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EVALUATION OF CO-ADMINISTRATION OF *MURRYA KOENIGII* LEAVES AND *MOMORDICA CHARANTIA* FRUITS ON ANTIULCER ACTIVITY IN EXPERIMENTALLY INDUCED ULCERS IN RATS

Tayum Yana [*1] and Savi Biswakarma [2]

Faculty of Pharmaceutical Sciences [1], Apex Professional University, Pasighat - 791102, Arunachal Pradesh, India.
Department of Pharmacology [2], Krupanidhi College of Pharmacy, Bengaluru - 560034, Karnataka, India.

Keywords:

Murraya koenigii, Momordica charantia, Ethanol; ranitidine, Peptic ulcer, Cytoprotection and histopathology

Correspondence to Author:
Tayum Yana

Lecturer,
Faculty of Pharmaceutical Sciences,
Apex Professional University,
Pasighat - 791102, Arunachal Pradesh, India.

E-mail: yanatayum2@gmail.com

ABSTRACT: In this study, the preventive and protective potentials of *Murray koenigii* (MK) leaves and *Momordica charantia* (MC) fruit extracts against experimentally induced peptic ulcers in Wistar rats were evaluated. Rats were divided into six experimental groups – normal (saline), control (ulcers), pretreatment with MC extract (fresh fruit juice, 500ml/kg), MK extract (400mg/kg)or both, and pretreatment with standard drug ranitidine (RTD). Parameters assessed in the study were -ulcer score, ulcer index, free acidity, total acidity, percentage of ulcer inhibition, pH of gastric juice, gastric volume, C-reactive protein, macroscopic evaluation of the stomach, and histopathological studies. The results of the pharmacological studies on the co-administration of MK extract and MC juice showed a significant decrease in ulcer score, ulcer index, gastric volume, free acidity, total acidity, and CRP value when compared to positive control. The pH and percentage of ulcer protection were significantly increased in the co-administration extracts treatment group comparably with the standard ranitidine group. Hence, the study revealed that pre-treatment with the combined administration of *Murraya koenigii* leaves and *Momordica charantia* fruit extracts improves the amelioration of gastric ulcers & proved to be cytoprotective and inhibits the occurrence of peptic ulcers in rats.

INTRODUCTION: Gastrointestinal (GIT) disease is a serious condition that affects individuals and can lead to death in severe cases. Patients with GIT disease experience discomfort, indigestion, morbidity, and mortality. Peptic ulcers are one of the GIT disease conditions [1]. These ulcers are characterized by breaks, wounds, hemorrhage, and holes in the segment of the gastrointestinal mucosa part due to non-equilibrium between aggressive forces like acid, pepsin, and defensive factors.

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The imbalance is mainly caused by *Helicobacter pylori* bacterial infection, non-steroidal anti-inflammatory drugs (NSAIDs), consumption of alcohol, stress, smoking, and poor diets, resulting in mucosal damages [2]. Standard treatments for peptic ulcer disease include antacids, proton pump inhibitors (PPIs), histamine (H2) receptor blockers, and anti cholinergic.

Chronic use of conventional medications results in unwanted side effects, unfavorable consequences, relapses, numerous drug-drug interactions, and in some circumstances, changes in biochemical pathways [3]. In providing primary healthcare around the world, traditional medicine plays a significant role with fewer side effects; numerous medicinal plants and their chemical compounds are useful and have claimed antiulcer activity vide therapeutic and

preventive potential (4). MK and MC are Medicinal plants traditionally used for the treatment of ulcers in Indian Ayurveda medicine [5, 6]. Chemical analysis of the MK extract has revealed that it is high in alkaloids, polyphenols, flavonoids, and chlorophyll. The aqueous extract of this leaf is effective in protecting against peptic ulcers, and constituent alkaloids like girinimbine reduce glutathione, non protein sulphydryls levels, and Nitric oxide (NO) concentration in the plasma and elevate Prostaglandin E2 (PGE_2) accompanied with a decreased level of Interleukin 6 (IL-6) [7]. Studies have been conducted previously to evaluate the ameliorative and cytoprotective effects of MC fruit extracts on peptic ulcers. MC was stated to comprise several flavonoids, alkaloids, and several other chemical components. The constituents were accountable for the antiulcer effect of the methanolic extract of MC, which considerably reduced the total acidity and the free acidity, whilst enhancing the mucus content, indicating that it has both antisecretory and cytoprotective effects on the stomach [8]. Since, there are no scientific reports on the combined effect of MK leaves and MC fruit extracts on the treatment of ulcers, the present research makes a novel effort to assess the preventive antiulcer activity of MK and MC pre-treatment on experimentally-induced ulcers in rats.

MATERIALS AND METHODS:

Collection of Plant Materials: The fresh MK leaves and fruit MC were bought from the native market of Bengaluru, Karnataka, India. Identification and verification of the plant material were done by Dr. P. E. Rajesekharan (taxonomist) at the Indian institute of horticultural research, Hesssarghatta lake post, Bengaluru-560089, with the herbarium reference number- PCOL/MK-MC 434/LEAF/KCP/2021-2022.

