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GASTROPROTECTIVE ACTIVITY OF A SIDDHA DRUG *KADUKKAI CHOORANAM* AGAINST INDOMETHACIN AND PYLORUS LIGATION INDUCED GASTRIC ULCER IN RATS

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Abstract: Gastric ulcer is major health problem, which can affect large number of populations in all geographical regions in developing countries like India, the major reason for ulcer development is stress, which plays a huge role in excess secretion of acid and thus may lead to ulcer. *Kadukkai chooranam(KC)* an herbal based Siddha formulation is administered for *Gunmam* (Acid peptic diseases) by many Siddha physicians popularly. Anti ulcerogenic activity of the KC has been studied against Indomethacin plus Pylorus ligation induced gastric ulcer model (4-groups/method, n=6, Indomethacin 48mg/kg, KC 150mg/kg at different period of days) in wistar albino rat models. Animals were sacrificed and their stomachs were subjected to macroscopic and microscopic ulcer index findings. Statistical data were analyzed by one way ANOVA followed by student's t-test. This study concluded that KC has significant anti-ulcer effects in experimental animals with ulcer induced by Indomethacin and pylorus ligation; it showed a dose dependent protection against Indomethacin (mg/kg body weight) induced ulcers in rats and it produced a significant reduction of ulcer index in the dose of mg/kg bodyweight. KC showed a statistically significant P value < 0.05 and <0.01 at dose level mg/kg and 150 mg/kg respectively as compared to control. Acute toxicity study was carried out as per standard guidelines and the LD50 was found to be greater than 2500mg/kg body weight with no pathological changes in the experimental animals.

Keywords: Kadukkai, chooranam, ethanol, indomethacin, pylorus

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INTRODUCTION

Peptic ulcer is one of the major gastro-intestinal disorders, which occur due to an imbalance between the offensive (Gastric acid secretion) and defensive (gastric mucosal integrity) factors[1].Consequently, reduction of gastric acid production as well as re-inforcement of gastric mucosal production has been the major approaches for therapy of peptic ulcer disease. As a result, more and more drugs, both herbal and synthetic are coming up offering newer and better options for treatment of peptic ulcer. The type of drugs varies from being proton-pump inhibitor to H_2 receptor antagonist or a cytoprotective agent. At the same time, each of these drugs confers simpler to several side effects like arrhythmias, Impotence, Gynaecomastia, Enterochromaffin-like cell (ECL), Hyperplasia and Haemopoeitic changes[2]. There are evidences for the participation of reactive oxygen species in the etiology and pathophysiology of human disease, such as Neurodegenerative disorders, Inflammation, Viral infections, Autoimmune gastrointestinal inflammation and Gastric ulcer[3] Drugs with multiple mechanism of protective action, including antioxidant activity, may be highly effective in minimizing tissue injury in human diseases. It has been demonstrated that many drugs and formulations possess potent antioxidant activity and are effective in the treatment of ulcers[4, 5].

Prevalence of Ulcers:

Gastric ulcer is major health problem, which can affect large number of populations in all geographical regions in developing countries like India, the major reason for ulcer development is stress, which plays a huge role in excess secretion of acid and thus may lead to ulcer. Ulcer prevalence increases with age and the most affected people fall in the age group of 40 to 60. Ulcer incidence is not affected by gender as both males and females are equally affected. Gastric Ulcer occur most commonly in elderly people, frail women, ill people, un skilled laboring classes and chronic administration of NSAID (non steroidal anti inflammatory drugs) in association with the development of gastric ulceration and severe complication, which limit their widespread clinical use[6]. A number of etiological factors are associated with the development of ulcer and the most prominent are helicobacter pylori, dietary factors, cigarette, alcohol, stress and NSAIDS.

Materials and Methods

Preparation of the Test Drug

Kadukkai *(Terminalia chebula)* is one of the main ingredients of many Siddha formulations used in clinical practice. The raw drug is purchased from the authenticated market and made in to fine powder to prepare the extract.

Experimental animal:

Healthy albino rats of Wistar strain (150-170gm) were selected for the study. The rats were procured from Tamil Nadu veterinary University Chennai. The rats were housed in well ventilated colony cages in the departmental animal house. Coprophagy was prevented by keeping the animals in cages with gratings as the floors. The animals were maintained on sterile, standard pellet diet and water *ad Libitum*.

