Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(2):451-468

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Anti-ulcer and gastro protective effects of fenugreek, ginger and peppermint oils in experimentally induced gastric ulcer in rats

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ABSTRACT

Gastric ulcer is one of the serious problems and diseases for many of patients that cause a lot of sufferings to these patients and its cause may be either due to stress or by using of a lot of anti-inflammatory drugs or even due to higher acidity of the stomach. So the present study was extended to investigate the anti-ulcer capacities of ranitidine drug alone and in combination with fenugreek, ginger and peppermint oils on both stress and indomethacin induced gastric mucosal ulcer. Gastric mucosa was evaluated, some serum antioxidant parameters were measured and GC-Ms analysis was performed for the three oils. One hundred and ten male albino rats were group into 2 main groups one normal control group and five groups in which stress gastric ulcers were induced experimentally and five indomethacin induced gastric ulcer groups. The data indicated that both stress and indomethacin induced gastric ulcer induced oxidative stress. The results revealed that both ginger and fenugreek oils have an antiulcer and gastro protective properties. Surprisingly, the stress induced ulcer model showed that ginger and fenugreek oils were superior to ranitidine in treatment of gastric ulcer. Ginger and fenugreek oils showed a promising antioxidant effect represented by increased catalase (CAT) and Super oxide dismutase (SOD) activities and decreased malondialdehyde (MDA) values. Peppermint oil on the other hand has lower properties of being an antiulcerogenic or a gastroprotective agent despite its importance as herbal medicine with so many benefits.

Keywords: Fenugreek oil, ginger oil, peppermint oil, Indomethacin, antiulcerogenic activities.
Abbreviations: *MDA (MAlondialdhyde), SOD (Super oxide dismutase), CAT (Catalase), GR (Glutathione reductase),GPx(Glutathione peroxidase).*

INTRODUCTION

In recent times, focus on plant research has increased all over the world and large evidences have been provided to show immense potential of medicinal plants used in various traditional systems Dahanuka et al. (2002). Although there is a large number of products that have been used as antiulcerogenic, most of these products produced several side effects including arrhythmias, impotence, gynecomastia and hematopoietic changes Ariyoshi et al.(1986). Therefore, there is a need for potent and less toxic antiulcerogenic agents. Plant extracts are the most attractive source since long time and a large number of plants have been shown to produce promising antiulcerogenic effects (Alkofahi and Atta 1999).

Fenugreek (Trigonella Feonum graecum) is an herbal medicine used in many parts of the world. Its leaves are used for the cooling properties and its seeds are used for their carminative, tonic and aphrodisiac effects Chopra et al.(1982). Fenugreek is reported to have nutritive properties and stimulate digestive process. It has been used to treat a number of gastro-intestinal disorders Pandian et al.(2002). Much work have been done on the beneficial effects of fenugreek on diabetes (Tahiliani and Kar 2003) stimulatory effect on immune function Bin-Hafeez et al.(2003), anti-inflammatory Sur et al.(2001) and hypercholesterolemia states (Ravikumar and Anuradha1999). This plant is known

to contain flavenoids (Kamel and Yandav 1999), alkaloids (Jain and Madhu 1988), nicotinic acid Rajalakshmi et al.(1964) and salicylate Swain et al.(1985).

Ginger (Zingiber officinal) family Zingiberaceae has been used in traditional medicine to aid in digestion and treat stomach upset, diarrhea, nausea and arthritis for centuries. In addition to these medicinal uses, ginger continues to be valued around the world as an important cooking spice and is believed to help the common cold, flu-like syndrome, headache and even painful menstrual periods. Some researchers showed that ginger oil has dominative protective effect on DNA damage induced by H_2O_2 and might act as a scavenger of oxygen radical and could be used as antioxidant, Lu et al. (2003).

Ginger is used as spice in food and beverages and in traditional medicine as carminative, antipyretic and in the treatment of pain, rheumatism and bronchitis Afzal et al. (2001). Its extracts have been extensively studied for a broad range of biological activities including antibacterial, Azu et al.(2007), analgesic and anti-inflammatory Azu et al.(2007), antiangiogenesis and antitumor Grzanna et al.(2005). It is also used for the treatment of gastrointestinal disorders including gastric ulcerogenesis Kim et al. (2005)

Peppermint (Mentha piperita), Family Lamiaceae is an ancient spice known to Chinese, Greek and Arab physicians. In Egypt and other Middle Eastern countries, peppermint tea is customarily used as a substitute for black tea refreshing drink. Apart from its use as condiment and as a flavoring agent, various medicinal properties are attributed to this tiny spicy herb, which range from dyspepsia, flatulence, indigestion, biliousness and to check morning sickness, nausea and summer diarrhea. The oil of peppermint is also indicated for both external and internal use. The Food and Drug Administration (FDA) granted the oil of peppermint as "Generally Regarded As Safe" (GRAS) status. Recently, sixteen clinical trials (randomized double blind crossover) have been undertaken on peppermint oil in Irritable Bowel Syndrome (IBS) or recurrent abdominal pain in children and are found to be efficacious Moshen et al.(2006).

In another study, the oil exerted a spasmolytic and antispasmodic effect on the smooth vasculature of the intestinal tract (Grigoleit and Grigoleit 2005b). A multi herbal formulation, in which Mentha piperita is one of the ingredients, has shown antiulcerogenic property. Recently peppermint has been found to exhibit pronounced anti-oxidative activity Capecka et al. (2005). There is a great controversy regarding the consumption of spices. It is said that the use of spices leads to gastric derangement and even ulceration; on the other hand, when such situations occur people use certain spices or plants in order to relieve their discomfort.

So, The present study was carried out to investigate the antiulcer and gastroprotective properties of oils of fenugreek, ginger and Peppermint , identify their chemical constituents by GC-Ms analysis and to determine the suitable dose for each oil as a protective agent against gastric ulcers, on both stress and indomethacin induced ulcer models in rats and to determine their effects on some important serum antioxidant enzymes such as catalase, superoxide dismutase and malondialdehyde as indicator of oxidative stress.

EXPERIMENTAL SECTION

2.1 Chemicals and tested compounds:

Indomethacin (Liometacen) ® each ampoule contains 50 mg indomethacin (The NILE Co. for Pharm. & Chemical Ind.). The dose for rat was 18 mg/kg b.wt orally given once a day for 3 successive days to induce ulcer (Paget and Parnes, 1964).

Ranitidine (Zantac) ® each ampoule contains 50 mg ranitidine (Glaxo Smith Kline Industries.). The dose for rat was 2.7 mg/ 100 gm (0.12ml/100 gm) given orally at once Abdel Aal et al.(2002).

2.2 Methods of oil extraction:

The oils of fenugreek, ginger and peppermint were prepared in the faculty of science, Zoology department, Zagazig university by distillation of 400 g of each plant after grinding by using electrical mixer and then the plant powder was soaked in methanol for 48 hours and then we take the filtrate and obtain the oil by using distillation instrument with heat supply (Heater) for several hours for each oil and then the oil extracts were sent to Department of Pharmacognsy, Faculty of Pharmacy, Zagazig University to assure their identity and purity.

2.2.1 Fenugreek oil: Fresh fenugreek seeds were obtained from local market (Cairo, Egypt), washed, homogenized and then we extracted its oil by distillatory instrument for 8 hours and it was daily freshly prepared and it was administered at doses 1.0, 2.0ml/kg body weight of rats orally by stomach tube (0.1 ml/ 100 gm rat) Mi-Hyun et al.(2005).

2.2.2 Ginger oil: Fresh ginger plant (rhizomes) were obtained from local market (Cairo, Egypt), washed, homogenized and then we extracted it's oil by distillatory instrument for 10 hours and it was daily freshly prepared and it was administered at dose 3.3, 4.3 mg/100 gm rat orally by stomach tube Helmy et al. (2011).

2.2.3 Peppermint oil: Fresh Peppermint plant were obtained from local market (Cairo, Egypt), washed, homogenized and then we extracted it's oil by distillatory instrument for 6 hours and it was daily freshly prepared and it was administered at dose 0.81, 0.91mg/100 mg orally by stomach tube (Barclay, 2007).

2.3 Animals

One hundered and ten adult male albino rats weighting 100-120 gm were used in this experiment, they were kept under hygienic condition and fed on barley, milk and water was provided ad lib. After one week of acclimatization, animals were allocated into 11 equal groups, each of 10 animals.

