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Research Article

EVALUATION OF ANTI-ULCER ACTIVITY OF METHANOLIC EXTRACTS OF *KIGELIA AFRICANA, SOPHORA INTERRUPTA* AND *HOLOPTELEA INTEGRIFOLIA* LEAVES IN EXPERIMENTAL RATS

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ABSTRACT

Peptic ulcer disease (PUD) is a serious gastrointestinal disorder that requires a well targeted therapeutic strategy. *Kigelia africana (Bignoniaceae), Sophora interrupta (Papilionaceae), Holoptelea integrifolia (Ulmaceae)* are plants, reported for their variety of ethnic medicinal value. Hence we have planned to screen antiulcer activity of methanolic leaf extracts of these plants. LD50 studies for methanolic extract of *S.interrupta* were conducted up to the dose level of 5 g /kg by following OECD guidelines No.423. Anti ulcer activity was evaluated using pylorus ligated ulcer model in rats. Preliminary phyto - chemical studies revealed the presence of saponins, glycosides, alkaloids, flavonoids in the methanolic extracts of *K.africana, S.interrupta, H.integrifolia*. The acute toxicity studies considered safe dose of *S.interrupta* for further pharmacological screening as 4000mg/kg. The methanolic extracts at 200mg/kg (K.africana), 400 mg/kg (S.interrupta), 500mg/kg (H.integrifolia) per oral doses significantly ($P < 0.01$) reduced the ulcer score, ulcer number, ulcer index, volume of gastric juice, free acidity and total acidity in Pylorus ligation induced ulcer models in rats. The ulcer curative ratios of methanolic extracts were almost comparable to that of standard Omeprazole. The present study revealed the antiulcer activity of leaf extracts of *K.africana, S.interrupta, H.integrifolia*

Keywords: *K.africana , S.interrupta , H.integrifolia,* Pylorus ligation, Anti ulcer activity.

INTRODUCTION

Urban population with sedentary life styles resulting in increased gastric and peptic ulcers. Peptic ulcer is a lesion of the gastric mucosa and duodenal mucosa occurs at a site where the mucosal epithelium is exposed to acid and pepsin. Peptic ulcers occur due to imbalance between the gastric acid secretion and gastric mucosal integrity. The aggressive and protective factors in the stomach are acid pepsin secretion, mucosal barrier, blood flow, cellular regeneration, prostaglandins and epidermal growth factors. It is reported that prolonged anxiety, emotional stress, hemorrhagic surgical shock, burns and trauma are known to cause severe gastric irritation. Physical, chemical and psychological factors may lead to gastric ulceration in humans and experimental animals. Treatment of peptic ulcer aims at either inhibition of gastric acid secretion or reinforcement of gastric mucosal production.

Antiulcer drugs like H_2 –receptor blockers, proton pump inhibitors, antimuscuranic drugs produce adverse reactions such as hypersensitivity, arrhythmia, impotence and haemopoietic changes with a possibility of increased rate of ulcer recurrence within one year after cessation of the treatment. Because of the above mentioned demerits reported with the current antiulcer therapy there is a need for the search of newer therapeutic antiulcer agents from plant sources from the alternative therapy of Ayurvedha medicine. Some plant extracts produce promising and favorable reasons in the treatment of gastric ulcers. Several plants and herbs are advocated for the treatment of gastrointestinal disorders including gastric ulcers.

Kigelia africana (family: Bignoniaceae) known as "sausage tree" cultivated in many parts of India as an ornamental and roadside tree. *Kigelia africana* is a multipurpose medicinal plant with many attributes and considerable potentials. The plant has traditional uses which include anticancer, antiulcer, anti-aging, antioxidant and antimalarial activities. It is also widely applied in the treatment of genital infections, gynecological disorders, renal ailments, fainting, epilepsy, rheumatism, sickle-cell anemia, psoriasis, eczema, central nervous system depression, respiratory ailment, skin complaint, body weakness, leprosy, worm infestation and tumors etc.[1]

Sophora interrupta Bedd belongs to the family, Fabaceae (Leguminaceae, Papilonaceae) is commonly called as *Edwardsia maderaspatana* Wight, Pili Girgoli. There are approximately 219 species in this genus *Sophora*. They were investigated to posses abortifacient, antibacterial, anticholesterolemic, anti-inflammatory, antispasmodic, diuretic, emetic, emollient, febrifuge, hypotensive, purgative, styptic, and tonic properties[2].