The study was designed and conducted according to the ethical norms approved by Institution Animal ethics committee (IAEC Vide reference no.A30.2.GT.0.70/72). Before beginning the experiments, the animals were allowed to acclimatize to animal house condition for a week period. The rats were divided into several groups depending on the nature of the study and each group consisted of 6-10 rats.

Experimental design[7, 8, 9]:

Gastric Ulceration, according to the method described by al shabanah was induced in 36 h fasted rats by the oral administration of an ulcerogenic drug, indomethacin (48mg/kg b.w) and a necrotizing agent, one ml of absolute ethanol. Groups of ten animals each were pretreated with *KC* 100, 150 and 200mg/kg, oral administration) 1h before the ulcerogenic procedure. The rats were killed 6h after indomethacin and 1h after the ethanol administration by an overdose of ether. The stomachs were removed and opened along the greater curvature of stomach and the ulceration was scored. The Ulcer index was evaluated according to severity and ulcer scores.

Oral Toxicity test:

Group of six rats were given graded doses of *KC* extract (500- 2500 mg/ kg b.w orally) and were continuously observed for two hours to detect changes in various anatomical and behavioural responses, any mortality during the study and following successive seven days has also been recorded.

Dosage and administration:

150 mg of *KC* extracts/kg body weight was administered to both ethanol induced and indomethacin induced Ulcer rats for 5,10 and 15 days, the rats were sacrificed at the end of treatment period and stomach was opened along the greater curvature and the ulceration was scored using hand lees. Ulcer index was calculated from the Ulcer scores. The rats were grouped as follows.

Ethanol induced Ulceration:

Group I - Control

Group II- Ethanol induced

Group III-Ulcer rats treated with *KC* extract for 5 days

Group IV- Ulcer rats treated with *KC* extract for 10 days

Group V- Ulcer rats treated with *KC* extract for 15 days

Pyloric ligated Ulceration[10] :

Group I - Control

Group II- rats treated with *KC* extract (150mg/kg for 5 days)

Group III- rats treated with *KC* extract (150 mg kg for 10 days)

Group IV- rats treated with *KC* extract (150mg/kg for 15 days)

At the end of the experiment period, control and tested groups of rats were subjected to pylorus ligation. The operative procedure adopted as a modified one[11]. Feed was with held one day prior to the operative procedure. The rats were anaesthetized with ether and the abdomen was opened through a mid- line incision. The pylorus was secured and ligated with skill sutures; care was taken not to ligate the blood vessels. The abdominal walls were closed and the animals were allowed to recover from anaesthesia. After pyloric ligation drinking water was with held and the gastric juice was allowed to collect for a period of 4h. The rats were then killed by an over dose of ether and the stomach removed after clamping the oesophagus.

The gastric mucosa was washed with 3ml of lukewarm distilled water and collected in graduated centrifuge tubes. The gastric juice and washing were homogenized and centrifuged at 5000 rpm/min for 15 minutes. The volume of gastric juice was measured and expressed as ml/100g body weight of the rat. Total acid output was determined by titrating suitably diluted gastric juice with 0.01N NaOH using phenolpthalein as indicator. Data is expressed as

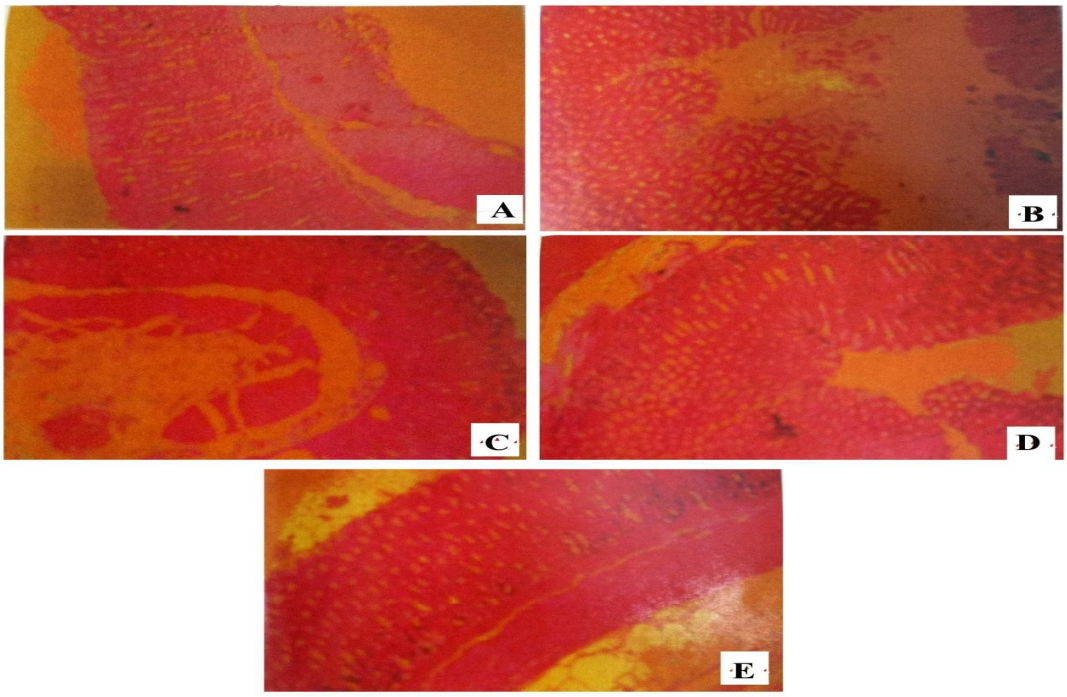
m Eq/L of gastric juice[12].