Experimental design:

2.3.1 Induction of stress:

Stress-induced ulcer was made by restraint (immobilization) Abdel Al-Aziz et al.(1996). Animals in groups: 2nd group (Stress), 3rd group (Ranitidine), 4th group (Fenugrek oil), 5th group (Ginger oil) and 6th group (Peppermint oil) were fasted for 12 hr, but allowed water ad-lib overnight. Immediately after 12hr fasting period rats of third group were orally administered a dose of ranitidine as previously described, rats of the fourth group were orally administered fenugreek oil while rats of fifth and sixth groups were given ginger and peppermint oils orally respectively as previously described. After 1 hour, rats were immobilized in supine position on a wooden board for 24 hrs. The four limbs of the rat were fixed at the 4 corners of the wooden board in a manner sufficient to prevent animal from turning or wedging itself, without hindrance of respiration. After 24 hr immobilization, the rats were sacrificed by slaughtering.

2.3.2 The first experiment (Stress- induced ulcer):

The 1st group (Control group): was considered as control and received only saline 1ml/100 gm.
Other rats in this experiment were allocated into 5 equal groups, each of 10 animals as follows.

The 2nd group (Stress- induced ulcer):- Rats in this group were exposed to immobilization stress for ulcer induction.

The 3rd group (Ranitidine +Stress group): Rats in this group received ranitidine orally at dose of 2.7 mg/100gm one hour before induction of ulcer by immobilization stress.

The 4th group (Fenugreek oil (0.1, 0.2 mg/kg) + Stress group): Rats in this group orally received fenugreek oil at its two doses at dose of 0.1, 0.2 mg/100 gm one hour before induction of ulcer by immobilization stress.

The 5th group (Ginger oil (3.3, 4.3 mg/100 gm) +Stress group): Rats in this group orally received ginger oil at its two doses of 3.3, 4.3 mg/100 gm one hour before induction of ulcer by immobilization stress.

The 6th group (Peppermint oil (0.81, 0.91 mg/100 gm) +Stress group): Rats in this group orally received peppermint oil at its two doses of 0.81, 0.91 mg/100 gm one hour before induction of ulcer by immobilization stress.

The ulcer score was calculated according to the 1 to 5 scoring system table.1 Brodie et al.(1962). As in the following

2.3.3 Second experiment (Indomethacin-induced ulcer):

Rats in this experiment were allocated into 5 equal groups and will receive oral doses of indomethacin for induction of ulcer, each of 10 animals as follows.

The 7th group (Indomethacin- induced ulcer): Rats in this group orally received indomethacin for 3 days for induction of ulcer.

The 8th group (Ranitidine+Indomethacin group): Rats in this group were orally given ranitidine orally during 3 day of ulcer induction by indomethacin.

The 9thgroup (Fenugreek oil (0.1,0.2 mg/100 gm)+Indomethacin group): Rats in this group were orally given fenugreek oil at its two doses at dose of 0.1, 0.2 mg/100 gm during 3 day of ulcer induction by indomethacin.

The 10th group (Ginger oil (3.3, 4.3 mg/100 gm) +Indomethacin group): Rats in this group were orally given ginger oil at its two doses of 3.3, 4.3 mg/100 gm during 3 day of ulcer induction by indomethacin.

The 11thgroup (Peppermint oil (0.81, 0.91 mg/100gm) +Indomethacin group): Rats in this group were orally given peppermint oil at its two doses of 0.81, 0.91 mg/100 gm during 3 day of ulcer induction by indomethacin.

3.2.4 Examination of stomach for gastric ulcers:
Four hours after the last dose of indomethacin and other drugs used in this experiment, the animals were sacrificed by slaughtering. The abdominal cavity of each animal was opened and the stomach was removed, opened along the greater curvature, followed by gentle wash with water to remove gastric contents and examined for ulceration as described previously The number and severity of discrete areas of damage in glandular mucosa were scored by 3 trained independent observers unaware of the drug treatment.

3.2.5 Blood samples collection:
Blood samples were collected in centrifuge tubes, left to clot for serum separation, centrifuged at 3000 r.p.m. for 15 minutes and the clear non-hemolysed sera was used for assay of antioxidants activity: CAT (Radwan and West ,1971), SOD (Radwan and Ghaleb ,1974)as well as determination of malondialdehyde level concentrations (Aebi,1984).

3.2.6 GC–MS analysis:
Determination of constituents of ginger, Fenugrek and Peppermint oil by GC/MS Ginger oil were analyzed chemically by gas chromatography and mass spectrometry (GC/MS; Model CP-3800-1200L Quadrupole MS/MS). Briefly, compounds were separated on a Factor Four capillary column VF-5ms (30 m × 2.25 mm ;0.25μm) using the carrier gas helium (0.7 ml/min). The injector temperature was 250 °C and the column temperature was maintained at 40 °C for 5 min and then programmed at 4 °C/min to 250 °C. The spectrometers were operated in electronionization (EI) mode at 70 eV ionization energy; the scan range was 35–400 amu. The detector was set as fixed voltage at 1200 V and the scan rate was 0.5 s per scan. The ionization source temperature was 250 °C. The identification of the major compounds was performed by comparing their mass spectra with the Wiley Registery of Mass spectra 8th edition library available in the instrument and confirmed by comparing with standards.

3.2.7 Statistical analysis:
The obtained data were analyzed using the statistical package for social science (SPSS, 15.0) for obtaining means and standard error. The total variation was analyzed by performing the statistical design T-test. Probability levels of less than 0.05 were considered significant (Snedecor and Cochran, 1982).

RESULTS AND DISCUSSION

3.1 Stress-induced ulcer:
Peptic ulcer is one of the major gastrointestinal disorders, which occur due to imbalance between the offensive (gastric acid secretion) and defensive (gastric mucosal integrity) factors Nishikimi et al.(1972). Although a number of antiulcer drugs such as anti-acids, H2 receptor antagonists, proton pump inhibitors and cytoprotectives are available for treatment of ulcer, all these drugs have side effects Esterbauer et al.(1982). Natural products are well known as a promising source for the discovery of major new pharmaceuticals. Many natural products are used in folk medicine for treatment of gastric ulcer.

Immobilization of rats for 24 hr Fig.1 and Table. 2 resulted in high incidence of ulceration (100%), extensive number of gastric mucosal lesions (4.2± 0.20) and high ulcer index (420) in the glandular segments of the stomach as indicated in table.1 (categories of ulcer score). These results may be attributed to physical or psychological stress which is one of the common causes of upper gastrointestinal ulceration (Hoogerwerf and Pasricha 2006). Although the pathogenesis of gastric lesions is not completely understood, the production of oxygen free radicals via the xanthine-xanthine oxidase system and neutrophils and lipid peroxidation initiated by the produced reactive oxygen species (ROS) have been investigated to explain the mechanisms of acute gastric lesion formation associated with stress Kumar et al.(2011). Immobilization stress induced ulcer within 24 hrs in glandular area of rat's stomach which was in agreement with Jiang et al. (2005).

Ginger oil treated group with dose 3.3mg/100gm showed the lowest ulcer score amongst all groups undergoing stress (2.0±0.21) with the lowest ulcer index (200) in the glandular segments of the stomach with a preventive index of 52.38% as indicated in table.2 and fig.2. Fenugreek oil administration was the second best herbal antiulcer agent used in this experiment recording (2.2±0.20) mean ulcer score, (220) ulcer index and preventive index of 47.62% as recorded in table.2 and fig.2. Our reference drug amazingly showed a less potency than ginger and fenugreek oils, ranitidine oral administration in its therapeutic dose revealed a relatively high mean ulcer score (2.6±0.40), with a relative high ulcer index (260) and its preventive index was 38.15% which is lower than that of ginger and fenugreek oils. Peppermint oil was the worst herbal used when compared with other medicinal compounds used, the

mean ulcer score was (3.0±0.54), ulcer index (300) and the preventive index was 28.57% as indicated in table.2 and fig.2.

Ginger has been used as an ingredient of Chinese traditional stomach medicines for thousands of years. The anti-ulcerative effects of ginger have previously been investigated in experimental gastric ulcer models Khushtar et al.(2009). However, the mechanism underlying the protective effects of ginger against gastric damage is unclear. In the present study, the anti-ulcerative effects of ginger powder were investigated in stress-induced gastric ulcer model rats. Stress and other chemicals have been reported to reduce the gastric juice pH and increase the volume of gastric juice (Wang et al., 2007), or decrease the volume of gastric juice and its acid output Jainu et al.(2006). In the present study, the volume of gastric juice and acid output/100 g body weight for 4 h reduced by induction of stress and recovered by the co administration of ginger after stress induction. 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 stress are not a major factor in ulcer formation and revealed the protective effects of ginger powder seen in these experimental ulcer model rats .The obtained data about ginger oil were in agreement with (El-Nabtity, 1996) who suggested that zingiberene, terpenoid and 6-gingerol are important constituents in stomach medications containing ginger and these compounds have been identified in our ginger samples.