Holoptelea integrifolia belongs to the family of Ulmaceae. It is an important pollen allergen of India and sensitizes almost 10% of the atopic population in Delhi[3]. Until today; some recent explorations have been reported on this plant in which antiviral activity[4], antioxidant, antimicrobial & wound healing activity[5] and antiemetic activity[6] is important. Ethno medically, the leaves and stem bark of this plant were used by local people for skin diseases,[7] obesity, cancer[8], and leaves decoction were used in the management of diarrhea[9]. Hence, the present study was under taken to evaluate the antidiarrhoeal activity of methanolic extract of leaves of *Holoptelea integrifolia* in mice model subjected to its traditional claim.

In pylorus ligation induced ulcer model gastric ulcers are due to over production of gastric acid or decrease in gastric mucous production. In this model an increase in acid-pepsin accumulation due to pylorus obstruction and subsequent digestion of the mucous was reported. The cause of gastric ulcers after pylorus ligation are due to an increase in gastric HCl secretion, stasis of acid due to stress and over production of gastric acid increased volume is also an important factor involved in ulcer formation as the unprotected lumen of the stomach is exposed to the accumulated acid.

No scientific data is available in support of gastro protective effect of *K.africana , S.interrupta , H.integrifolia.* Based on the traditional uses the aim of present study was to investigate the antiulcer activity of leaf extract of *Kigelia africana, sophora interrupta, holoptelea integrifolia* in pylorus ligated rat model.

METHODS AND MATERIALS

Plant materials

The fresh leaves of *K.africana , S.interrupta , H.integrifolia* were collected from Dr. K. Madhava Chetty, Assistant professor, Department of Botany, Sri venkateswara University, Tirupati, Andhra Pradesh, India, in june 2010[10]. The plant was identified by a Botanist, and voucher specimen was deposited in Sri venkateshwara University, Department of Botany and a copy has been preserved for the future reference at the herbarium of the institute T.R.R.C.P. (1447/PO/a/11/CPCSEA). After authentification, the plants were cleaned and shade dried and milled into coarse powder by a mechanical pulverizer.

Preparation of the plant extract

The powdered material (2 kg) was defatted with petroleum ether (60- 80°C) in a soxhlet extraction apparatus and marc was extracted with methanol (1000 ml) overnight, at room temperature with constant stirring. The extract was filtered and the filtrate was concentrated at 30°C under reduced pressure in a rotary evaporator. Extract was dried in dessicator. The crude extract was suspended in 1% Tween 80 to required concentrations and used for the experiments.

Experimental animals

Swiss albino mice 20-22g and wister rats 160-200g of either sex were purchased from NIN, Hyderabad, India. They were housed in polypropylene cages in a controlled room temperature 22± 1ºC and relative humidity of 60-70. They were kept under standard conditions of 12/12 h light and dark cycle. The animals were maintained with standard pellet diet and water ad libitum. The animals were acclimatized to laboratory condition for seven days before commencement of experiment. The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethical Committee and was cleared by the same before starting.

Preliminary phytochemical screening

The phytochemical examination of the MEKA, MESI and MEHI was performed by the standard methods[11].

Acute oral toxicity studies

Acute oral toxicity study of methanolic extract of Sophora interrupta was carried out in swiss albino mice of either sex 20-22 g according to OECD guidelines No. 423. MESI at different doses up to 5000 mg/kg p.o was administered and animals were observed for behavioral changes, toxicity and mortality up to 48 h [12].

Formulation

Suspensions were formulated of required concentrations 200mg/kg[13] (K.africana), 400 mg/kg (S.interrupta), and 500mg/kg[14] (H.integrifolia) by using 1% Tween 80 and double distilled water. The formulated suspensions were compared for various evaluation parameters.