Histological studies:

A portion of the ulcer region in the stomach tissues was dissected out and fixed in 10% buffered neutral formalin solution for histological observation. After fixation tissues were embedded in paraffin, solid sections were embedded in paraffin; solid sections were cut at

5mm and stained with hematoxylin and eosin[13] . The sections were examined with the help of a qualified pathologist under the light microscope and photo micrographs were taken (Fig.1).

Fig.1 Histopathological appearance of stomach section



A- Control
B- Indomethacin induced
C- Ethanol induced
D – KC EXTRACT+INDOMETHACIN
E – KC EXTRACT+ETHANOL

Determination of degree of ulceration[14]:

The surface area (A) mm^2 covered by each section was measured and the sum of erosion areas per rat stomach was calculated. Percentage of ulcerated surface (US) was calculated.

$$\%US = \frac{\text{Total area covered by Ulcers}}{\text{Total corpus mucosal surface}}$$

Ulcer index was calculated from percentage of ulcerated surface

The following score was used in order to calculate ulcer index[15],

Ulcer index : O.No Ulcer; 1.Us < 0.5; 2. 05 ≤ 2.5; 3. 2.5 ≤ 5; 4. 5 ≤ 10; 5. 11 ≤ 15;

6. 15≤20; 7. 20 ≤ 25; 8. 30; 9. 30 ≤ 35; 10. US> 25≤ 35

Results and Discussion

Acute oral toxicity studies:

Acute oral toxicity studies revealed that administration of ethanolic extract of *KC* extract in increasing doses upto 2500mg/kg b.w did not elicit any sign of toxicity and mortality in rats when observed for 7days after oral administration. The drug did not produce any perceptible changes in the autonomic and behavioural responses in rats. Oral administration of the *KC* extract did not show any significant changes on food or water consumption, either on body weight nor relative weight of the organs. The animals did not show any Macroscopic changes in their lives, kidney, lungs or spleen and did not present any symptoms of diarrhoea, seizures and sleepiness. Though *KC* extract is commonly referred as laxative it did not cause any excessive defecation even at the highest single dose tested in the present study. Thus toxicity studies carried out in rats indicate no lethal effect at least up to an oral dose of 2.5g/kg body weight indicating that LD_{50} if any should be higher than this dose.

Acid neutralization effect of *KC extract*

The amount of acid in fresh lyophilized powder of *KC* extract was too little to be determined because its P^H was almost equal to the end point of the filtration using 0.01N NaOH. The volume of 0.01 N NaOH used for the filtration of fresh *KC* extract to end point was less than 0.01ml.The acid consuming capacity of 1 gm of *KC* extract to neutralize 1N HCl in 5.94 mEq while the acid consuming capacity of Magnesium trisilicate is 5.0 mEq. Each 1ml of 1N HCl is equivalent to 1mEq of acid consumed. Thus the results of this study indicate that the observed decrease in the number of lesions in both indomethacin and ethanol induced Ulcer rats may partially be due to the acid neutralizing capacity of *KC* extract.

