

## Therapeutic role of ginger and chamomile aqueous extract for peptic ulcer using experimental rats.

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**Abstract:** This work was designated to clear out the effect of aqueous extracts of ginger (*Zingiber Officinale*) family zingiberaceae and chamomile (*Matricaria chamomilla*) a member of Asteraceae family on healing aspirin induced ulcer in male albino rats. Thirty six male albino rats (150±10g b.wt. each) were used and disparted into 6 groups (n= 6 rats), one of them used as control-ve group while other 5 groups orally given aspirin (200mg/kg b.wt.), one of these groups left as control +ve and the rest groups orally administrated with aqueous extracts of ginger and chamomile at two doses of 200 and 400 mg/kg b.wt., for each of them for seven days. The length of gastric ulcer, the volume of gastric juice, pH, total acidity and histopathological changes were examined. The results concluded that oral administration with all doses of aqueous extracts of ginger and chamomile reduced the length of gastric ulcer, total acidity, volume of gastric juice and histopathological changes. On the other hand, all aqueous extracts increased pH value of gastric juice. According to the results, ginger and chamomile could be used for healing acute gastric ulcer disease especially ginger and chamomile water aqueous extract at doses of 400 mg/kg b.wt., for each of them.

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**Keywords:** Peptic ulcer, aqueous extract, ginger, chamomile, histopathological changes.

### 1.Introduction

Peptic ulcer disease is a common disorder that affects millions of people worldwide (Lockrey and Lim, 2011). Concepts of peptic ulcer disease have undergone radical change over the last twenty years (Evertiart *et al.*, 2014). The word 'peptic' derives from the Greek term 'peptikos,' meaning related to digestion (Pahwa *et al.*, 2010).

Peptic ulcer is a group of disorders characterized by the presence of ulcers in any portion of gastrointestinal tract (GIT) exposed to acid in sufficient concentration and duration. Although these ulcerations most commonly occur in the stomach (gastric ulcer) or small intestine (duodenal ulcer), this disease also includes barrett ulcer of the esophagus (Barrett's esophagus or Barrett's metaplasia) and other upper GI ulcers. It is develops when aggressive factors overcome the protective mechanisms (Borrelli and Izzo, 2000). *Helicobacter pylori*, non-steroidal anti-inflammatory drugs and acid-pepsin hyper-secretion are the major factors that disrupt this equilibrium (Calam and Baron, 2001). It is the cause of significant morbidity with patients having a low health-related quality of life (Barkun and Leontiadis, 2010).

The principles of management for peptic ulcer disease is accurate diagnosis, usually endoscopic to exclude malignancy and assess *H. Pylori* status and treating the cause, i.e. treat *H. Pylori* if present and cease causative drug (Lockrey and Lim, 2011).

Herbal medicines are now used by up to 50% of the western population in a number of instances 10% for the treatment or prevention of digestive disorders (Langmead and Rampton, 2011). Considering the morbidity caused by peptic ulcer disease and dyspepsia over the world cheap and easily available treatments will always be in demand especially for the people of non-industrialized countries (Minaiyan *et al.*, 2006).

Ginger is among the 20 top-selling herbal supplements in the USA (Borrelli *et al.*, 2004). Ginger is a widespread herbal medicine mainly used for the treatment of gastrointestinal (GI) disorders including: dyspepsia, nausea and diarrhea. Aromatic, spasmolytic, carminative and absorbent properties of ginger are probably responsible for the therapeutic applications in digestive tract ailments (Minaiyan *et al.*, 2006). Several studies have shown that ginger extract, essential oils and glycolipids possess a number of pharmacological actions which at least in part for some of them anti-ulcerogenic or ulcer preventive efficacy may be suggested (Afzal *et al.*, 2001).