Pharmacological Studies: Anti-ulcer Activity

Pylorus ligation induced ulcer model [15]

Wistar rats weighing between (160-200 gm) were divided into 5 groups of 6 rats in each. They were under fasting for 24 hrs with water ad libitum prior to experiment in individual cages with measures taken to avoid coprophagy. Group A was served as normal control given with vehicle only. Group B with standard i.e., Omeprazole and groups C, D and E were treated with doses of MEKA, MESI and MEHI respectively. The various groups were treated with vehicle/drug/ extracts 30 min prior to pylorus ligation and the details of the protocol was given below: Group A: Normal animals treated with vehicle only; Group B: Standard i.e., Omeprazole (20 mg/kg p.o); Group C: MEKA (200 mg/kg); Group D: MESI (400 mg/kg); Group E: MEHI (500 mg/kg)

Experimental procedure

Under light ether anesthesia, the abdomen was opened and the pylorus ligation performed and then sutured. 4 h after pylorus ligation all the animals were sacrificed with excess of anesthetic ether and the stomach of each rat was dissected out. Gastric juice collected into centrifuge tubes was centrifuged at 1000 rpm for 10 min and volume was noted. The pH of the gastric juice was recorded by pH meter. The gastric content was subjected for analysis of free and total acidity. The stomachs were washed under running tap water and then focused under microscope to note the ulcers in the glandular portion. The number of ulcers per stomach shown in figure 1 was scored and the scoring is done as per standard procedure.

Scoring of ulcer[16]

0 = Normal stomach

0.5 = Red coloration

1 = Spot ulcers

1.5 = Hemorrhagic streaks

2 = Ulcer > 3 mm but > 5 mm

3 = Ulcers > 5 mm.

Ulcer index = UA+US+UP/10

Where,

UA=Average number of ulcers per animal, US=Ulcer severity score,

UP=Percentage of animals with ulcers (UP=Total ulcers in a group/total number of animals x 100).

The formula for calculation of Percentage ulcer inhibition –

Percentage inhibition=UIC-UIT/UIC X 100

Where

UIC=Ulcer index of control group,

UIT= Ulcer index of test group.

Reagents for biochemical estimations of free and total acidity[15]

a. Freshly prepared 0.01N oxalic acid solution (BDH) was used to standardize sodium hydroxide.

b. Freshly prepared 0.01N sodium hydroxide

c. Topfer's reagent. It is dimethylaminoazobenzene 0.5% in absolute ethanol available in 100 ml package.

d. Freshly prepared 1% Phenolphthalein (BDH) solution prepared in 50% absolute ethanol.

Methods for biochemical estimation of free and total acidity

Collection of gastric juice

Gastric content collected from pylorus ligated rats was centrifuged and the volume of gastric juice as well as pH of gastric juice was noted. The gastric juice was subjected to biochemical estimations as follows

Determination of free and total acidity

1 ml of gastric juice was pipette into a 100 ml conical flask, 2 or 3 drops of Topfer's reagent was added and titrated with 0.01N Sodium hydroxide until all traces of red colour disappears and the colour of the solution turns to yellowish orange. The volume of alkali added was noted. This volume corresponds to free acidity. Then 2 or 3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge appears. Again the total volume of alkali added was noted now this volume corresponds to total acidity.

Acidity was calculated by using the Formula

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH}}{0.1} \times 100 \text{ meq /lt/ 100g}$$

Statistical Analysis

The results were shown in the Table No.1 & 2. The values expressed as mean ± SEM from 6 animals. The results were subjected to statistical analysis by using one way ANOVA followed by Dunnett's-'t'- test to verify the significant difference if any among the groups. P<0.01*and P<0.001**were considered significant.

RESULTS

Phytochemical screening

The results of preliminary phytochemical screening of the MEKA, MESI and MEHI revealed that presence of flavonoids, carbohydrates, glycosides, steroids,phenols. The constituents present in the extracts are shown in Table No. 3

Acute toxicity Studies

The results of acute toxicity studies indicate that the extract did not produce any behavioral changes, toxic reaction and mortality up to the dose level of 2500 mg/kg body weight in mice even after 48 h, but produced toxic symptoms of mortality with dose level of 5000mg/kg and hence the safe dose for further pharmacological screening was considered as 4000mg/kg.

Anti-ulcer activity

Pylorus ligation induced ulcer model in rats

In pylorus ligation induced ulcer model in rats a significant increase in ulcer index (11.29+0.03) are noted. In the same model a significant increase in gastric volume (9.38+0.28ml), free acid (78.22+1.08mEq/L) and total acid (105.2+1.36mEq/L) are noted. Standard i.e., Omeprazole (20 mg/kg) treatment has significantly reduced ulcer index (0), gastric volume (3.04+0.11ml), free acid (27.05+0.58mEq/L) and total acid (56.98+0.92mEq/L).