Preparation of Extracts:

MK Leaves Extract: At room temperature, the MK leaves were shade dried, followed by crushing into a powder form with uniform fine particles. The methanolic extraction method utilizing the Soxhlet apparatus was followed. Specifically, 100 gm of powder was weighed and placed inside the Soxhlet extractor. It was first defatted using petroleum ether (300 ml) which was placed in the circular-bottom flask that was connected to the Soxhlet apparatus and condenser. The solvent was then heated using a heating mantle for 6 hours at 60ºC, and then shade-dried, succeeded by extraction with methanol (500ml) for 36-72 hours (until the colour of the extract turns transparent from green). A water bath was utilized to evaporate the solvent. The final percentage yield of MK extracts was 7.58% w/w which was then refrigerated at 5 °C in airtight containers [9].

MC Fruit Extract: The fresh MC fruit was ground by using a mixer blender and using Whatman filter paper, it was filtered, and the filtrate was then kept in a clean sterile container at 4 ºC.

Dose selection was done according to the toxicity studies of OCED 423 guidelines, previous studies and research articles on MK and MC.

Phytochemical Screening: Preliminary phytochemical investigation of both plants extracts MC and MK for the presence of various phytoconstituents [10].

Experimentally Animals: Wistar rats weighing 150-200g were procured from Krupanidhi College of Pharmacy, Bengaluru, India. They were housed & acclimatized in a well-ventilated animal house. Laboratory conditions were maintained for 15 days earlier to the experimentation in a temperature (25 ± 5ºC) and relative humidity (50 - 70) % with a 12-hour cycle of light and darkness and unlimited food and water as per the recommendations of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The Institutional Ethical Committee (IEC) accepted the experiment protocol by the number KCP/IAEC/PCOL/77/2021. According to the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA) guidelines, the animals were kept in an animal home under conventional conditions.

The rats were Grouped as follows:

Group 1: Normal group (1ml normal saline).

Group 2: Control group (ulcers were induced by ethanol and swimming stress model).

Group 3: MC extract500 ml/kg b.w, p.o.

Group 4: MK extract 400mg/kg b.w, p.o.

Group 5: Combined extracts of MC500ml/kg + MK extract 400mg/kg p.o.

Group 6: Standard drug ranitidine 20mg/kg body weight i.p/p.o.

Animals were fed via an oral tube once daily for seven days in a row with all compounds.

Methodology: The research used two experimental models.

Ethanol-induced Ulcer Model: Prior to the ulcer induction, the animals were kept for 24 hours without food. On the 7th day of extracts and standard treatment 2 ml of ethanol per body weight given, via the oral route, the ulcer was induced in this manner after ulcer induction rats were euthanized 1 h later. The stomach was cut out, intestinal juice was collected tube and parameters were estimated [11].

Swimming Stress-induced Ulcer Model: Prior to the ulcer induction, the animals were kept for 24 hours without food. On the 7th day of extracts and standard treatment, A glass or plastic cylinder with a height of 50 cm and a diameter of 30 cm, filled with water up to a height of 40 cm, and maintained at 20-25 °C for 4 hours, was used to forcefully induce the ulcer. For 4 hours, the animals were permitted to swim in the water then sacrificed with a large dosage of carbon dioxide and each animal's stomach was taken out and the greater curvature opened. Parameters were estimated [12].

Parameters Estimated: The following variables parameters were estimated in both models in the study:

Ulcer Score and Ulcer Index:

- An ulcer scoring
- 0 indicates a ordinary-coloured
- 0.5 Intense red-coloured
- 1 visible ulcer
- 1.5 severe ulcer stripes
- 2 sore greater than 3 but less than 5
- 3 ulcers are greater than 5.

After the stomachs of the experimental rats were removed, the ulcer index (UI) was determined using their ulcer scoring.

$$UI = 10 / X,$$

Where, X = whole stomach mucosal surface/whole ulcerated surface.

Percentage of Ulcer Inhibition:

$$\text{Percent inhibition (\% I)} = (USC - UST) / USC \times 100$$

USC= Ulcer score of control and UST = Ulcer score of the test animal.

Gastric Volume and Gastric Juice's pH: From rats used in experiments, gastric juice was obtained and the volume of the gastric contents was determined using the supernatant fluid, which was centrifuged at 2000 rpm for 10 minutes, The gastric juice's pH was also evaluated using a digital pH metre.

Free Acidity and Total Acidity: The supernatant fluids of gastric contents were collected in a small conical flask and Topfer's reagent 2 drops were added. A solution of 0.01N sodium hydroxide was poured into a burette and wait till the flask's color shifted to yellow during the trituration process. Then 2 drops of phenolphthalein were use and titrated until the orange coloration was obtained [13].

C-reactive Protein: Through the retro-orbital method, blood was collected aseptically in tubes, and centrifugal techniques were used to separate the serum. Prior to use, gently pipette-mix the latex reagent into the tube, the instrument's auto analyser has assay parameters specified. Incorporate well, aspirate, and calibrate [14].

Histopathological Examination: The stomach tissues will be embedded in paraffin after being fixed in 10% formalin for 24 hours. To evaluate the development of ulcers, small sections (3-6 m) were sliced, marked with hematoxylin and eosin (H&E) dye, and then seen via a light microscope with a 10 magnification [15].

Statistical Analysis:

- Utilizing the GraphPad Prism programme, the statistical significance between the groups was evaluated.
- The data were compared using a one-way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test.

- For nonparametric comparisons of CRP, ulcer score, ulcer index, free acidity, total acidity, percentage of ulcer protection, volume of gastric juice, and acidity of gastric juice.
- The values were presented as mean ±SEM, and p value less than 0.05 was regarded as significant.