Chamomile is one of the most widely used as medicinal plants. It has been included in the pharmacopoeia of 26 countries. Amino acid polysaccharides, fatty acids, essential oils, minerals, elements, flavonoids, and other phenolic compounds are the main constituents of chamomile (McKay and

Blumberg, 2006). Chamomile used in modern medicine primarily for their spasmolytic antiphlogistic, antibacterial properties and as a multipurpose digestive to treat gastrointestinal disturbances (Shikov *et al.*, 2008), including digestive disorders, "spasm" or colic, upset stomach, flatulence (gas), ulcers and gastrointestinal irritation (Kroll and Cordes, 2006). Chamomile had anti-inflammatory and spasmolytic effects on the stomach and duodenum, therefore, it is thought to heal ulcers. Previous study reported that chamomile flower extract has a complex effect on the luminal and mucosal environment of the stomach and duodenum. Some of these actions are important in healing the ulcers and others are important in preventing subsequent ulcer relapse (Rezq and Elmallh, 2010). Therefore this investigation aimed to study the possible therapeutic role of ginger and chamomile aqueous extract for peptic ulcer using experimental rats.

## 2. Materials and Methods

### a- Materials:

#### 1- Investigated Samples:

The first plant used was ginger (*Zingiber officinale*), family of zingiberaceae and the second was chamomile (*Matricaria chamomilla*), a member of the daisy family. All these plants were purchased as crude dried material from local market of Holy Makkah, KSA.

#### 2- Rats:

Thirty six male albino rats (150±10g *b.wt.*.) of Sprague Dawley Strain were purchased from King Fahd medical Research Center (KFMRC), Jeddah, KSA.

#### 3- Drugs:

Aspetic injectable was purchased and prepared by dissolving one vial in 25ml distilled water to obtain solution. A volume of 1ml of this solution was orally given (at the level 200mg/kg body weight) for one day to induce acute gastric ulcer in male albino rats according to the methods described by Agrawal *et al.*, (2000).

### b- Methods:

#### 1- Preparation of A queous Extracts:

One gram of powdered ginger or chamomile mixed with 100 ml distilled water were boiled for 10 min and then cooled for 15 min. Thereafter, the aqueous extract was filtered using a Millipore filter (Millipore 0.2 mm, St. Quentin en Yvelines, France) to remove particulate matter. The filtrate was then freeze-dried and the desired dose (200 and 400 mg of lyophilised aqueous extract of ginger and chamomile per kg body weight) was then prepared and reconstituted in 1.5 ml of distilled water. The aqueous extracts were prepared daily, just before administration. The extracts obtained were then given orally to different groups of rats at a dose of 200 and

400 mg/kg body weight. The ginger extract was green coloured but chamomile was yellow coloured with a percent yield of 14%, its average osmolarity was 60 mOsm, pH 6.5, and with a very low viscosity. Lemhadri *et al.*, 2004.

#### 2- Experimental Design:

Thirty six Sprague - Dawley male albino rats, each weighing 150±10 g., were housed in special cages under controlled conditions. Every day animals were observed for the external appearance, shape, colour and distribution of hair and physical activity. All rats were fed on the control diet which consisted of casein (12.5%), corn oil (10%), choline chloride (0.2%), vitamin mixture (1%) (Campbell, 1963), cellulose (5%), salt mixture (4%), sucrose (22%) and corn starch (up to 100%) (Hegested *et al.*, 1941) for 7 consecutive days before the beginning of the experiment for adaptation. Diets were presented to rats in special non-scattering feeding cups to avoid loss of food and contamination. Tap water was provided to rats by mean of glass tubes projecting through wire cages from inverted bottles supported to one side of the cage. Rats disparted into 6 groups (n= 6 rats), one of them used as control-ve group while other 5 groups orally given aspirin (200mg/kg *b.wt.*), one of these groups left as control +ve and the rest groups orally administrated with aqueous extracts of ginger and chamomile at two doses of 200 and 400 mg/kg *b.wt.*, for each of them for seven days.

#### 3- Biochemical Analysis:

The length of gastric ulcer was carried out according to the method of Akhtar and Ahmad (1995), the volume of gastric juice was measured by graduated cylinder and expressed as ml., total acidity was determined by titration of 1ml gastric juice in 10ml of distilled water with 0.01N NaOH using two drops of phenolphthalein as an indicator. Data were expressed as percentage and the pH degree was determined by pH meter.