In pylorus ligation induced ulcer model, the extracts MEKA, MESI and MEHI have significantly reduced the ulcer index (7.41+0.24, 5.37+0.16and 3.43+0.06) and the ulcer formation (34.36%, 52.39% and 69.61 %) is significantly reduced, shown in Figure 2. Similar to the above a significant reduction in gastric volume (4.65+0.07, 4.19+0.12and 3.85+0.08ml), shown in Figure 3, free (56+1.65, 43.5+1.25 and 34.67+0.88 mEq/L), shown in Figure 4 and total acid (89.17+1.42, 79.83+1.07and 69.17+1.01mEq/L) is noted and shown in Figure 5.

Table 1: Antiulcer effect of MEKA, MESI, MEHI in pylorus ligated ulcer model in rats

Groups	Dose	Volume(ml)	P^H	Free Acidity (Eq/l)	Total Acidity (Eq/l)
Control	1ml/kg BW	9.38+0.28	2.56+0.12	78.22+1.08	105.2+1.36
Omepra-zole	20mg/kg BW	3.04+0.11*	6.51+0.11*	27.05+0.58*	56.98+0.92*
MEKA	200mg/kg BW	4.65+0.07*	3.49+0.08*	56+1.65*	89.17+1.42*
MESI	400mg/kg BW	4.19+0.12*	4.35+0.07*	43.5+1.25*	79.83+1.07*
MEHI	500mg/kg BW	3.85+0.08*	4.91+0.11*	34.67+0.88*	69.17+1.01*
F		251	198.5	304.9	246.5

n = 6, Values are Mean + SEM, Statistical significant test for comparison was done by one ANOVA (dF = 29) followed by dunnet's't' test. Symbols statistical significant: P < 0.01 Vs control. MEKA-Methanolic extract of K.africana, MESI- Methanolic extract of S.interrupta, MEHI – Methanolic extract of H.integrilfolia.*

Table 2: Antiulcer effect of MEKA, MESI, MEHI in pylorus ligated ulcer model in rats

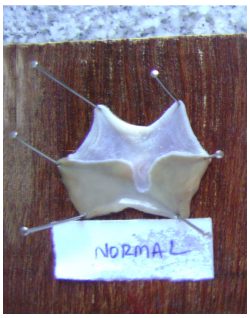
Groups	Dose	Ulcer Index (Mean+SEM)	% of Protection
Control	1ml/kg	11.29+0.03	
Omeprazole	20mg/kg BW	0**	100
MEKA	200mg/kg BW	7.41+0.24*	34.36
MESI	400mg/kg BW	5.37+0.16*	52.39
MEHI	500mg/kg BW	3.43+0.06*	69.61

n = 6, Statistical significant test for comparison was done by one ANOVA (dF = 29, F=962) followed by dunnet's't' test. Symbols statistical significant: P < 0.01 and P<0.001** Vs control. Significant at P < 0.01* and P<0.001**, MEKA-Methanolic extract of K.africana, MESI- Methanolic extract of S.interrupta, MEHI – Methanolic extract of H.integrilfolia.*

Table 3: The Phytochemical profile of the leaf extracts

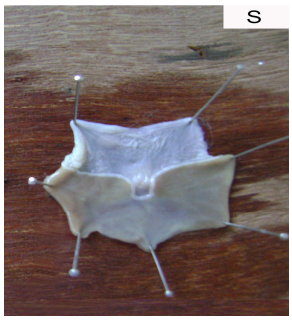
Phytoconstituents	Presence/ Absence		
	MEKA	MESI	MEHI
Sugar	+	+	+
Tannin	+	-	+
Alkaloid	-	-	-
Flavonoid	+	+	+
Saponin	+	+	+
Steroid	+	-	+
Cardiac glycoside	+	+	+
Ester	+	+	+
Proteins	-	-	-
Amino acid	-	-	-
Carbohydrates	+	+	+

'+' indicates presence and '-' indicates absence



Normal control

Toxicant control



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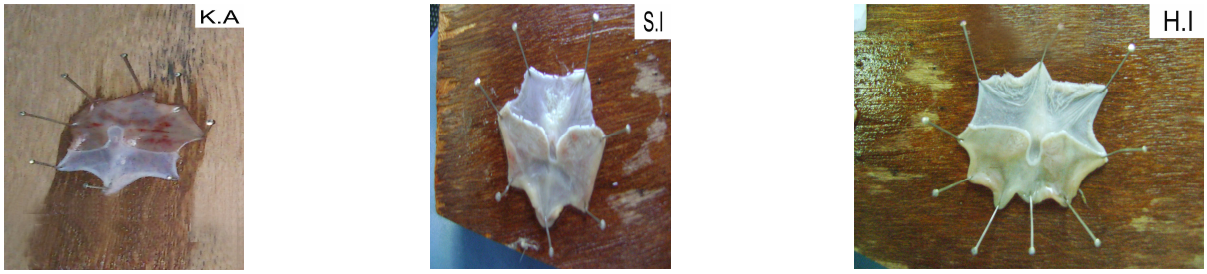