Pre-treatment studies:

Pre-treatment with a single dose of *KC* extract 100, 150 and 200 mg/K b.w orally) resulted in significant ($p<0.05$) and dose dependent decrease in the intensity of gastric mucosal damage by ethanol, a powerful necrotizing agent (Table 1) when compared to ulcer control group rats. The observed decrease in ulcer index in *KC* extract treated group of rats may be due to its anti secretory or cytoprotective properties or both. Though the mechanism of Ulcer formation by

ethanol is quite different, the efficacy of the drug was found to be the same in controlling the gastric ulceration.

Pre-treatment with *KC* extract was found to inhibit the necrosis induced by ethanol. The stomach of the animals with higher dose of extract *KC* (150 and 200mg) showed more or less normal appearance. These findings confirm the cytoprotective nature of the *KC* extract. The gastric mucosal coat is considered to be important both in preventing damage to the gastric epithelium as well as facilitating its repair. Since there was a marginal difference exhibits between the two higher doses (150 and 200 mg/Kg b.w) tested.

Table 2 shows percentage of ulcerated surface and ulcer index of control and tested groups of rats in both the ulcer models. A high degree of ulcer index and ulcerated surface were obtained in both the ulcer induced grown rats than ethanol induced ulcer rats. Though the treatment with *KC* extract *for* 5 to 10 days exhibited decrease in ulcer index, the decrease in severity of ulcer was more marked in ulcer rats treated with *KC* extract for 15 days.

Ulcer induced group:

Ulcer index and Ulcer Severity were found to be comparably higher in indomethacin induced ulcer rats than ethanol induced ulcer rats. Through treatment with *KC* extract for 8 and 10 days exhibited decrease in ulcer index, the decrease in severity of ulcer was more marked in ulcer rats treated with *KC* extract for 15 days.

Pylorus ligation:

The results of pylorus ligated studies are summarised in Table 3. The result is evident that oral administration of *KC* extract at a concentration 150mg/kg b.w for 15days significantly reduced ($p<0.05$) the gastric ulceration in a duration dependant manner. It is important to note that there is no significant alteration in total volume, acidity and pH in the *KC extract* treated rats when compared to control group of rats. Thus it may be concluded that the anti ulcerogenic property of *KC* extract may be mainly due to its cytoprotective effect, an interesting finding in this study is the ability of the extract to completely prevent the formation of pylorus ligated ulcer in a stomach environment containing acid concentrations near to ulcerated control. Usually in shay ligated rat, gastric acid levels between 40 and 65 mEq/l are capable of causing severe ulceration of the rat gastric mucosa. Since the accumulated acid requires the presence of active pepsin for the formation of pylorus ligated ulcers, Products capable of rendering the peptic secretions inactive may also prevent mucosal ulceration. The conversion of pepsinogen to pepsin occurs at pH 2, while pepsin inactivation occurs at about pH 6. The gastric pH values, observed in the study suggest that the gastric pepsin formation could have been limited by the extract since the extract treated and control values are closely similar.

The pre-treatment dosage schedule and pylorus ligation reveals, it is evident that administration of *KC* extract at a concentration of 150 mg/kg b.w/rat day for 15days significantly decreased ($P<0.05$) ulceration in both indomethacin and ethanol induced ulcer rats. Hence the same dosage at the level of 150mg/kg b.w for 15 days produced maximum efficacy at a minimum dosage.

The *KC* extract drastically reduced both indomethacin and ethanol induced ulceration in rats in a time dependent manner. The Pre-treatment and dosage fixation studies revealed that the incidence of ulcer in both the models is predominant in the glandular part of stomach. The phytochemical screening revealed the presence of tannis, saponins, flavonoids, glycosides and sterols in *KC* extract which are known to protect the integrity of mucous membranes may have strengthened the mucosal barrier[16].

Histological studies

The results of histological observations made on stomach of control and tested rats are presented in plates (Fig.1 A-E). Plate A shows the histological observations made on the gastric mucosa of control rats showing normal architecture. Plate B represents stomach tissue of indomethacin induced Ulcer rats with severe Ulcer lesions on the gastric mucosa. Plate C illustrates alcohol induced ulcer rats with prominent lesions and ulcerated surface on the gastric mucosa Plate D and E represent *KC* extract (150mg/kg b.w) treated indomethacin and ethanol induced ulcer rats respectively. Administration of *KC extract* was found to protect the gastric surface against ulceration, which is evident by decrease /absence of lesions in both the ulcer- induced models.

Ulcer index:

The numbers of lesions present on the gastric mucosa are indicative of the severity of ulcer disease. The diameter of the lesion is used for the determination of ulcer index, a measure of ulcer in the gastric mucosa.