Ginger contains a number of pungent constituents and active ingredients. Steam distillation of powdered ginger produces ginger oil, which contains a high proportion of sesquiterpene hydrocarbons, predominantly zingiberene (Mustafa et al., 2001). The major pungent compounds in ginger, from studies of the lipophilic rhizome extracts, have yielded potentially active gingerols which can be converted to shogoals, zingerone and paradol as previously reported by Yamahara et al.(1988).

These results were reinforced recently by (Govindarajan ,1982) who mentioned that these compounds are some of the extensively studied phytochemicals and account for the antioxidant, anti-inflammatory, antiemetic, and gastroprotective activities.

Another study carried out by Baliga et al.(2011) who suggested that ginger performs the antiulcer effect by eliminating the Helicobacter pylori bacteria whose secretions of ammonia in the stomach are responsible for many ulcers, especially of the duodenum and also for other stomach problems like gastritis. The plant also neutralizes the excess gastric acid in the stomach which causes other forms of ulcers.

Cells in general contain a large number of antioxidants to prevent or repair the damage caused by ROS. Reactive oxygen species are produced in many aerobic cellular metabolic processes; they include various intracellular targets including lipids, proteins and DNA Cerutti et al.(1985). Although ROS generated during normal aerobic metabolism, the biological effect of ROS on these intracellular targets are dependent on their concentration and increased levels of these species are present during oxidative stress. The increased levels of ROS are cytotoxic while lower levels are necessary for the regulation of several key physiological mechanisms. However, increased levels can also result in ROS induced damage including cell death, mutations, chromosomal aberrations and carcinogenesis.

Stress induced ulcer groups showed a significant decrease in catalase and superoxide dismutase activities with a significant increase in malondialdehyde concentration as reported in table .3. The decreased activities of catalase, superoxide dismutase may be due to exhaustion of these enzymes in catalyzing the overproduction of free radicals due to exposure to oxidative stress which induce lipid peroxidation that causes malondialdehyde formation, that was in agreement with Tandon et al.(2004) who stated that there were an increase in rat gastric mucosal lipid peroxidation and a decrease in catalase level in cold restraint stress-induced gastric ulceration, and in clinical peptic ulceration an increase in serum lipid peroxidation and a tendency to decrease in catalase and superoxide dismutase levels were observed.

Concerning the results obtained about ginger oil administration as a pretreatment before induction of ulcers in rats, a significant increase in catalase and superoxide dismutase enzymes and a significant decrease in MDA values when compared with those observed in stress induced ulcer group as shown in table.3 and fig.3. The antioxidant effects of ginger may modulate the oxidative stress. A similar data were obtained by Kondeti et al.(2010)who found that SOD, CAT, glutathione peroxidase (GPx) and glutathione reductase (GR) parameters were increased in experimentally diabetic rats and MDA values were decreased.

A significant increase in CAT and SOD activities with a significant decrease in MDA levels of fenugreek oil treated rats when compared to the stress induced ulcer rats as indicated in table.3 and fig.3 and these results were confirmed by Sur et al.(2001)who found that aqueous extract of fenugreek oil showed a significant ulcer protective effects. The cytoprotective effect of the seeds seemed to be not only due to the anti-secretory action but also due to the effects on mucosal glycoproteins. The fenugreek seeds also prevented the rise in lipid peroxidation induced by ethanol presumably by enhancing antioxidant potential of the gastric mucosa thereby lowering mucosal injury. These observations show that fenugreek seeds possess antiulcer potential.

3.2 Indomethacin-induced ulcer:

Oral administration of indomethacin (18mg/kg b.wt.) fig.4,5 and table.4 for three consecutive days resulted in high incidence of gastric ulceration (100%), mean ulcer score (3.0±0.27) and high ulcer index (300). These results were confirmed by De-Barros et al.(2007) and Dursu et al.(2009 a) who stated that indomethacin caused several gastric lesions.

Ranitidine administration in its therapeutic schedule orally for 3 consecutive days exhibited superior results in treatment of gastric ulcer induced by indomethacin when compared with any other drug used as indicated in table.4 and fig.4,5. Our reference drug showed 80% incidence of gastric ulceration, mean ulcer score and this was the lowest value of incidence of gastric ulceration among all tested drugs (1.0±0.32) as well as ulcer index (80). Preventive index score recorded the highest levels compared to other drugs (66.66%). These obtained data are confirmed recently by Sakat et al.(2012) who stated that ranitidine is a gastroprotective and antiulcer drug after using it as a standard agent for evaluation of gastroprotective activity of Oxalis corniculata.

Indomethacin administration elicited a significant decrease in catalase and superoxide dismutase activities with a significant increase in malondialdehyde level in rat's serum (table.5 and fig.6) when compared to normal group. This was in agreement with Dursu et al.(2009b) who mentioned that MDA (malondialdehyde) and MPO (myeloperoxidase) were significantly increased resulting from indomethacin application. They stated that indomethacin causes gastric damage by not only inhibiting cyto-protective PG synthesis but also by affecting oxidant and antioxidant mechanisms. Experimental studies showed that the catalase activity decreases were due to the indomethacin-induced stomach damage Basivireddy et al. (2003). Also, a study carried out by Motawi et al. (2008) stated a reduction in superoxide dismutase activity post indomethacin administration in rat.

Pretreatment of rats with ranitidine in its therapeutic dose for 3 successive days induced a significant increase in the activity of catalase and superoxide dismutase while malondialdehyde levels decreased significantly compared to indomethacin-induced ulcer group. In the same line, Sathish et al.(2011) found a significant decrease in MDA values in ranitidine treated rats when compared with either aspirin or absolute alcohol induced ulcer rats.

Ginger oil treatment during indomethacin administration (18mg/kg b.wt.) for 3 consecutive days induced a significant decrease in mean ulcer scores (1.4±0.24), a significant decrease in ulcer index (140) with a significant increase in the preventive index (53.33%) as reported in table.4 and fig.4,5.

Oral administration of fenugreek oil produced gastroprotective effect in indomethacin-treated group; the protective effect was the same as in ginger oil treated group. There were no significant changes in lesion score of ranitidine treated group and both of ginger and fenugreek oils. Peppermint oil administration reduced mean ulcer score when compared with rats received only indomethacin but it was less effective than other drugs used as indicated in table.4 and fig.4, 5.

Fenugreek oil administration resulted in significant increase in the values of catalase and superoxide dismutase but the values of malondialdehyde were significantly decreased when compared with indomethacin induced ulcer group. Our obtained results strongly confirmed with (Radwan and Ghaleb 1974) who showed a significant increase in serum GSP and SOD values and a significant decrease in MDA values in rats treated with fenugreek oil, extract and powder when compared with rats received aspegic drug that cause gastric mucosal damage as indicated in table.5 and fig.6. In recent studies, fenugreek had received much scientific attention as a potential source of antioxidant properties that could be linked to its gastroprotective effect. It appears to influence free radicals formation and associated with increased antioxidant enzyme activities Kaviarasan et al.(2007). Aqueous extract of fenugreek seeds possess significant antioxidant activities in-vitro (Anuradha and Ravikumar1998).

Ginger oil administration resulted in significant increase in the values of catalase and superoxide dismutase but the values of malondialdehyde were significantly decreased when compared with indomethacin induced ulcer group as indicated in table.5 and fig. 6. A study carried out by Cao et al. (1993) strongly confirmed our results as the authors suggested that the decline in SOD may be a response to increased production of H_2O_2 and O_2^* or the products of its

decomposition. After ginger administration the SOD activity elevated as its antioxidant compounds like gingerols, shogoals and ketone compounds were responsible for scavenging the superoxide anion radicals.

Our results are reinforced by Yamahara et al., 1989; Huang et al., 1991 who carried out an experimental study on isolated Guinea pig ileum, several compounds in ginger (e.g., [6]-gingerol, [6]-shogaol, and galanolactone) have been shown to have anti- serotonin (5-hydroxytryptamine) effects and this may possibly suggest that the anti-emetic action of either ginger or some of its constituents may be mediated centrally via 5-HT3 receptors, as these constituents have small molecular weights and could easily cross the blood brain barrier and this compound (6-gingerol) is indicated in our table .6 and Fig. 7in the GC-Ms analysis of Ginger oil .

Parallel to our results, (Sharma et al., 1997) who indicated that Cisplatin treatment causes nausea and vomiting in man and animals. Acetone and 50% ethanolic extracts of ginger at oral doses of 25, 50, 100 and 200 mg/kg exhibited significant protection, while aqueous extract at these doses was ineffective against cisplatin emesis in dogs, and rats (Sharma and Gupta, 1998).but in contrary to our results Ernst and Pittler (2000) reviewed the evidence for the usefulness of ginger against nausea and vomiting from six clinical studies. Three on post-operative nausea and vomiting were identified and two of these suggested that ginger was superior to placebo and equally effective as metoclopramide.