#### 5- Histopathological Investigation:

Specimens from stomachs were collected from rats of all experimental groups at the end of the experimental period, fixed in 10% neutral buffered formalin (pH=7.0), dehydrated in ethyl alcohol, then cleared in xylol and embedded in paraffin; 4-6 microns thickness, sections prepared and stained with heamtoxylin and eosin for examining both fore and glandular parts of the stomach (Carleton, 1976).

#### 6- Statistical Analysis:

The obtained data were statistically analyzed using computerized SPSS (Statistic Program Sigmastat, statistical soft-ware, SAS Institute, Cary, NC). Effects of different treatments were analyzed by one way ANOVA (Analysis of variance) test using Duncan's multiple range test and  $p < 0.05$  was used to

indicate significance between different groups (Snedecor and Cochran, 1967).

### 3. Results and Discussion

#### 1. Effect of aqueous extracts on the length of gastric ulcer in rats:

Data listed in table (1) show the effect of ginger (*Zingiber Officinale*) and chamomile (*Matricaria chamomilla*) aqueous extracts at two doses on aspirin induced gastric ulcer in rats.

**Table (1): Effect of ginger (*Zingiber Officinale*), and chamomile (*Matricaria Chamomilla*) aqueous extracts at two doses on the length of gastric ulcer in rats.**

Groups		Aspirin and Extracts	Doses (mg/kg <i>b.wt.</i> )	Gastric ulcer length (mm.)
				Mean $\pm$ SE
Control -ve	1	-	-	0.00
Control +ve	2	Aspirin (Asp)	200	7.11 $\pm$ 0.075 a
Treated Groups With Aqueous Extracts	3	(Asp) + Ginger	200	4.63 $\pm$ 0.023 c
	4	(Asp) + Ginger	400	2.05 $\pm$ 0.020 e
	5	(Asp) + Chamomile	200	5.93 $\pm$ 0.040 b
	6	(Asp) + Chamomile	400	3.01 $\pm$ 0.017 d

- Values denote arithmetic means  $\pm$  standard error of the means
- Means with different letters (a, b, c, d) in the same column differ significantly at  $p \leq 0.05$ .
- Using one way ANOVA test, while those with similar letters are non-significant.

It could be observed that the length of gastric ulcer in control +ve group was 7.11 $\pm$ 0.075 mm., compared with zero in control -ve group (normal rats). This mean that there were significant increase in gastric ulcer length in control positive group when compared to control negative one. All aqueous extracts of ginger 200 & 400 mg/kg body weight and chamomile 200 & 400 mg/kg body weight showed significant decrease in gastric ulcer length as compared to control positive group which were 4.63 $\pm$ 0.023, 2.05 $\pm$ 0.020, 5.93 $\pm$ 0.040, 3.01 $\pm$ 0.0173 and 7.11 $\pm$ 0.075 mm, respectively. Rats administered with ginger 400 mg/kg body weight showed the highest significant decrease in the length of gastric ulcer compared to all treated groups.

These data were in agreement with Mahady *et al.*, (2003) who concluded that gingerols inhibit the growth of some *H. pylori* strains experimentally and this activity contribute to its preventive effects. Meanwhile, Rezaq and Elmallh, (2010) reported that chamomile flower extract has a complex effect on the luminal and mucosal environment of the stomach and duodenum. Some of these actions are important in healing the ulcers and others are important in preventing subsequent ulcer relapse. In addition, Khushtar *et al.*, (2009) concluded that the rats which were treated with ginger oil had significantly ( $p < 0.01$ ) increased the mean gastric wall mucus thickness (barrier mucus).

Credence for this speech, Bombik and Saba (2002) concluded that the extracts of some herbs including chamomile increased the concentration of zinc, copper and iron in blood serum which was helpful for the flowing of blood to the stomach mucosa causing treatment of the pain, according to this action, these herbs were useful for healing of gastric ulcer.

#### 2. Effect of aqueous extracts on volume of gastric juice in rats:

Table (2) show the effect of water plant extracts of ginger and chamomile at doses of 200 and 400ml on the volume of gastric juice in rats.