RESULTS:

Phytochemical Screening: The methanolic extract of leaves MK revealed the existence of alkaloids, flavonoids, terpenoids & phenol. MC juice extract revealed the existence of protein, carbohydrates, tannin, phenol and flavonoids.

Ethanol-induced Ulcer Model: Effect of Co-administration of MK leaves and MC fruit on antiulcer activity in ethanol-induced ulcers in rats the details results are represented in **Table 1-2**, as well as the graphic representations in **Fig. 1-3.**

TABLE 1: EFFECT OF ADMINISTRATION OF MC AND MK EXTRACTS IN THE FOLLOWING PARAMETERS

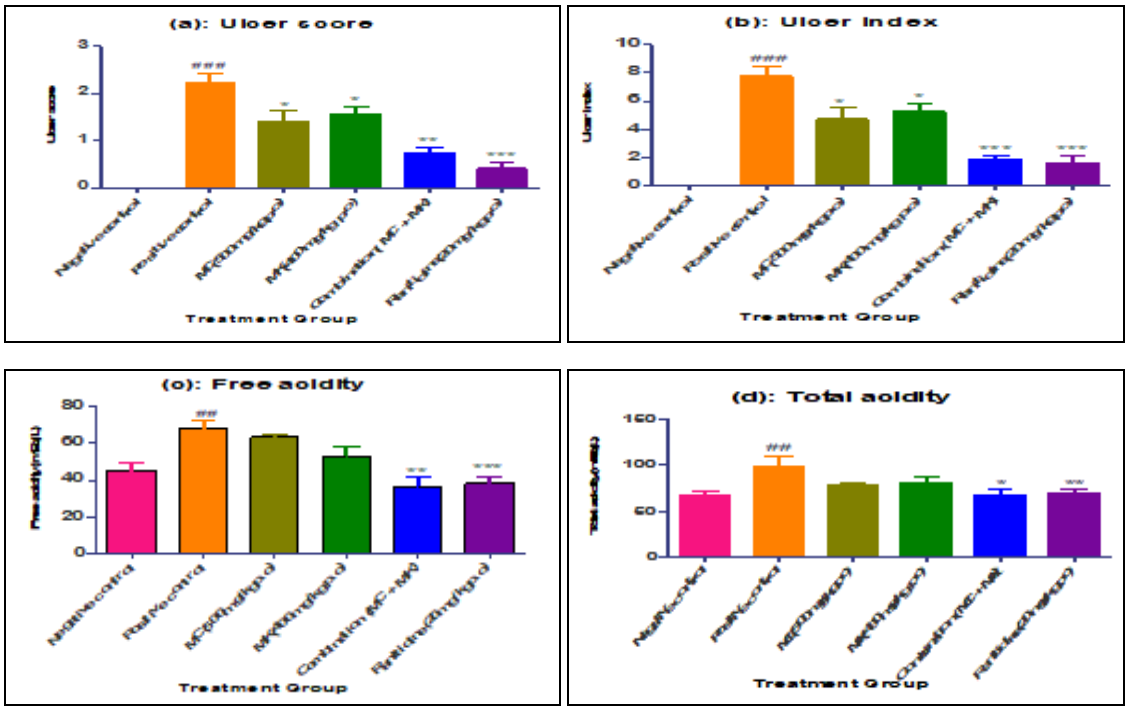
Treatment	Ulcer score	Ulcer index	Free acidity (mEq/L)	Total acidity (mEq/L)
Negative control	00.00±0.00	00.00±0.00	45.0 ± 4.28	68.33±3.07
Positive control (EtOH1ml/kg)	2.23±0.21$^{###}$	7.78±0.69$^{###}$	68.33±4.01	98.33±11.38$^{##}$
MC500mg/kg	1.41 ± 0.23*	4.72 ± 0.79*	63.33±2.10	78.33±3.07
MK400mg/kg	1.58 ± 0.15*	5.28 ± 0.51*	53.33±4.94	81.67±6.54
Combination (MC+MK)	0.75±0.11**	1.94±0.27***	36.67 ±4.94**	68.33±4.77*
RNT20mg/kg	0.41±0.15***	1.67±0.42***	38.33 ±3.07***	70.00±3.65**

All values are mean ± SEM, n = 6. $^{##}$p<0.01&$^{###}$ p<0.001 negative control versus positive control. *p<0.05, **p<0.01 &***p<0.001 All treated group versus positive control.

TABLE 2: RESULT OF ADMINISTRATION OF MK AND MC EXTRACTS IN THE FOLLOWING PARAMETERS

Treatment	Percentage inhibition	pH	Gastric volume (ml)	CRP(mg/dL)
Negative control	00.00±0.00	8.23 ± 0.08	2.53 ± 0.23	8.01±0.58
Positive control (EtOH1ml/kg)	0.00 ± 0.00	2.61±0.11$^{###}$	3.26 ± 0.30	25.83 ±2.52$^{###}$
MC500mg/kg	55.57±5.56*	6.08 ±0.09**	2.93 ± 0.21	15.12±0.68**
MK400mg/kg	61.13±7.04*	4.08 ± 0.06*	2.75 ± 0.17	11.55 ±0.31***
Combination (MC+MK)	86.17 ±2.76***	7.00±0.05***	2.18 ±0.13**	7.19±0.53***
RNT20mg/kg	88.93 ±3.50***	7.40±0.15***	2.21 ±0.22**	7.85±0.46***

All values are mean ± SEM, n = 6. $^{##}$p<0.01&$^{###}$ p<0.001 negative control versus positive control. *p<0.05, **p<0.01 & ***p<0.001 All treated group versus positive control.