Table 4 and 5 indicate the ulcer index and ulcerated surface in control and tested groups of both the ulcer models. A marked increase was observed in ulcerated surface and ulcer index of both the ulcer induced groups of rats. A decrease in ulcer severity was evident by decrease ulcer index in *KC extract* treated ulcer rats. Ranitidine, reference drug was found to significantly decrease the ulcer index only in indomethacin- induced ulcer rats but not in ethanol induced ulcer rats.

Indomethacin potentiates the stress, 2-deoxy-D- glucose and Vagal-stimulated gastric secretion and produced severe gastric lesions. Further, it was also reported that indomethacin increased both stress- induced ulceration and gastric acid secretion[17, 18].

Administration of *KC* extract enhanced the mucosal resistance and thus resulted in decrease in ulcer index and ulcerated surface. The secretory drug ranitidine also markedly inhibited the indomethacin induced gastric lesions. These results suggest that the antiulcer activity of *KC* extract against indomethacin induced ulcer might also be related to its anti secretory effect.

Ranitidine did not overcome the mucus depletion induced by ethanol since it acts via blocking of H_2 receptors[19] on contrary *KC* extract administration result in decreased ulcer index. The mucus depletion by ethanol was overcome by *KC* extract which underlines its cytoprotective nature.

It may also be proposed that the decrease in ulcer severity *KC* may be attributed to its active ingredients with gastric protective nature. The phytochemical analysis revealed the presence of tannins, saponins and flavonoids, substance known to affect the integrity of mucous membranes. Tannins are known to protect the outermost layer of mucosa and to render it less permeable and more resistance to chemicals and mechanical injury or irritation and thus prevent ulcer development. Flavonoids have also been reported to offer same protection in ulcer development by increasing capillary resistance and improving micro circulation[20].

Acidity plays an important role in the pathogenesis of indomethacin induced gastropathy. Gastric mucosal damage induced by indomethacin is augmented by the presence of high concentration of acid into the gastric lumen[21]. Indomethacin induced damage to rat mucosa is markedly dependent on luminal pH Gastric acidity may potentially facilitate indomethacin induced mucosal damage by two mechanisms[22] (i) by enhancing gastric absorption of these drugs.(ii) by amplifying mucosal injury once mucosal defences have been impaired by the decrease in prostaglandin synthesis. Stimulation of mucus secretions such as glycoprotein and mucin by *KC extract* helps in decreasing the volume of acidity of gastric juice. Further hyposecretory nature of *KC extract* in ulcer induced rats may further help in decreasing the volume pH and acidity of gastric juice towards near normal levels. Thus normalization of gastric juice acidity may indirectly help in healing of ulcer lesions in *KC extract* treated ulcer rats.

DISCUSSION

Treatment with *KC* extract antagonizes the aggressive factors which play a crucial role in the pathogenesis of gastric lesions and augment defensive factors to protect the gastric mucosa from ulceration. The increase in the levels of group, particularly sialic acid and hexosamine in the extract treated groups indicate the increase in the production of mucus there by possibly protects the gastric mucosa in both ulcer models. The efficacy of the extract was more or less same in both the models and showed promising antiulcer activity more than that of ranitidine.

Tannins are of the constituent of *KC extract* from a pellicle over the lining of gastric mucosa to resist the attack of proteolytic enzymes. Thus resistance to proteolysis may help in the restoration of Glycoprotein moiety of gastric mucosa in *KC* treated ulcer rats. Further flavanoids have been reported to be present in *KC extract*. Flavonoids might play a role in stabilizing the antioxidant status of the gastric mucosa which may have maintained its glycoprotein moiety. Thus *KC* extract may have ameliorated glycoprotein abnormalities through its action on pepsin mediated proteolysis. Thus this study establishes the ulcer protective effect of *Kadukkai chooranam* extract and the protective effect is mediated by defensive mucosal factors.