**Table (2): Effect of ginger (*Zingiber Officinale*), and chamomile (*Matricaria Chamomilla*) aqueous extracts at two doses on the volume of gastric juice in rats.**

Groups		Aspirin and Extracts	Doses (mg/kg <i>b.wt.</i> )	Volume of gastric juice (mL.)
				Mean $\pm$ SI
Control -ve	1	-	-	0.31 $\pm$ 0.017 <sup>f</sup>
Control +ve	2	Aspirin (Asp)	200	0.87 $\pm$ 0.006 <sup>a</sup>
Treated Groups With Aqueous Extracts	3	(Asp) + Ginger	200	0.61 $\pm$ 0.012 <sup>c</sup>
	4	(Asp) + Ginger	400	0.49 $\pm$ 0.005 <sup>d</sup>
	5	(Asp) + Chamomile	200	0.73 $\pm$ 0.004 <sup>b</sup>
	6	(Asp) + Chamomile	400	0.41 $\pm$ 0.005 <sup>e</sup>

- Values denote arithmetic means  $\pm$  standard error of the means
- Means with different letters (a, b, c, d) in the same column differ significantly at  $p \leq 0.05$ .
- Using one way ANOVA test, while those with similar letters are non-significant.

It is clear from the table that ulcerated rats (Control positive group) showed significant increase ( $p \leq 0.05$ ) in the volume of gastric juice in rats when compared to normal rats which were 0.87 and 0.31 ml, respectively. All experimental groups which treated with all aqueous extracts showed significant decrease in the volume of gastric juice as compared to control positive group. Oral administration with water extracts of chamomile at a dose of 400mg/kg *B.wt* showed the highest decrease in the volume of gastric juice in rats which was  $0.41 \pm 0.005$  ml., when compared to control positive group which was  $0.87 \pm 0.006$  ml.

These results agreed with Khayyal *et al.*, (2001) who revealed that the extracts of some plants including (*Matricaria recutita*) reduce acid output and increase mucin secretion and prostaglandin E<sub>2</sub>, but released and decreased leukotrienes. They found that these extracts were anti-ulcerogenic agents and also confirmed histologically. Whereas Brzozowski *et al.*, (2005) who reported that the volume of gastric juice and acid output/100 g body weight for 4 h reduced by aspirin and recovered by the coadministration of ginger with aspirin. The acidity of gastric juice was not significantly changed by any

treatments. Our results suggest that the changes in the volume of gastric juice and acid production induced by aspirin are not a major factor in ulcer formation or the protective effects of ginger powder seen in these experimental ulcer model rats. Also, Karbalay-Doust and Noorafshan, (2009) Revealed that oral administration of chamomile extract at 400 mg/kg can be effective in preventing gastric ulceration in mice and does not produce toxic effects in doses up to 5000 mg/kg confirmed these results. Meanwhile, Zhongzhi *et al.*, (2011) who investigated the mechanism underlying the protective effects of ginger against gastric damage induced by aspirin in rats. Gastric mucosal lesions were produced by orally administering 200 mg/kg aspirin suspended in 1% carboxymethylcellulose solution to pyloric-ligated male Wistar rats. Ginger powder (200 mg/kg) markedly reduced the aspirin-induced gastric hemorrhagic ulcer area.

### 3. Effect of water plant extracts on the pH of gastric juice in rats:

The effect of water plant extracts on the pH of gastric juice listed in table(3).

**Table (3): Effect of ginger (*Zingiber Officinale*), and chamomile (*Matricaria chamomilla*) aqueous extracts at two doses on the pH of gastric juice in rats.**

Groups	Aspirin and Extracts	Doses (mg/kg <i>b.wt.</i> )	pH of gastric juice
			Mean $\pm$ SE
Control -ve	1	-	$5.87 \pm 0.040^a$
Control +ve	2	Aspirin (Asp)	$3.44 \pm 0.012^e$
Treated Groups With Aqueous Extracts	3	(Asp) + Ginger	$4.67 \pm 0.023^c$
	4	(Asp) + Ginger	$5.78 \pm 0.040^{ab}$
	5	(Asp) + Chamomile	$4.19 \pm 0.011^d$
	6	(Asp) + Chamomile	$5.35 \pm 0.024^b$

- Values denote arithmetic means  $\pm$  standard error of the means
- Means with different letters (a, b, c, d) in the same column differ significantly at  $p \leq 0.05$ .
- Using one way ANOVA test, while those with similar letters are non-significant.