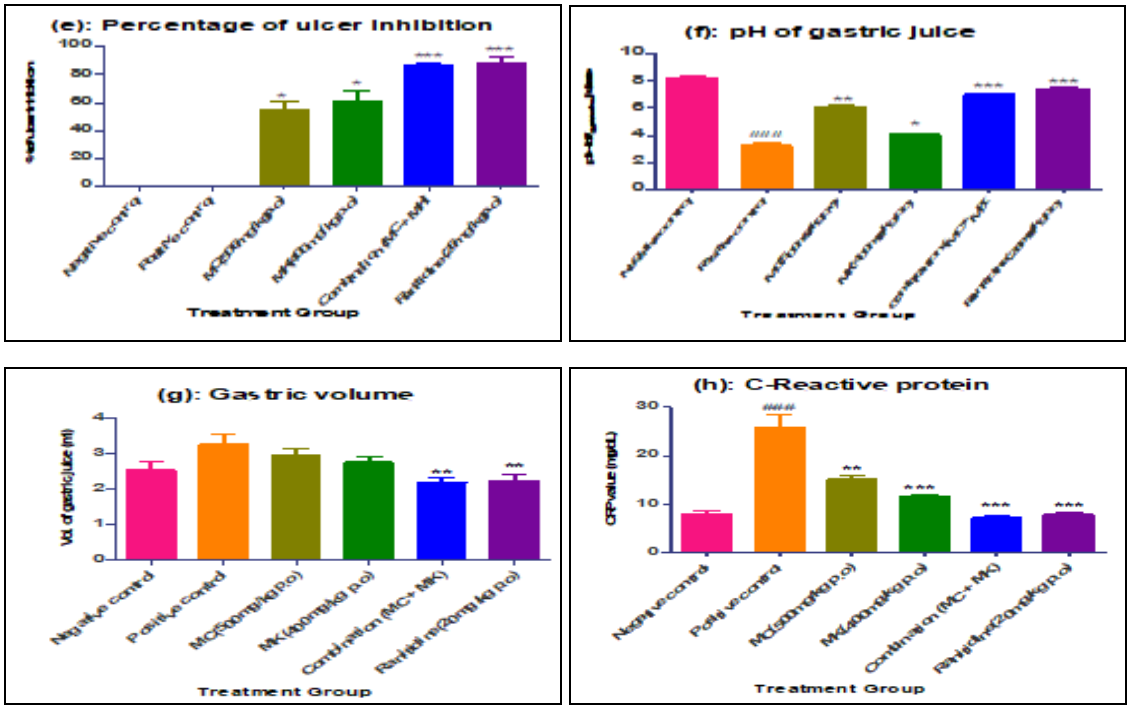


FIG. 1: GRAPH SHOWING THE EFFECT OF COMBINATION EXTRACTS AND STANDARD (RANITIDINE) ON THE (A) ULCER SCORE, (B) ULCER INDEX, (C) FREE ACIDITY, (D) TOTAL ACIDITY, (E) PERCENTAGE OF ULCER INHIBITION (F) PH, (G) GASTRIC VOLUME AND (H) CRP OF ETHANOL-INDUCED ULCERS IN RATS. All values are mean ± SEM, n = 6.$^{\#\#}p < 0.01$ &$^{\#\#\#}$ $p < 0.001$ negative control versus positive control and *$p < 0.05$, **$p < 0.01$ & ***$p < 0.001$ All treated group versus positive control.