MEKA **MESI** **MEHI**

Fig. 1: Effect of MEKA, MESI and MEHI on pylorus ligation induced ulcer model in rats

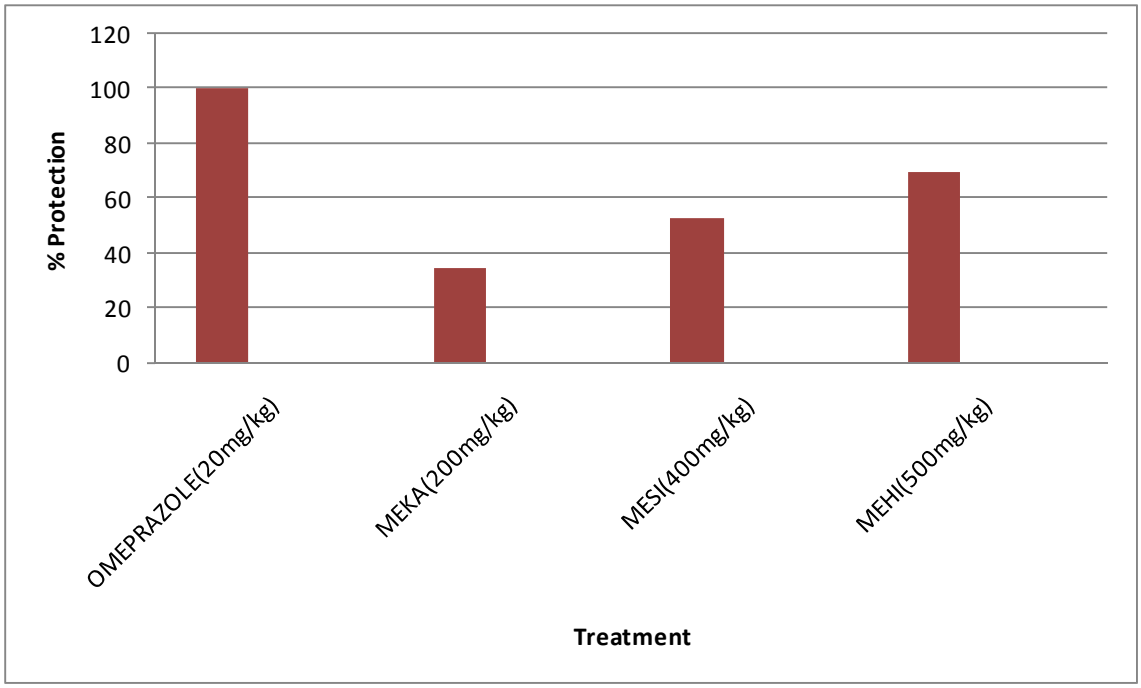

Fig. 2: Antiulcer effect (% protection) of MEKA, MESI and MEHI in pylorus ligation induced ulcer model in rats.