Confirming to our results ,Mahady et al. (2003) were the first to provide evidence that the active constituents of ginger (gingerols) are effective in vitro against Helicobacter pylori, the primary etiological factor associated with dyspepsia, peptic ulcer disease and development of gastric and colon cancer. This was further confirmed by Mahady et al. (2005) and Nostro et al. (2006). O'Mahony et al. (2005) tested the bactericidal and anti adhesive properties of ginger and several other culinary and medicinal plants against H. pylori and found that ginger was highly effective in killing H. pylori, but had lesser ability in inhibiting the adhesion of this bacterium to stomach sections.

More recently, Siddaraju and Dharmesh, (2007) reported that ginger-free phenolic and hydrolyzed phenolic fractions of ginger were both potent inhibitors of gastric cell proton potassium ATPase activity and H. pylori growth, and suggested that the two fractions could be inexpensive multistep blockers against ulcer.

Polyphenolic compounds represent a highly diverse class of secondary metabolites distributed widely in the plant kingdom. Recently, they are a subject of considerable scientific and therapeutic interest mainly due to their antioxidant properties and related health promoting benefits. The evidences strongly support the contribution of polyphenols in the prevention of cardiovascular diseases, cancers, osteoporosis, neurodegenerative diseases, and diabetes mellitus. They exhibit several biological activities in the gastroprotective area, including anti-secretory, cytoprotective, and antioxidant actions. Zingiber officinal possessing polyphenolic compounds that have shown gastroprotective and antiulcer properties as indicated in table. 6 and fig.7.

These polyphenolic compounds protect the gastrointestinal mucosa from lesions produced by various experimental ulcer models, against different necrotic agents. Moreover, these can be utilized as an alternative or an additive agent to the current therapy. Therefore, our obtained data about ginger oil as an antiulcer and gastroprotective agent strongly go hand to hand with Sabiha et al.(2011). Ginger has a more effective and less toxic therapeutic potential for the treatment of peptic ulcers. In the same line, Zhangzhi et al.(2011) suggested that ginger can protect the stomach against ulcer formation induced by aspirin by reducing inducible nitric oxide synthetase (iNOS) activity in the gastric mucosa and inflammatory cytokine (TNF-α and IL-1β). These effects of ginger seem to be derived from the action of gingerols and shogaols, the main ingredients of ginger.

Fenugreek oil is a waxy liquid pressed or distilled from the seeds of Trigonella feonum graecum. It was used for centuries across Asia and the Mediterranean area. This essential oil is rich in phytic acid, saponins and trigonelline as indicated in our results and identified compunds. These nutrients along with antioxidants endow fenugreek oil the power to fight viruses, cancers and free radicals which lead to aging as previously reported by Sauvare et al.(2000).
The incidence of gastric ulceration, mean ulcer score and ulcer index were significantly decreased in group treated with fenugreek oil in dose 0.1 mg/100 gm and these results were more effective than group treated with a dose of 0.2ml/100gm and there was a significant increase of the preventive index. These results were similar to Atta et al.(2005) who investigated the effect of fenugreek oil, seeds and powder in experimentally induced ulcer in rats and found a significant improvement in the volume of gastric juice, total acidity of gastric juice and lesion score and this may be attributed due to its chemical constituents as reported in table. 8 and fig.8.

There were no significant improvements in peppermint oil treated groups either with a dose of 0.81, 0.91 mg/100gm when compared with control group. The incidence of gastric ulceration, mean ulcer score and ulcer index remained high with low preventive index. Peppermint oil contains menthol, menthone and cineol as reported in table.7 and

fig.8 and these compounds may work by relaxing smooth muscle of gastrointestinal tract. Peppermint oil also may reduce lower esophageal sphincter pressure. It offers mild efficacy for symptoms of irritable bowl syndrome (IBS) and may improve colonic spasm (Barclay, 2007). Another study (found that Mentha microphylla (peppermint) despite it decreases the ulcer index but still not as curative as other herbs used. In coincidence with our results Ibrahim et al.(2006) showed that peppermint possesses antiulcerogenic principles which protect against gastric mucosal damage induced by indomethacin and noxious chemicals, through inhibition of basal gastric acid secretion.

Fenugreek oil was as effective as ginger oil. It resulted in significant decrease in mean ulcer score (1.6± 0.41), ulcer index (160), with high preventive index (46.66%). Similar findings that have been obtained by (Radwan and Ghaleb ,1974) who showed a significant decrease in gastric ulcer index, gastric juice and high curative index in group of rats received aspegic to induce gastric ulceration and then co treated with fenugreek oil.

Table (1): Ulcer scoring system

Lesions	*Score*
1 or 2 minute, sporadic, punctuate lesion	1
Several small lesions.	2
One extensive lesion or multiple moderate-sized lesion	3
Several large lesions.	4
Several large lesions with stomach perforation.	5

Ulcer index "U.I" (stomach ulceration) (Wilhelmi A. and Menasse).
U.I = means ulcer score of animals similarly treated X %of ulcerated animals of the group.

Preventive index "P.I." (The preventive effect of the any antiulcer agents used against the severity of ulceration)**.**

$$P.I = \frac{\text{U.I control} - \text{U.I treated}}{\text{U.I control}} \times 100$$

Table 2: Effect of oral administration of fenugreek, ginger and peppermint oils in stress induced ulcer rats by immobilization on incidence of gastric ulceration, mean ulcer score, ulcer index and preventive index

Group	*Incidence of gastric ulceration (%)*	*Mean ulcer score*	*Ulcer index*	*Preventive index (%)*
Stress induced ulcer G2	100	4.2 ± 0.20[a]	420	zero
Ranitidine + Stress treated group	100	2.6 ± 0.40[b]	260	38.15
Fenugrek oil (0.1 mg/100gm) + Stress treated group	100	2.2 ± 0.20 [b]	220	47.62
Fenugrek oil (0.2 mg/100gm) + Stress treated group	100	2.4 ± 0.10 [b]	240	44.32
Ginger oil (3.3 mg/100 gm) + Stress treated group	100	2.0± 0.21 [b]	200	52.38
Ginger oil (4.3 mg/100 gm) + Stress treated group	100	2.2± 0.11 [b]	220	50.42
Peppermint oil (0.81 mg/100 gm) + Stress treated group	100	3.0± 0.54 [ab]	300	28.57
Peppermint oil (0.81 mg/100 gm) + Stress treated group	100	3.4± 0.51 [ab]	330	30.11

Means within the same column carrying different superscripts are significant at P≤0.05 (M±SE) (n=10).

Table 3: Effect of oral administration of fenugreek, ginger and peppermint oils in stress induced ulcer rats by immobilization on catalase activity, super oxide dismutase activity and malondialdehyde concentrations

Group	Catalase activity(U/L)	Super oxide dismutase Activity(U/ml)	Malondialdehyde concentrations nmol/ml
Control group	549 ±37.6 [bc]	126.30±12.08 [bc]	26.85±1.25 [b]
Stress induced ulcer G2	339 ±31.3 [d]	85.92±7.76[d]	40.68±1.16 [a]
Ranitidine + Stress treated group	666 ±39.5 [b]	133.18±9.87[bc]	16.50±0.98 [d]
Fenugrek oil (0.1 mg/100gm) + Stress treated group	953±58.6 [a]	203.14±21.34[a]	17.94±1.07 [d]
Fenugrek oil (0.2 mg/100gm) + Stress treated group	933±45.4 [a]	200.01±20.34[a]	19.65±1.00 [d]
Ginger oil (3.3 mg/100 gm) + Stress treated group	815±48.8 [ab]	171.54±13.21 [ab]	18.10±0.88 [d]
Ginger oil (4.3 mg/100 gm) + Stress treated group	790±38.2 [ab]	145.22±14.11 [ab]	20.10±0.68 [d]
Peppermint oil (0.81 mg/100 gm) + Stress treated group	669±43.3 [b]	131.60±11.40 [bc]	22.20±0.96 [c]
Peppermint oil (0.91 mg/100 gm) + Stress treated group	680.09±11.3 [b]	141.90±14.00 [bc]	20.20±0.38 [c]

(n=10). *Means within the same column carrying different superscripts are significant at P≤0.05, (M ±SE)*

Table 4: Effect of oral administration of fenugreek, ginger and peppermint oils during 3 day ulcer induction by indomethacin in rats on incidence of gastric ulceration, mean ulcer score, ulcer index and preventive index