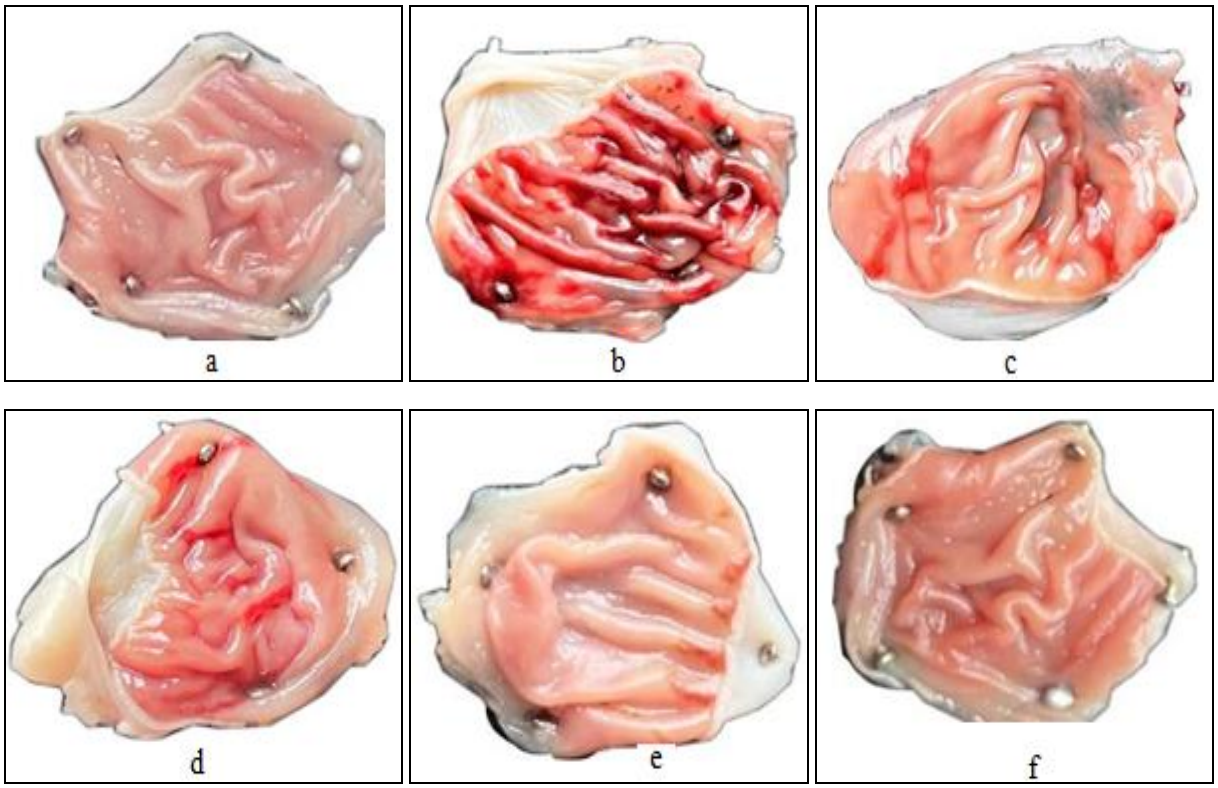


FIG. 2: MICROSCOPIC EVALUATION OF STOMACH IN ETHANOL-INDUCED ULCERS IN RATS (A) NEGATIVE CONTROL, (B) POSITIVE CONTROL (ETOH1ML/KG), (C) MC500MG/KG, (D) MK400MG/KG, (E) COMBINED DOSE (MC+MK), AND (F) RTD20MG/KG

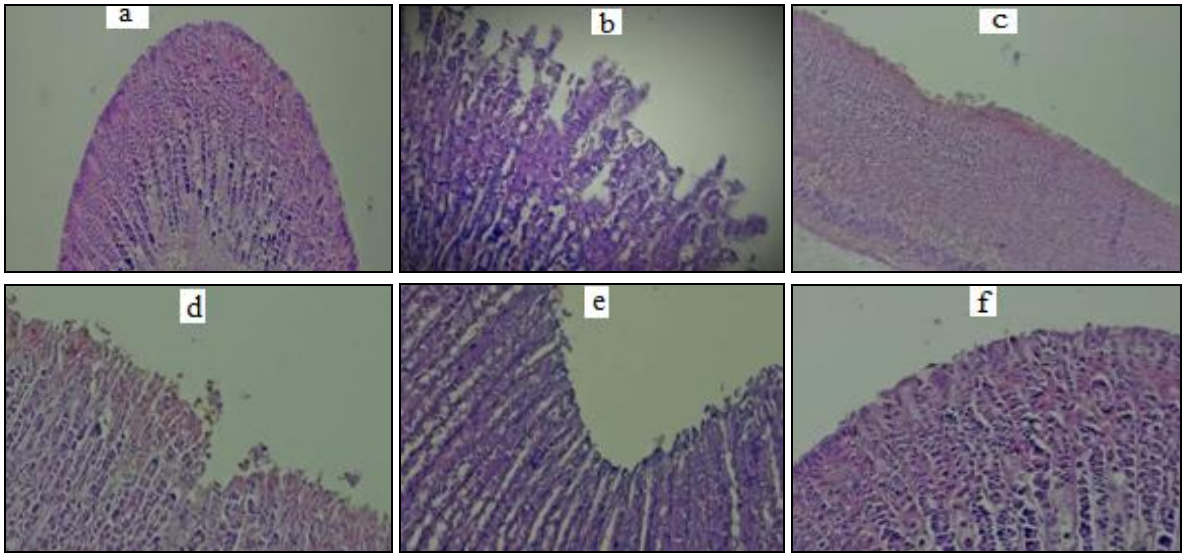


FIG. 3: STOMACH HISTOPATHOLOGICAL EXAMINATION OF RATS TREATED WITH RANITIDINE, MC, MK AND COMBINE DOSE IN ETHANOL ULCER MODEL (A) NEGATIVE CONTROL, (B) POSITIVE CONTROL (ETOH1ML/KG), (C) MC500MG/KG, (D) MK400MG/KG, (E) COMBINED DOSE (MC+MK), AND (F) RTD20MG/KG

Swimming Stress-induced Ulcers in Rats: Effect of Co-administration MK leaves and MC fruits on antiulcer activity in swimming stress-induced ulcers in rats the details results are represented in **Table 3-4**, as well as the graphic representations in **Fig. 4- 6.**