It could be noticed that oral administration of aspirin at a dose of 200mg/kg *B.wt.*, (control +ve) decreased the pH of gastric juice which was  $3.44 \pm 0.012$  compared with  $5.87 \pm 0.040$  for normal rats (control -ve). Oral administration with all aqueous extracts showed significant increase in the pH of gastric juice as compared to control positive group.

Oral administration with water extracts of ginger and chamomile at a dose of 400mg/kg *b.wt.*, for each of them showed the highest increase in the pH of gastric juice in rats when compared to control positive group which were  $5.78 \pm 0.040$ ,  $5.35 \pm 0.024$  and  $3.44 \pm 0.012$ , respectively. Meanwhile, oral administration with water extracts of ginger at a dose of 400mg/kg *B.wt.*, showed no significant change in pH as compared to control positive group (normal

rats) which were  $5.78 \pm 0.040$  and  $5.87 \pm 0.040$ , respectively.

Similar results were obtained by Al-Hindawi *et al.*, (1989) who studied some Iraqi plants including (*Matricaria chamomilla*), they found that *M. Chamomilla* didn't reflect any decrease in the pH value, so this herb has anti-inflammatory activity for healing gastric ulcer disease. Whereas, Rezaq and Elmallh, (2010) studied the increase in pH values of gastric juice of treated rats with cinnamon and chamomile aqueous extracts was more detectable with increasing the dose. Meanwhile, Abid, *et al.* (2012) studied the protection effects of Licorice and Chamomile extracts may be due to its antioxidant contain and protective effect on the lining mucosa by enhancing the protection factors. Moreover, EL-

Metwally *et al.* (2014) studied the ginger, clove and castor oils possess antiulcer potential due to its antioxidant and anti-inflammatory. The healing activity may be due to its cytoprotectivity effect coupled with anti-secretory activity.

#### 4. Effect of water plant extracts on the total acidity of gastric juice in rats:

Data listed in table (4) show the effect of ginger (*Zingiber Officinale*) and chamomile (*Matricaria chamomilla*) aqueous extracts at two doses on the total acidity of gastric juice in rats.

**Table (4): Effect of ginger (*Zingiber Officinale*), and chamomile (*Matricaria chamomilla*) aqueous extracts at two doses on the total acidity of gastric juice in rats.**

Groups	Aspirin and Extracts	Doses (mg/kg <i>b. wt.</i> )	Total acidity (%)
			Mean $\pm$ SE
Control -ve	1	-	0.19 $\pm$ 0.005 <sup>f</sup>
Control +ve	2	Aspirin (Asp)	1.47 $\pm$ 0.011 <sup>a</sup>
Treated Groups With Aqueous Extracts	3	(Asp) + Ginger	0.82 $\pm$ 0.010 <sup>c</sup>
	4	(Asp) + Ginger	0.37 $\pm$ 0.003 <sup>e</sup>
	5	(Asp) + Chamomile	1.03 $\pm$ 0.017 <sup>b</sup>
	6	(Asp) + Chamomile	0.43 $\pm$ 0.007 <sup>d</sup>

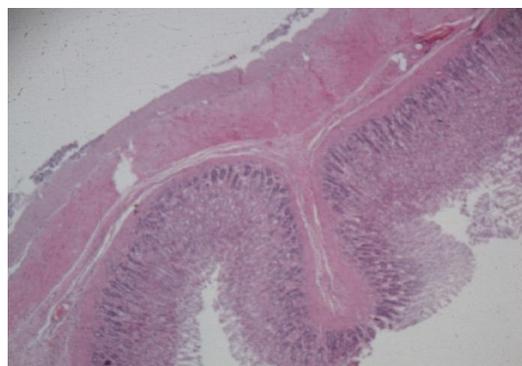
- Values denote arithmetic means  $\pm$  standard error of the means
- Means with different letters (a, b, c, d) in the same column differ significantly at  $p \leq 0.05$ .
- Using one way ANOVA test, while those with similar letters are non-significant.