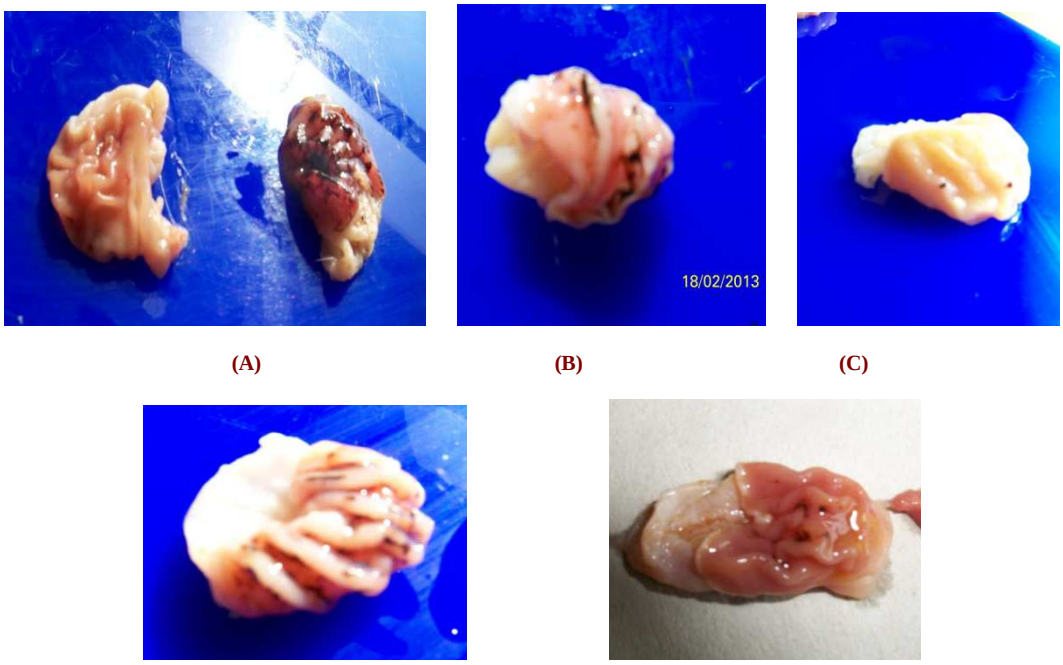
Group	*Incidence of gastric ulceration (%)*	*Mean ulcer score*	*Ulcer index*	*Preventive index (%)*
Indomethacin induced ulcer G7	100	3.0± 0.31 [a]	300	zero
Ranitidine+Indomethacin treated group	80	1.0± 0.32 [c]	80	66.66
Fenugrek oil (0.1 mg/100gm)+Indomethacin treated group	100	1.6± 0.41 [bc]	160	46.66
Fenugrek oil (0.2 mg/100gm)+Indomethacin treated group	100	1.9± 0.21 [bc]	140	41.11
Ginger oil (3.3 mg/100 gm)+Indomethacin treated group	100	1.4± 0.24 [bc]	140	53.33
Ginger oil (4.3 mg/100 gm)+Indomethacin treated group	100	1.7± 0.31 [bc]	170	48.10
Peppermint oil (0.81 mg/100 gm)+Indomethacin treated group	100	2.0± 0.31 [b]	200	33.33
Peppermint oil (0.91 mg/100 gm)+Indomethacin treated group	100	2.4± 0.21 [b]	240	37.98

(n=10). Means within the same column carrying different superscripts are significant at P≤0.05 (M ±SE)

Table 5: Effect of oral administration of fenugreek, ginger and peppermint oils during 3 day ulcer induction by indomethacin in rats on catalase activity, super oxide dismutase activity and malondialdehyde concentrations

Group	*Catalase activity (U/L)*	*Superoxide dismutase (SOD)(U/ml)*	*Malondialdhyde (MDA)(nmol/ml)*
Stress induced ulcer G2	418±21.2 [d]	70.10±6.5 [d]	60.60±1.58 [a]
Ranitidine + Stress treated group	618±45.5 [bc]	157.76±11.34 [b]	16.30±1.54 [b]
Fenugrek oil (0.1 mg/100gm) + Stress treated group	833±40.6 [a]	242.50±20.14 [a]	18.64±1.05 [b]
Fenugrek oil (0.2 mg/100gm) + Stress treated group	820±37.3 [a]	239.20±18.01 [a]	20.32±1.01 [b]
Ginger oil (3.3 mg/100 gm) + Stress treated group	859±54.2 [a]	199.22±12.67 [ab]	18.08±1.67 [b]
Ginger oil (4.3 mg/100 gm) + Stress treated group	827±34.1 [a]	190.11±10.22 [ab]	20.06±1.11 [b]
Peppermint oil (0.81 mg/100 gm) + Stress treated group	719±31.2 [b]	115.84±6.46 [c]	21.14±1.33 [b]
Peppermint oil (0.91 mg/100 gm) + Stress treated group	732±29.4 [b]	119.66±4.26 [c]	24.06±1.11 [b]

Means within the same column carrying different superscripts are significant at P≤0.05.



Fig(1): (A) Significant lesions in stress induced ulcer group (G2) compared to control (G1) with severe ulceration in the glandular segments. (B) Stress induced ulcer, treated with ranitidine (G3). (C) Stress induced ulcer, treated with fenugreek oil (G4). (D) Stress induced ulcer, treated with ginger oil (G5). (E) Stress induced ulcer, treated with peppermint oil (G6)

Table 6: Identification of chemical components in the essential oils of ginger

No.	Retention time (min)	Compounds	Retention indices	Relative contents	(%)	R.S.D. of MD–SPME (%)
				MD–SPME	SPME	
1	8.60	2-Heptanone	890	0.11	ND	3.3
2	8.89	2-Heptanol	902	0.21	0.11	3.6
3	9.49	Tricyclene	913	0.14	ND	5.8
4	9.66	Thujene	928	0.02	ND	3.6
5	9.85	α-Pinene	941	2.13	0.41	4.6
6	10.29	Camphene	953	7.30	1.70	4.7
7	11.09	α-Pinene	980	0.26	0.05	2.4
8	11.39	6-Methyl-5-hepten-2-one	987	3.52	1.68	6.3
9	11.50	α-Myrcene	991	1.56	0.27	7.4
10	11.55	2-Methyl-6-hepten-1-ol	994	0.74	0.39	3.8
11	11.87	α-Phellandrene	998	1.11	0.27	4.6
12	12.03	Octanal	1001	0.04	ND	3.1
13	12.21	Terpinene	1020	0.09	ND	6.2
14	12.44	*p*-Cymene	1026	0.19	0.05	4.4
15	12.61	α-Phellandrene	1030	22.84	ND	5.8
16	13.07	α-Ocimene	1040	0.03	ND	3.9
17	13.32	Terpinene	1052	0.33	0.12	2.7
18	14.17	Linalool	1088	0.94	0.39	4.7
19	14.23	2-Nonanone	1104	0.24	ND	5.4
20	14.46	*p*-Meth-2-en-1-ol	1124	1.17	0.94	5.8
21	15.68	Camphor	1144	0.82	0.48	6.7
22	15.78	Camphene hydrate	1150	0.11	0.35	5.2
23	16.00	Isoborneol	1163	0.12	0.09	7.3
24	16.25	Borneol	1167	4.81	4.91	5.2
25	16.50	Terpinen-4-ol	1177	0.53	0.28	3.7
26	16.84	α-Terpineol	1189	1.92	ND	5.4
27	16.99	2-Thujenal	1208	0.23	0.23	4.1
28	17.69	Nerol	1230	1.47	1.47	6.3
29	18.00	6-shogaol	1236	1.78	1.45	7.10
30	18.03	Neral	1244	2.03	ND	7.4
31	18.33	Geraniol	1253	1.75	1.70	6.7
32	18.75	Geranial	1273	5.25	7.96	5.7
33	19.13	Bornyl acetate	1288	0.86	0.79	3.5
34	19.21	2-Undecanone	1294	0.41	0.32	5.5
35	19.72	2-Methoxy-4-vinylphenol	1314	0.06	0.06	8.3
36	20.59	β-Cubebene	1357	0.05	ND	6.3
37	21.03	Cycloisosativene	1370	0.43	0.52	6.5
38	21.22	Copaene	1381	0.91	1.23	5.8
39	21.54	α-Elemene	1395	0.66	1.14	2.4
40	21.78	α-Caryophellene	1420	0.28	0.33	6.4
41	22.43	α-Elemene	1433	0.44	0.55	5.7
42	22.81	α-Farnesene	1460	0.22	0.31	4.3
43	23.09	Gurjuene	1475	0.19	ND	4.3
44	23.36	Germacrene D	1480	0.64	ND	3.7
45	23.43	Curcumene	1482	3.59	5.13	4.4
46	23.49	Valencene	1493	0.86	1.95	7.4
47	23.72	Zingiberene	1498	15.48	26.27	2.4
48	23.86	Qq`1-Bisabololene	1508	2.54	4.78	2.6
49	23.55	-Farnesene	1510	2.51	4.19	7.3
50	24.09	(+)-Epi-bicyclosesquiphell-andrene	1521	0.16	0.25	5.5
51	24.23	β-Sesquiphellandrene	1526	5.54	8.19	6.3
52	24.36	Germacrene B	1557	0.19	ND	5.8
53	24.69	Zingiberenol	1583	0.14	0.14	3.5
54	24.90	Eudesmol	1614	0.07	ND	6.3