TABLE 3: EFFECT OF ADMINISTRATION OF MC AND MK EXTRACTS IN THE FOLLOWING PARAMETERS

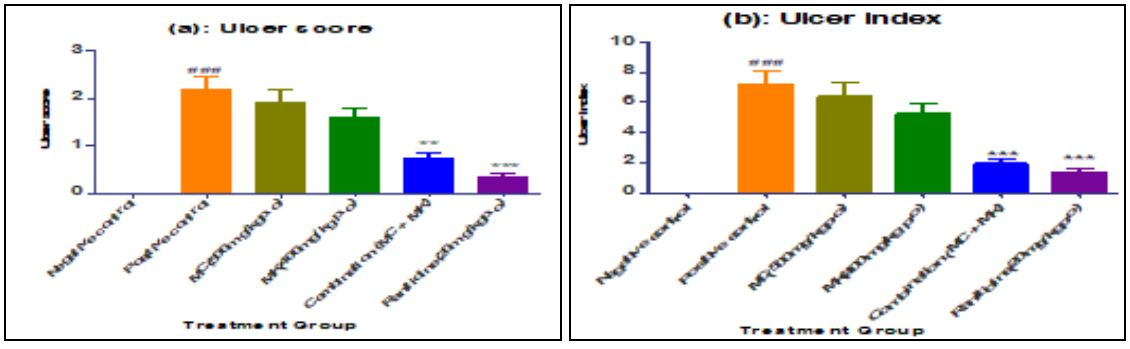
Treatment	Ulcer Score	Ulcer Index	Free acidity(mEq/L)	Total acidity(mEq/L)
Negative control	00.00±0.00	00.00±0.00	28.7 ± 1.15	40.00±1.39
Positive control	2.17±0.27###	7.23±0.92###	37.0 ± 1.73	80.67±1.85##
MC500mg/kg	1.92 ± 0.27	6.40 ± 0.90	32.8 ± 2.15	44.83±1.13**
MK400mg/kg	1.58 ± 0.20	5.28 ± 0.67	31.5 ± 2.05	45.17±1.167**
Combination (MC+MK)	0.75 ±0.11**	1.95±0.27***	27.5 ±1.38**	43.83 ±2.62***
RNT20mg/kg	0.33±0.10***	1.39±0.27***	24.2±2.17***	47.17 ±2.99***

All values are mean ± SEM, n = 6. ##p<0.01&### p<0.001 negative control versus positive control. *p<0.05, **p<0.01 & ***p<0.001 All treated group versus positive control.

TABLE 4: RESULT OF ADMINISTRATION OF MK AND MC EXTRACTS IN THE FOLLOWING PARAMETERS

Treatment	Percentage inhibition	pH	Gastric volume(ml)	CRP((mg/dL)
Negative control	000.00±0.00	3.63 ± 0.14	3.63 ± 0.14	2.73 ± 0.15
Positive control	00.00±0.00	1.86±0.11###	4.36±0.17###	30.08 ±1.33###
MC500mg/kg	55.57±5.56**	2.43 ± 0.13*	3.26 ± 0.08	14. 88±0.69**
MK400mg/kg	66.70±7.46**	2.87 ± 0.15	2.91 ± 0.14	12.60±0.61**
Combination (MC+MK)	91.70 ±3.71***	3.43 ±0.17**	2.36 ±0.11**	8.73±0.48***
RNT20mg/kg	94.47 ±3.50***	3.87 ±0.07**	2.617±0.15**	8.71±0.18***

All values are express as mean ± SEM, n = 6. ### p<0.001 negative control versus positive control. *p<0.05, **p<0.01 & ***p<0.001 All treated group versus positive control.



