1% protection of ulcer (Figure-4).

Histopathology

Tissue sample of stomach with hematoxylin and eosin staining in (Figure-5) demonstrated that, mucosal layer of normal saline treated disease control group animal (B) was deeply damaged anatomy of underlying tissues, mocusal perforation with blood capillaries congestion, mononuclear cells infiltration and inflammation was seen.

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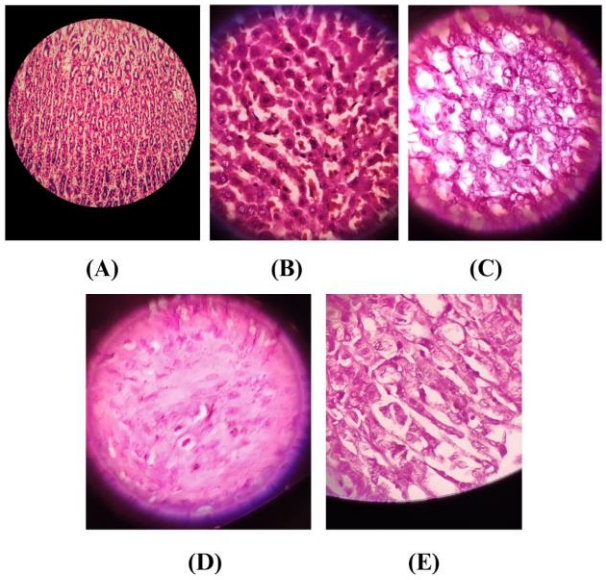

Figure-5: Histopathological results of excised rat stomach of different group of animals. Where, (A) represents normal control, (B) disease control, (C) standard animal group and (D) and (E) represents test group-1 and test group-2 respectively.

Standard group (C) treated with Omeprazole 20mg/kg having smaller mucosal damage with mild inflammation was observed. Test group animals Figure-5(D) was observed after treatment with MCPE extract at dose of 200mg/kg for minor mucosal damage with no sign of inflammation and few mononuclear cells infiltration. Figure-5(E) represents normal anatomy of stomach mucosa with no sign of inflation with minor mucosal damage. There are no mononuclear cells infiltration was observed in Figure-5(E) group. Figure-5(A) normal control group animal shows normal morphological characteristics stomach wall where, no damage to the mucosal layer was seen.

Discussion

Peptic ulcer disease remains a serious health issue worldwide, characterized by mucosal damage in the stomach and duodenum, often associated with factors such as *Helicobactor pylori* infection, NSAIDs, and alcohol consumption [16].

Ibuprofen induced peptic ulcer in rats are commonly used animal model for assessment of anti-ulcer activity of a drug. Oral administration of ibuprofen inhibits bicarbonate, gastric mucus, and nitric oxide in stomach and results in stomach mucosal damage and inflammatory cell infiltration [17]. Present study suggests that the anti-ulcer effects of both plants due to presence of alkaloids, flavonoids, tannins and phenolic compounds.

The gstroprotective effects of MCPE extract can be attributed to its constituent bioactive compounds. Alkaloids present in both Momordica charantia and Phyllanthus emblica extract may reduced the gastric acid secretion and increases mucosal protection mechanisms [18].

Conclusions

The results showed that, MCPE at a dose of 500mg/kg exhibited maximum ulcer protection of 84.5%, which was significantly higher than the standard drug Omeprazole (20mg/kg) which showed 78.1% ulcer protection($P<0.001$). Additionally, MCPE at dose of 200mg/kg showed 69.1% ulcer protection ($P<0.05$). Histopathological observation revealed that MCPE 500mg/kg treated animals having no gastric lesion or mucosal damage with significant ulcer protection.

These finding suggested MCPE possesses potent anti-ulcer activity, which may be attributed to the synergistic effect of bioactive compounds present in both plants. These findings are consistent and reliable with previous investigations, which have reported the anti-ulcer potential of Momordica charantia and Phyllanthus emblica individually.

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