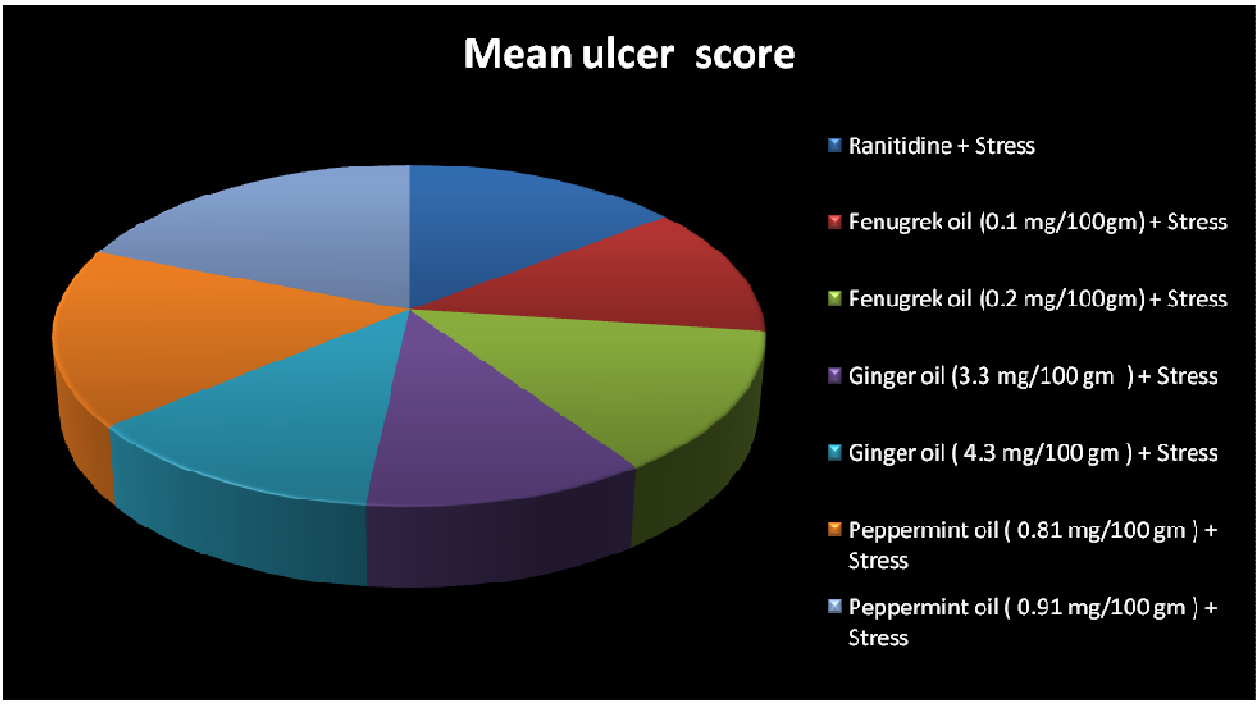
ND: Not detected

Table 7 Chemical composition of Peppermint oil by GC–MS

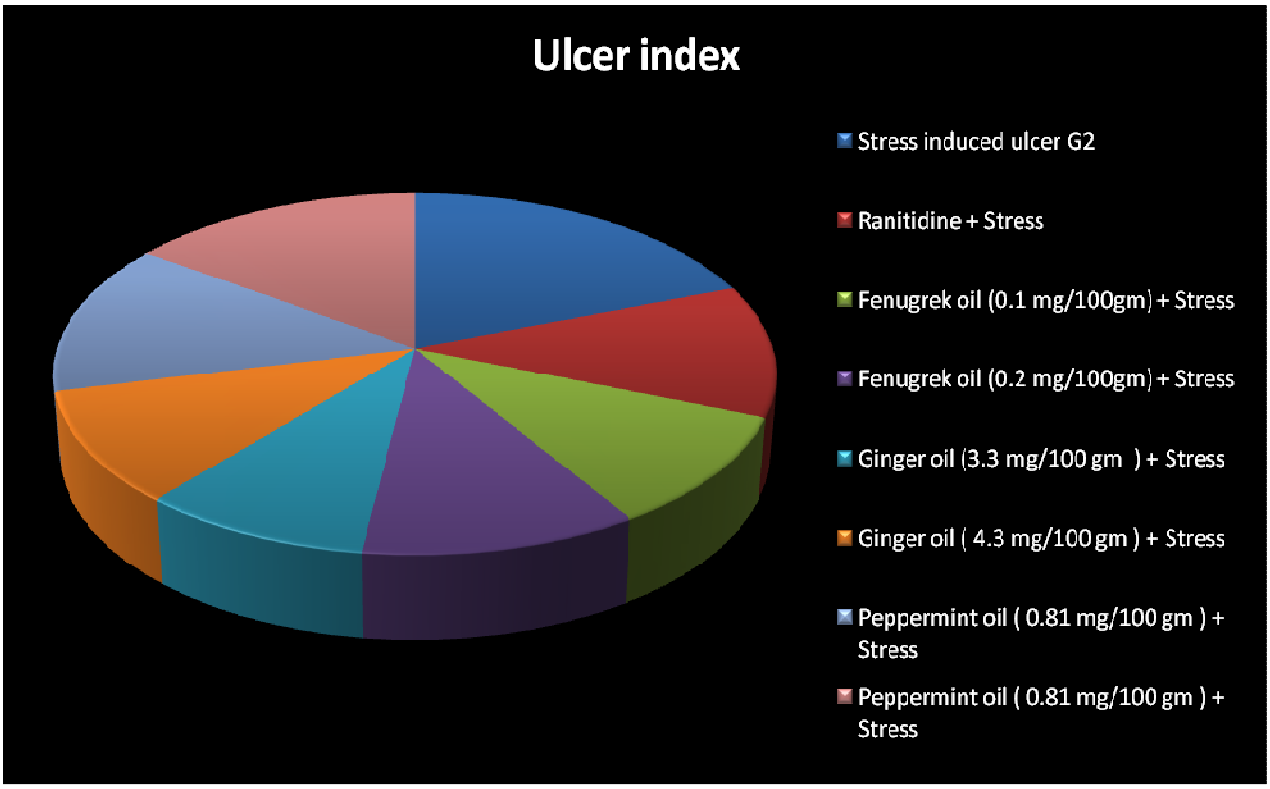
S.no	Compound name	S.no	Compound name	S.no	Compound name	S.no	Compound name	S.no	Compound name
1	Caryophyllene oxide	10	α -Pinene	19	Linalool acetate	28	Cis-Linalooloxid	37	(+)-Limonene
2	+/− Nerolidol	11	Menthyl acetate	20	-Terpineol	29	Terpineol	38	p-Cineole
3	Caryophyllene	12	Isopulegol	21	(+)-Sabinol	30	(+)-Isomenthone	39	-Ocimene
4	(+)-Isomenthol	13	Caryophyllene oxide	22	p-Allylanisole	31	Menthone	40	p-Cymene
5	Pulegone	14	+/− Nerolidol	23	Germacrene D	32	Menthofuran	41	Isovaleric acid
6	Farnesene	15	Caryophyllene	24	Piperitone	33	Menthyl acetate	42	2-Methylbutyl ester
7	Terpineol	16	(+)-Isomenthol	25	(+)-Carvone	34	d-Linalool	43	α -Terpinolen
8	(+)-Sabinol	17	Pulegone	26	Geraniol acetate	35	-Terpineol	44	1-Octenyl acetate
9	p-Allylanisole	18	Farnesene	27	Geraniol	36	Menthol	45	3-Octanol