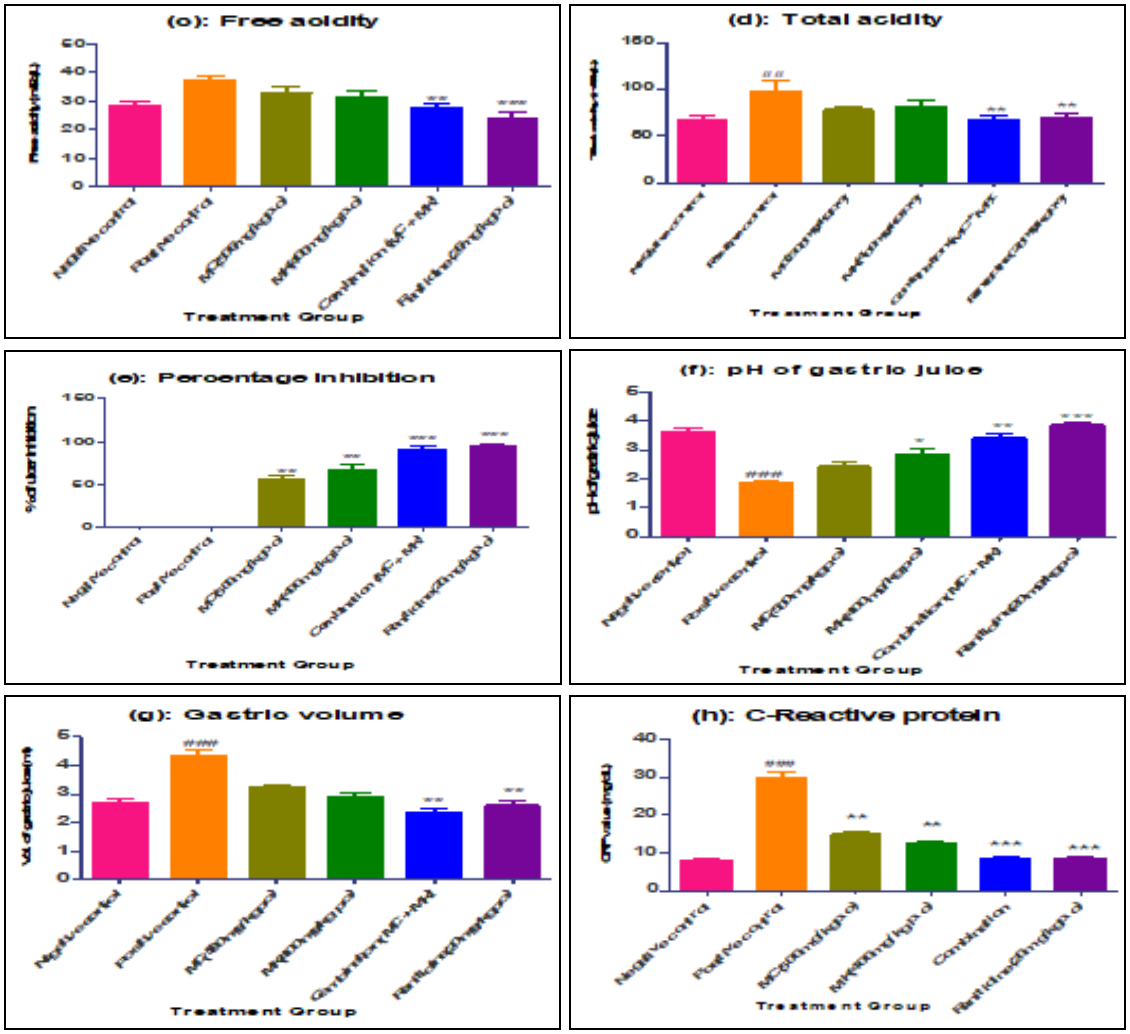


FIG. 4: GRAPH SHOWING THE EFFECT OF COMBINATION EXTRACTS AND STANDARD (RANITIDINE) ON THE (A) ULCER SCORE, (B) ULCER INDEX, (C) FREE ACIDITY, (D) TOTAL ACIDITY, (E) PERCENTAGE PROTECTION, (F) PH, (G) GASTRIC VOLUME AND (H) CRP OF SWIMMING STRESS-INDUCED ULCERS IN RATS. All values are mean ± SEM, n = 6.$^{\#\#}p < 0.01$ &$^{\#\#\#}$ $p < 0.001$ negative control versus positive control and *$p < 0.05$, **$p < 0.01$ & ***$p < 0.001$ All treated group versus positive control.

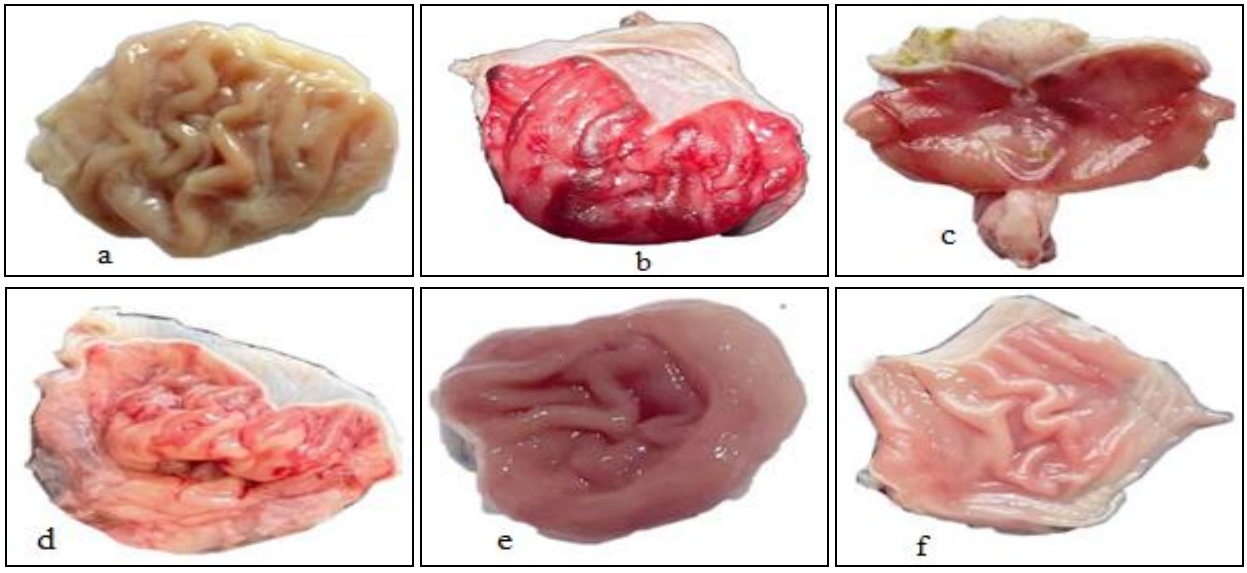


FIG. 5: MICROSCOPIC EVALUATION OF STOMACH IN RAT ULCERS CAUSED ON BY SWIMMING STRESS (A) NEGATIVE CONTROL, (B) POSITIVE CONTROL (SWIM STRESS), (C) MC500MG/KG, (D) MK400MG/KG, (E) COMBINED DOSE (MC+MK), AND (F) RTD20MG/KG