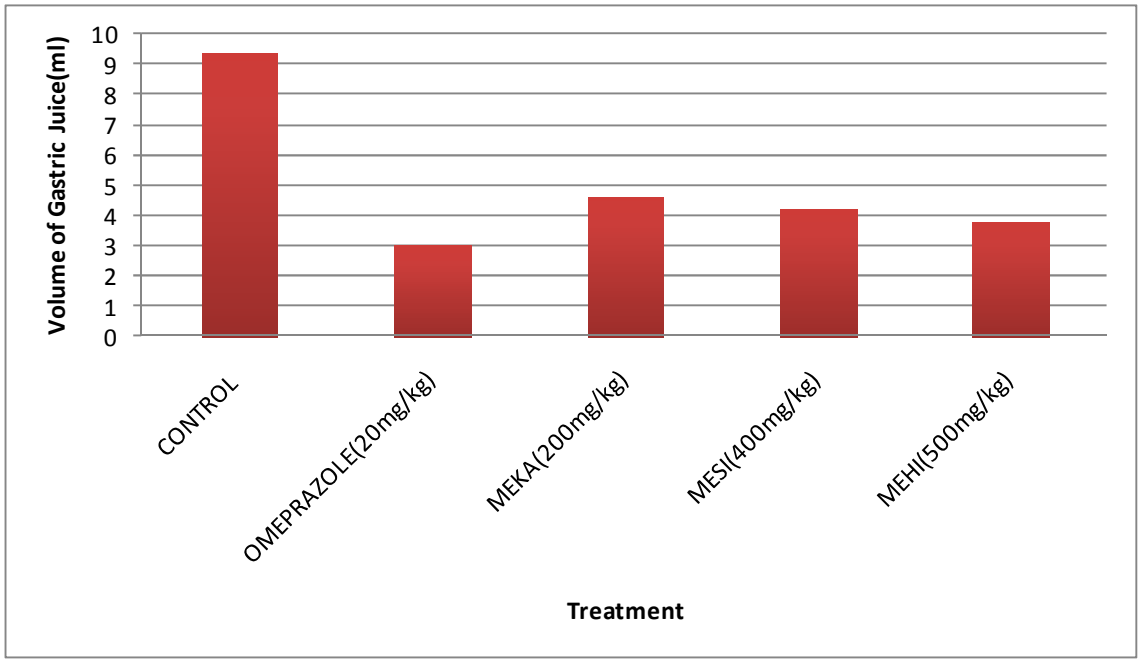

Fig. 3: Antiulcer effect of MEKA, MESI and MEHI on volume of gastric juice in pylorus ligation induced ulcer model in rats.

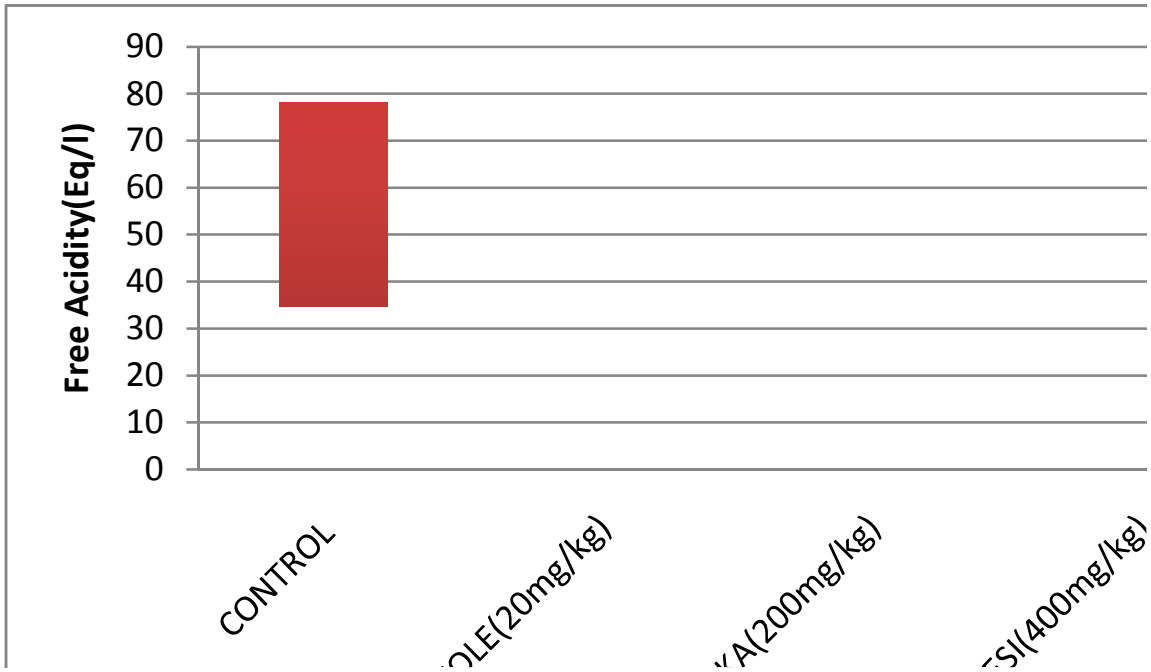

Fig. 4: Antiulcer effect of MEKA, MESI and MEHI on free acidity in pylorus ligation induced ulcer model in rats