Table 1. Effect of Pre treatment with *KC* extract on the ethanol induced ulcer

Group	Ulcerate surface (%)	Ulcer index
Control	0.0	0.0
Ethanol	20.8 ± 0.18 [a*]	7
Ethanol+ *KC Extract* (100mg).	7.6 ± 0.58 [b*]	4
Ethanol+ *KC Extract* (150mg).	2.1 ± 0.16 [b*]	2
Ethanol+ *KC Extract* (200mg).	2.0 ± 0.20 [b*]	2

Values are expressed as mean ± S.D for six animals in each group. One way ANOVA followed by post hoc test (LSD)

$P < 0.05$. comparisons are made between [a] control, [b] ethanol

Table 2. Effect *KC* extract treatment for 5, 10, 15 days ethanol induced ulcer rat

Group	Ulcerate surface (%)	Ulcer index
control	0.0	0.0
Ethnol	50.2 ± 4.6 [a*]	10
Ethanol+*T.chebula*(5 day)	14.6 ± 1.1 [b*]	5
Ethanol+*T.chebula*(10 day).	3.7 ± 0.28 [b*]	3
Ethanol+*T.chebula*(15 day).	0.60 ± 0.05 [b*]	2

Values are expressed as mean ± S.D for six animals in each group. One way ANOVA followed by post hoc test (LSD)

P < 0.05. comparisons are made between [a] control, [b] ethanol.

Table 3. Effect of *KC* extract on pylorus ligated ulcer model

Groups	Ulcerated surface	Ulcer index	Total gastric volume	Gastric acidity	pH
Control	10.2±0.90	4	4.5±0.3	47.3±3.8	3.26±0.25
KC extract (5 days)	5.1±o.26 [a*]	3	5.1±0.2	45.3±2.8	3.23±0.26
KC extract (10 days)	2.4±0.20 [a*]	2	4.7±0.5	43.1±3.2	3.86±0.3
KC extract (15 days)	0.5±0.12 [a*]	1	5.2±0.7	39.6±3.6	3.96±0.32

P<0.05 comparisons are made between [a] control rats.

Table 4. Effect of *KC* extract on the extent of ulceration in control and indomethacin induced ulcer group rats

Group	Ulcerate surface (%)	Ulcer index
GroupI – control	0.0	0.0
GroupII – indomethacin	81.3 ± 6.2 [a*]	10
GroupIII – indomethacin +*KC*	1.2 ± 0.09 [b*]	2
GroupIV – indomethacin + *KC*	1.4 ± 0.10 [b*]	2

P < 0.05. Comparisons are made between [a] control, [b] indomethacin

Table 5. Effect of KC extract on the extent of ulceration in control and ethanol induced ulcer group rats

Group	Ulcerate surface (%)	Ulcer index
Group I - control	0.0	0.0
Group II - Ethnol +*KC extract*	59.6 ± 4.8 [a*]	10
Group III - Ethanol +*KC extract*	1.5 ± 1.10 [b*]	2
Group IV - Ethanol +*KC extract*	27.6 ± 1.9 [b*]	8

P < 0.05. Comparisons are made between [a] control, [b] ethanol.

Table 6. Levels of gastric volume acidity and pH of control and tested groups on indomethacin induced ulcer rats

Groups	Total gastric volume (ml)	Gastric acidity (mEq/l)	P^H
Groups I- control	2.24 ± 0.16 [a*]	3.78 ± 0.42 [a*]	4.23 ± 0.32 [a*]
Groups II- indomethacin	4.34 ± o.21 [b*]	6.92 ±0.56 [b*]	2.3 ± 0.23 [b*]
Group III- indomethacin + *KC extract*	2.89 ± 0.42 [b*]	3.97±0.33 [b*]	4.01 ± 0.35 [b*]
Group IV- indomethacin + Ranitidine	3.01 ± o.33 [b*]	4.12 ± 0.43 [b*]	3.98 ± 0.36 [b*]

P < 0.05. Comparisons are made between [a] control , [b] indomethacin

Table 7. Levels of gastric volume acidity and pH of control and tested groups on ethanol induced ulcer rats

Groups	Total gastric volume (ml)	Gastric acidity (mEq/l)	P^H
Groups I- control	2.53 ± 0.24 [a*]	3.78 ± 0.42 [a*]	4.23 ± 0.32 [a*]
Groups II-Ethanol	4.34 ± o.21 [b*]	6.92 ±0.56 [b*]	2.3 ± 0.23 [b*]
Group III- Ethanol +*KC extract*	2.89 ± 0.42 [b*]	3.97±0.33 [b*]	4.01 ± 0.35 [b*]
Group IV- Ethanol+ Ranitidine	3.01 ± o.33 [b*]	4.12 ± 0.43 [b*]	3.98 ± 0.36 [b*]

$P < 0.05$. Comparisons are made between [a] control , [b] Ethanol

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