It is clear that in (C +ve) group there were significant increase in the total acidity in gastric juice in rats compared to (C -ve) group which were  $1.47 \pm 0.011$  and  $0.19 \pm 0.005$  %, respectively. Ulcerated rats and orally administered with all aqueous extracts showed significant decrease in the percentage of total acidity compared to control positive group. Oral administration with water extracts of ginger at a dose of 400mg/kg *b. wt* showed the highest decrease in the percentage of total acidity in rats when compared to control positive group which were  $0.37 \pm 0.003$  and  $0.19 \pm 0.005$ %, respectively. On the other hand, oral administration of chamomile at a dose of 200mg/kg *B. wt.*, showed the lowest decrease in total acidity of gastric juice compared to control positive group which were  $1.03 \pm 0.017$  and  $1.47 \pm 0.011$ %, respectively.

These results sustained by Nanjundaiah *et al.* (2011) who concluded that the phenolic content in aqueous extract of ginger is reported to have potential ulcer preventing ability, aqueous extract of ginger will also reduce free radicals damage during ulceration. Hence, ginger is used as ulcer preventive agent. The aqueous extract of ginger was able to protect the gastric mucosa and inhibited gastric acid secretion, probably by blocking H<sup>+</sup>, K<sup>+</sup>-ATPase action (Gastric H<sup>+</sup>, K<sup>+</sup>-ATPase located in the apical membrane of parietal cells, pumps protons into the gastric lumen, using energy derived from the hydrolysis of ATP, and is thus involved in gastric acid secretion), inhibiting growth of *Helicobacter pylori*, and offering antioxidant protection against oxidative stress properties in vitro.

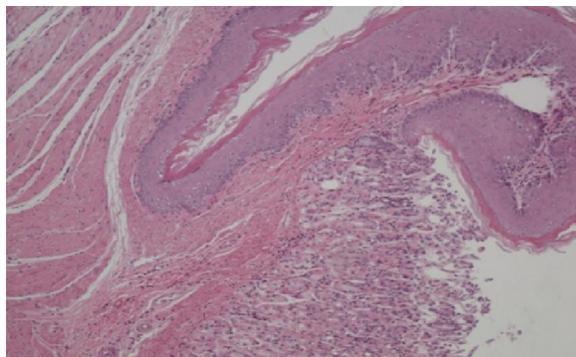
Whereas, khushtar *et al.* (2009) obtained that ginger oil has a protective action against gastric ulcer induced by aspirin plus pylorus ligation in wistar rat. The present investigation was performed in aspirin and pylorus ligation-induced ulcer model in wistar rats, in which ability of ginger oil to provide gastric protection was studied at two different doses 0.5 and 1 g/kg. gastric protection was evaluated by measuring the ulcer index, serum gamma-GTP levels, total acidity of gastric juice and gastric wall mucus thickness. Meanwhile, Jung *et al.* (2003) demonstrated that dried powder of chamomile have a large amounts of flavonoids, beta carotene, vitamin C, vitamin E and dietary fibers. All these components were useful for the stomach to treatment damage of mucosa membrane, so they can be used for healing gastric ulcer disease.