Table 8 Chemical composition of Fenugrek oil by GC-MS

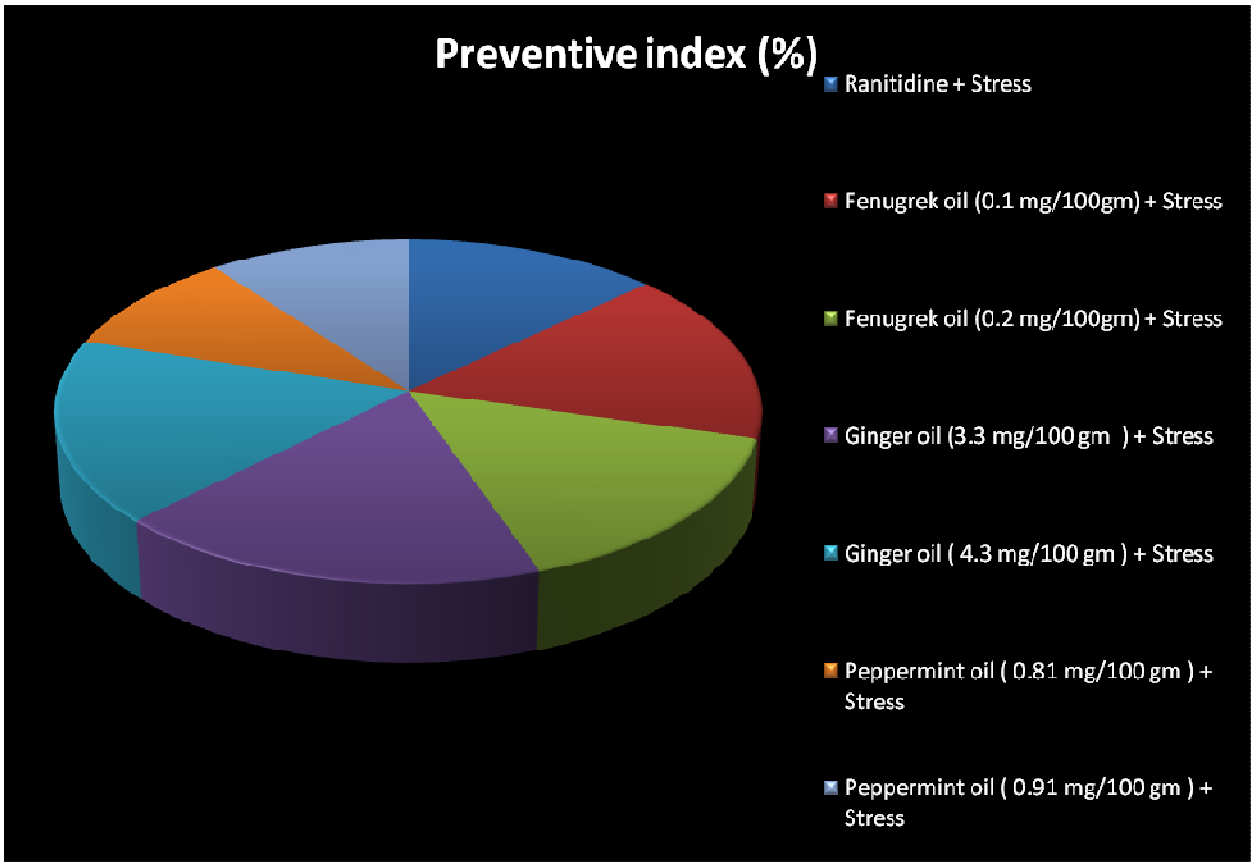
S.no.	Compound name	S.no.	Compound name
1	Danshensu	8	Medioresinol
2	Narirutin	9	.Prolithospermic acid
3	Apigenin-O-rutinoside	10	Salvianolic acid E
4	Rosmarinic acid	11	12-Hydroxyjasmonate sulfate
5	Salvianolic acid B	12	Diosmin
6	Myricetin-O-glucoside	13	Eriocitrin
7	Luteolin-O-glucuronide	14	Apigenin



(A) Mean ulcer score

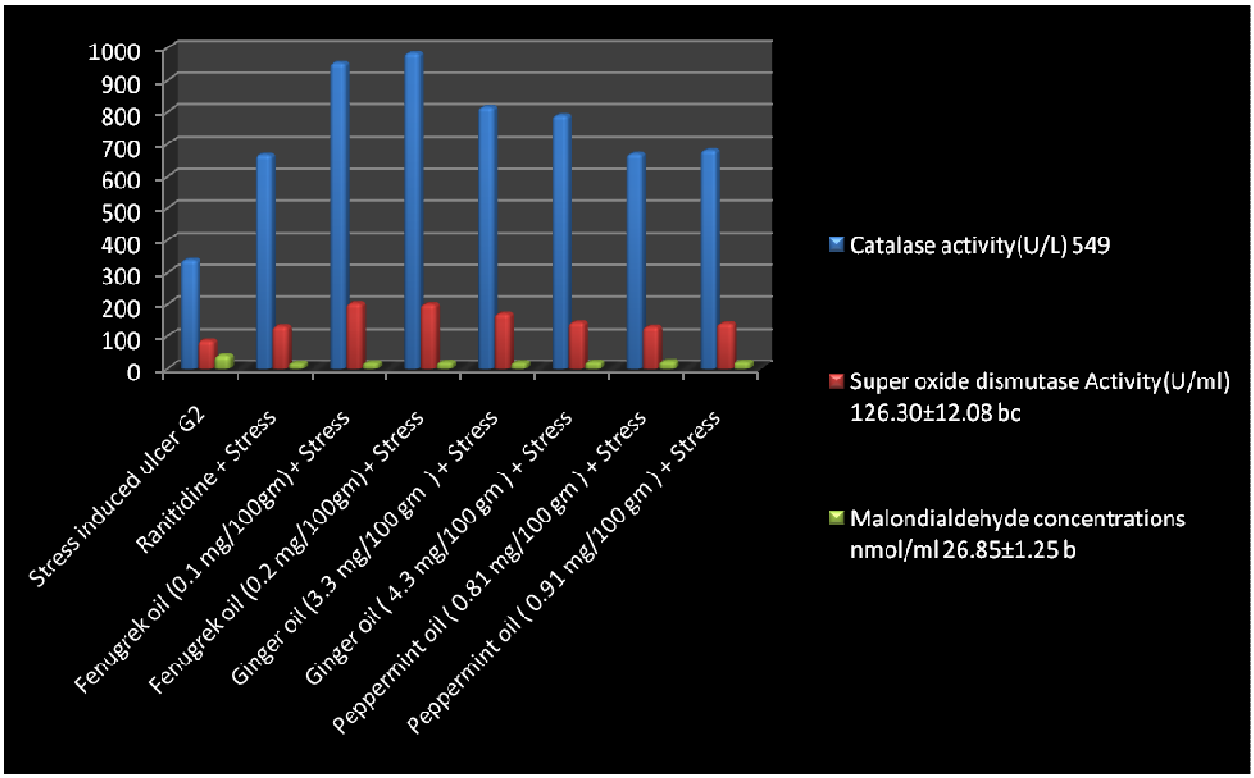


(B) Ulcer index

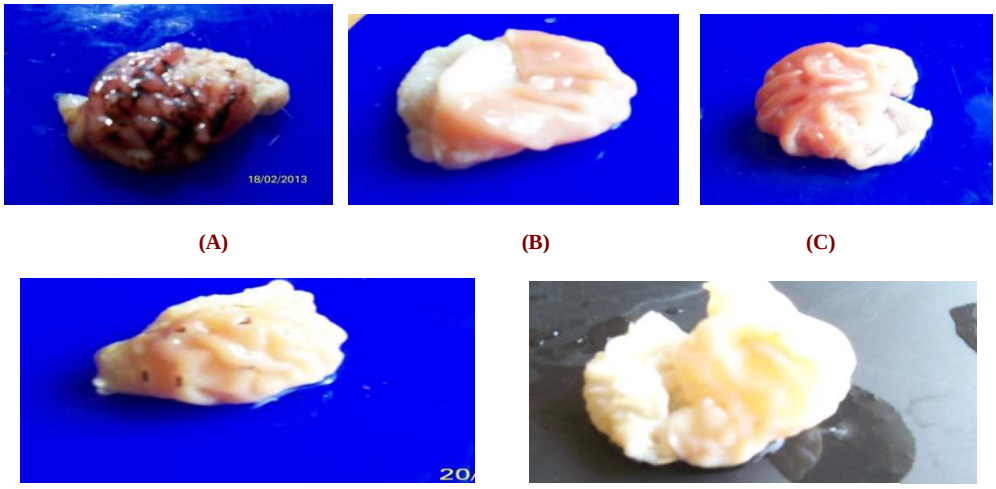


(C) Preventive index %

Fig (2): Effect of oral administration of fenugreek, ginger and peppermint oils in stress induced ulcer rats