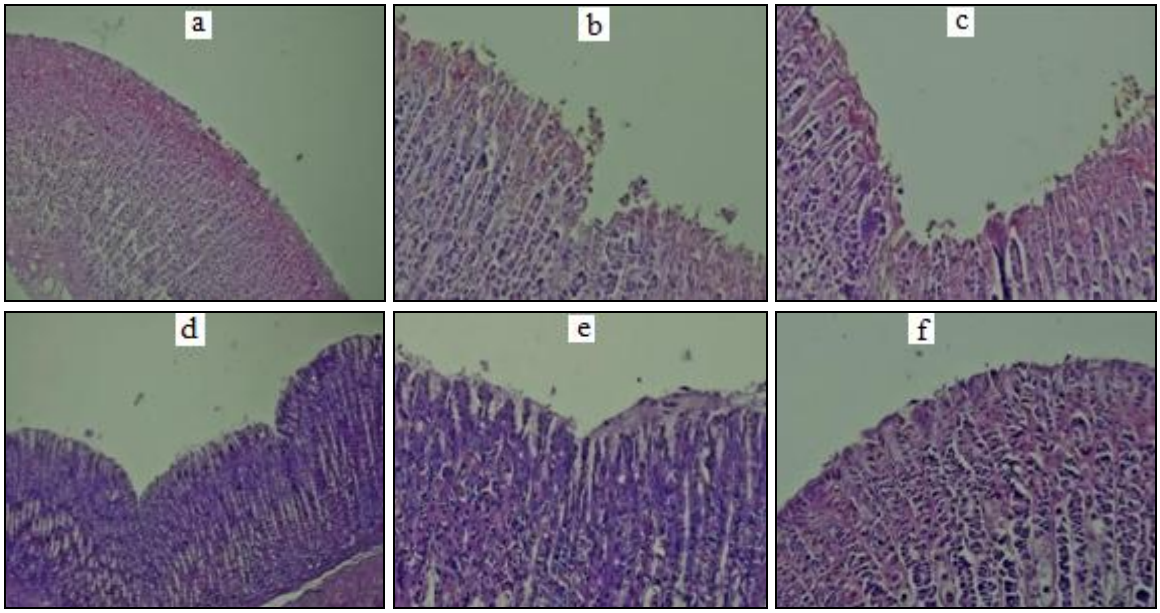


FIG. 6: STOMACHS HISTOPATHOLOGICAL EXAMINATION OF RATS TREATED WITH RANITIDINE, MC, MK AND COMBINE DOSE IN SWIMMING STRESS-INDUCED ULCER MODEL (A) NEGATIVE CONTROL, (B) POSITIVE CONTROL (SWIM STRESS), (C) MC500MG/KG, (D) MK400MG/KG, (E) COMBINED DOSE (MC+MK), AND (F) RTD20MG/KG

DISCUSSION: The present research was aimed to assess the preventive antiulcer potential of methanolic MK leaves extracts and MC fruit extracts, co administered for a period of week in Wistar rats that were then subjected to experimentally induced ulcers. In the ethanol induced ulcer model, the mucosa of the epithelium wall of stomach is damaged along with bleeding, lesion, and perforation due to the corrosive nature associated with free radical production [16]. In the swimming stress-induced ulcer model, it is considered that histamine release increases the production of hydrochloric acid and decreases the secretion of mucin, both of which lead to the formation of stomach ulcers [17].

The co-administration of MK (400mg/kg *b.w.p.o*) and MC (500mg/kg *b.w.p.o*) as pretreatment was effective in both models indicating that this extract possess a gastric cytoprotective effect. This activity was measured in terms of the observations reported in the present study. There was a substantial reduction in the ulcer score and ulcer index, in the groups pretreated with co-administration of MC and MK extracts. The free acidity and total acidity values were reduced by co-administration of MC and MK extracts and standard drug when compared with positive control in ethanol-induced as well as in swimming stress-induced models. Co-administration of MC and MK treated group significantly inhibited the formation of ulcers similar to the standard drug, which was comparatively higher than the MC of 500 mg/kg and MK of 400 mg/kg in both ethanol and swimming-induced model. MC and MK both combined extracts and standard drug neutralized the pH of gastric liquid by a significant rise in pH where the pH of the MC and MK extracts treated group had slightly acidic and the positive control pH was found to be highly acidic in nature. The gastric volume of the combination dose and standard drug also significantly decreased as compared with the positive control, MC, and MK treated groups in both models.

The combination, of MC and MK, treated groups and the standard-treated group showed a significant decrease in CRP when compared with the ulcerated group in both models. Macroscopic Examination of the stomach in the positive control had intense redness, a lesion, and streaks that indicate the severity of ulceration. The stomach of the treated group with MC and MK has shown less ulceration than the positive control, and the stomach of the combined dose and standard control has nearly the same morphology as the stomach of the negative control in both models. **Fig. 2 & 5.** Histopathological examination of this study of the gastric mucosa on the ulcerated control group of ethanol-induced and swimming stress-induced

ulcer models demonstrated severe ulceration with haemorrhage of the gastric mucosa. Moreover, the severity reduced significantly in treatment to the normal structural morphology by protection against the depletion of the stomach mucosa due to the cytoprotective effect of MC and MK co-administration treatment **Fig. 3 & 6.** The finding of this study and previous studies suggests the phytoconstituents present in MC & MK could be the reasons for chemicals involved in the healing of rat stomach ulcers. Further research is needed to identify the potential phytoconstituents that are capable of inhibiting ulcer activity.

CONCLUSION: The study found that both methanolic leaves extracts of *Murraya koeinigii* 400mg/kg b.wp.o. and fresh juice extracts of *Momordica charantia* 500mg/kg b.w p.o. have moderate substantial antiulcer activity, and their co-administration treatment exhibits highly significant and promising effects in decreasing ulcers, similar to the standard drug ranitidine, and proved to be cytoprotective.

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CONFLICTS OF INTEREST: NA

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