#### 5. Histopathological Evaluation:

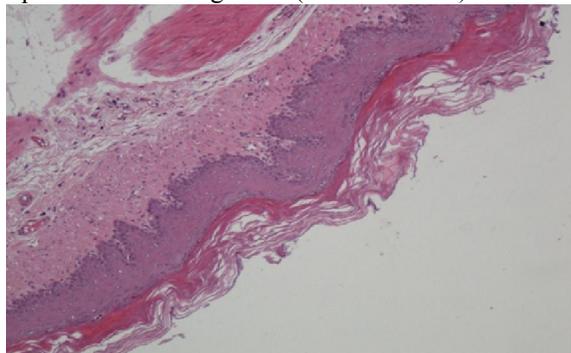


**Photo 1:** Stomach of rat from control negative group showing normal mucosal gastric glad's.

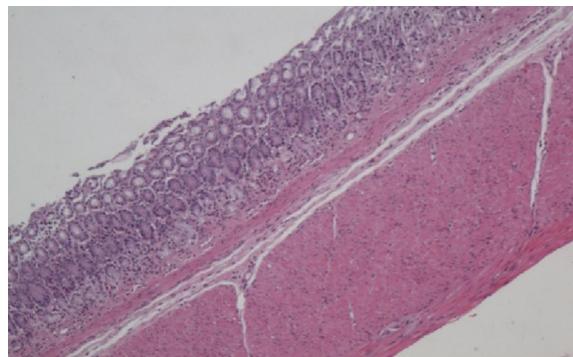
Microscopically, the stomach of rat from control negative group revealed gastro-esophageal wall showing normal mucosal gastric glad's (Photo 1). Meanwhile, the stomach of rat from control positive group showed gastro-esophageal wall the mucosal layers of the stomach was hyper plastic and associated with focal superficial necrosis gastric (photo 2). Stomach of rat from group 3 (ginger 200 mg/kg *b.wt.*) revealed gastric wall the mucosal showing partial atrophy of the mucosal glad's (photo 3). Moreover,



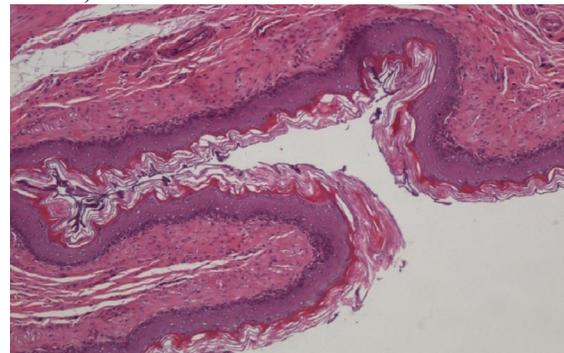
**Photo 2:** Stomach of rat from (C -ve) group showing that gastro-esophageal wall, the mucosal layers of the stomach, was hyper plastic and associated with focal superficial necrosis gastric. (H and E  $\times$  400)



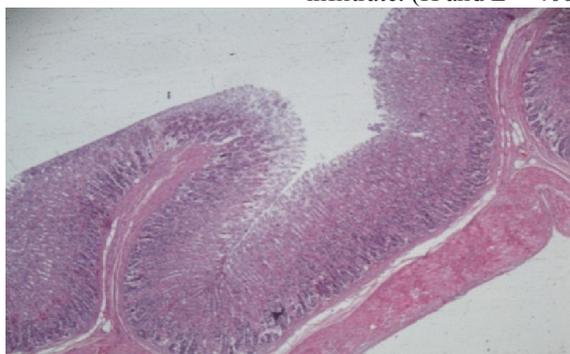
**Photo 4:** Stomach of rat from group 4 (ginger 400 mg/kg *b.wt.*) showing gastric wall with little focal superficial gastric mucosal glad necrosis and partial atrophy. (H and E  $\times$  400)



**Photo 3:** Stomach of rat from group 3 (ginger 200 mg/kg *b.wt.*) revealing gastric wall the mucosal showing partial atrophy of the mucosal glad's. (H and E  $\times$  400)



**Photo 5:** Stomach of rat from group 5 revealing gastro-esophageal wall with marked gastric mucosal glad necrosis and associated with lymphocyte cell infiltrate. (H and E  $\times$  400)



**Photo 6:** Stomach of rat from group 6 showing gastro-esophageal wall with unremarkable pathologic changes. (H and E  $\times$  400)

Stomach of rat from group 4 (ginger 400 mg/kg *b.wt.*) revealed gastric wall with little focal superficial gastric mucosal glad necrosis and partial atrophy (photo 4). Also, stomach of rat from group 5 revealed gastro-esophageal wall with marked gastric mucosal glad necrosis and associated with lymphocyte cell infiltrate (photo 5). As soon as, stomach of rat from group 6 showed gastro-esophageal wall with unremarkable pathologic changes (photo 6).

## Conclusion

Ginger and chamomile could be used for healing acute gastric ulcer disease especially ginger and chamomile water aqueous extract at doses of 400 mg/kg b.wt., for each of them.

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