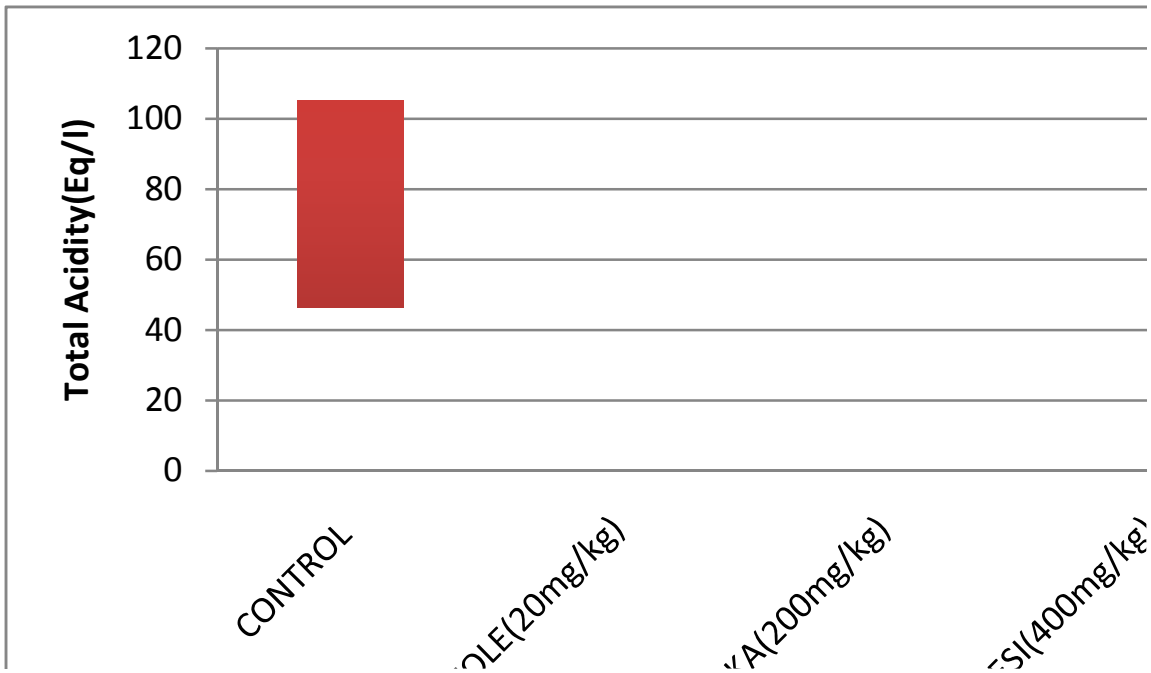

Fig. 5: Antiulcer effect of MEKA, MESI and MEHI on total acidity in pylorus ligation induced ulcer model in rats

DISCUSSION

The leaf extracts (MEKA, MESI and MEHI) of K.africana, S.interrupta and H.integrifolia were subjected for phytochemical investigation and LD50 studies were performed with MESI up to the dose level of 5000 mg/kg. Mortality was observed with methanolic extract of S.interrupta up to the maximum dose level of 5 g/kg. Doses namely medium (1/10th) were selected with respect to the LD50 dose i.e, 400 mg/kg (S.interrupta) body weight for the present study. The extracts were evaluated for their anti-ulcer activity in pylorus ligated ulcer models in rats.

It has been proposed that in pyloric ligation, ulcers are developed due to accumulation of gastric acid and pepsin, which leads to auto-digestion of gastric mucosa[17]. The Anti-ulcer property of MEKA, MESI and MEHI in pylorus ligation model is evident from its significant reduction in free acidity, total acidity and ulcer index. Because MEKA, MESI and MEHI treated animals significantly inhibited the formation of ulcers in the pylorus ligated rats and also decreased both the concentration and increased the pH, it is suggested that MEKA, MESI and MEHI can suppress gastric damage induced by aggressive factors.

The extracts produced a significant ($p<0.01$) anti-ulcer activity but a relatively better anti-ulcer activity was recorded with methanolic extract of Holoptelea integrifolia.

CONCLUSION

Leaf extracts of K.africana, S.interrupta, H.integrifolia exhibited a significant anti-ulcer activities in experimental animals rats.

Methanolic extract of Holoptelea integrifolia exhibited relatively better anti-ulcer activities than K.africana and S.interrupta extracts. The difference in the evaluated activities could be due to the number and the quantity of phytoconstituents present in the extracts.

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