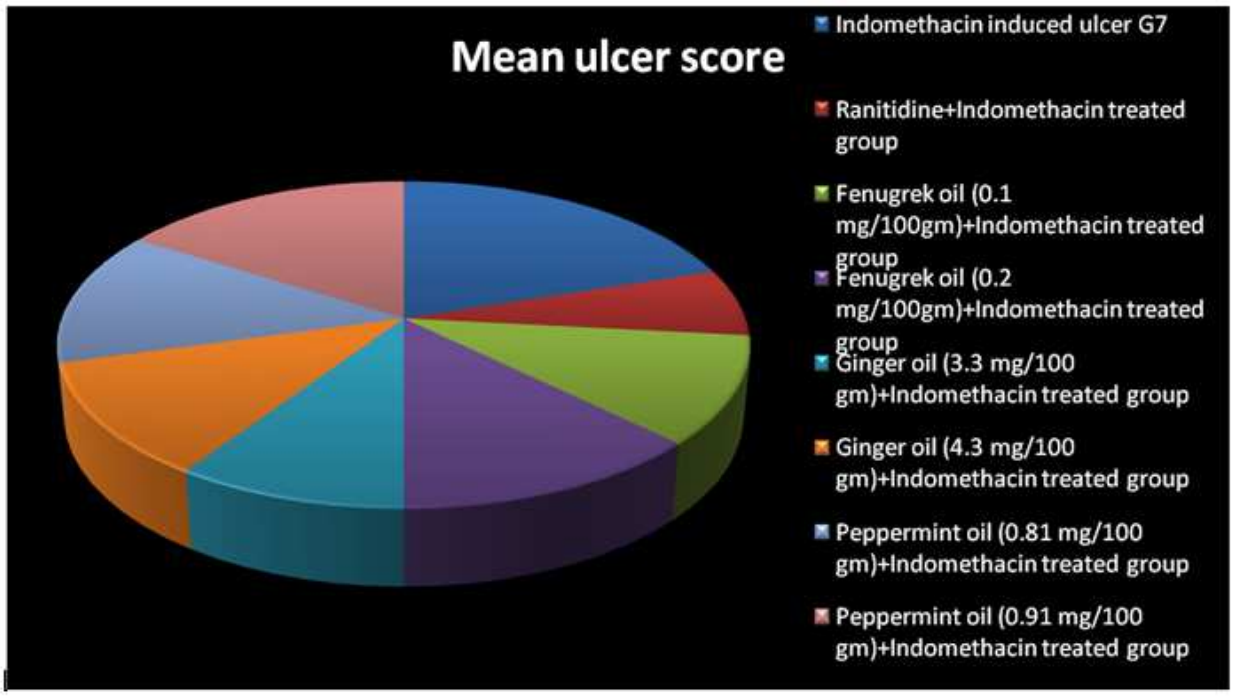
Fig(3): Effect of oral administration of fenugreek, ginger and peppermint oils in stress induced ulcer rats



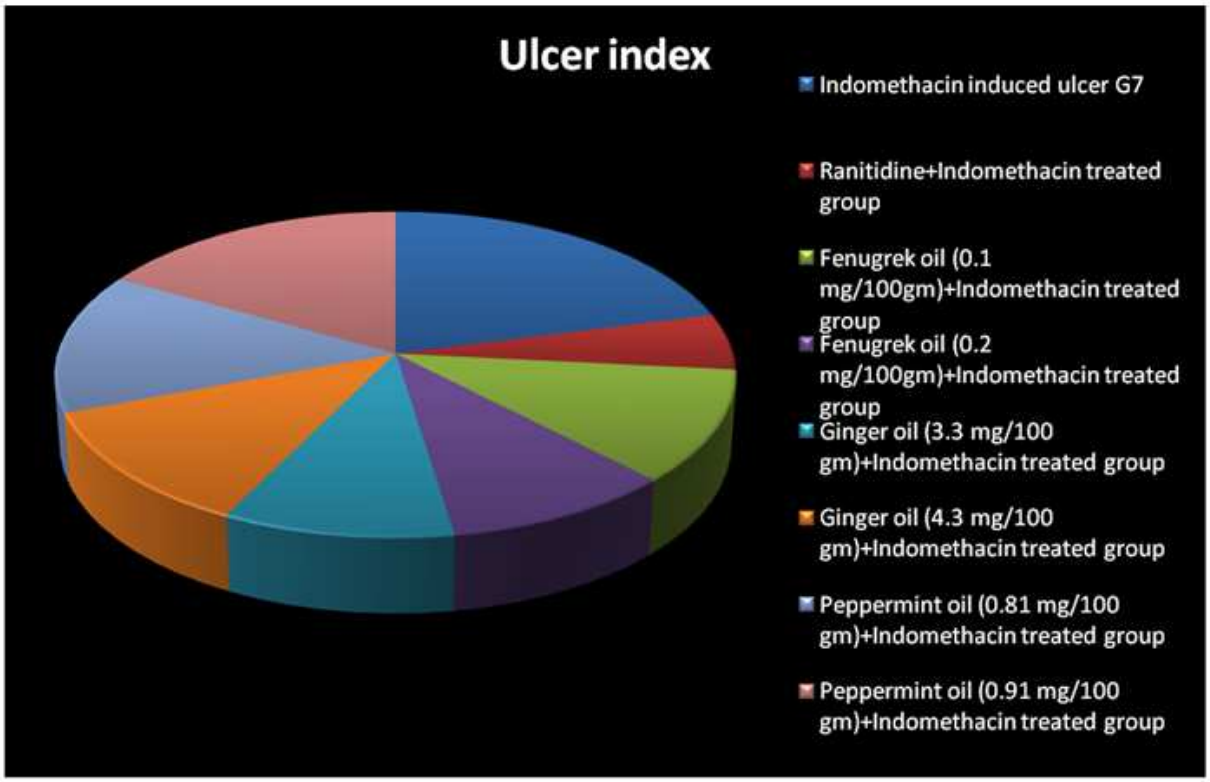
(A) **(B)** **(C)**

(D) **(E)**

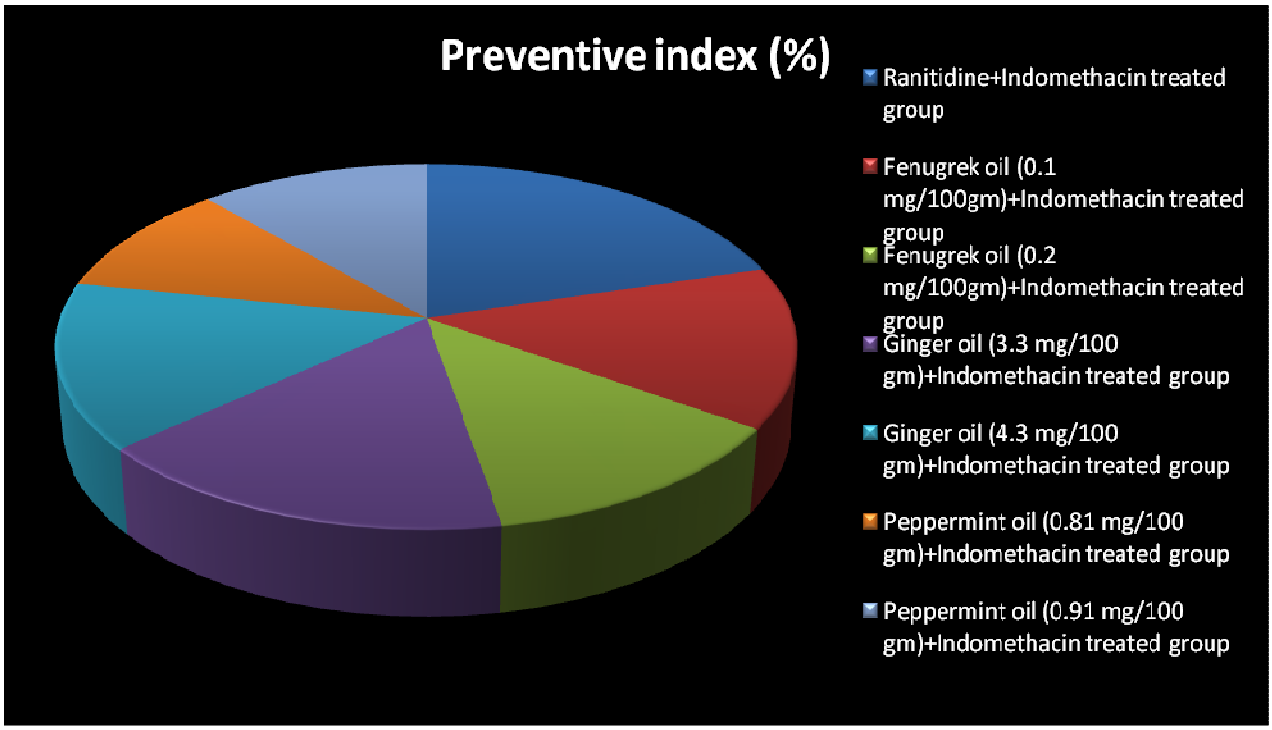
Fig (4): (A) Multiple lesions were observed with severe ulceration in indomethacin induced ulcer group *(G7).* (B) Indomethacin induced ulcer group, treated with ranitidine *(G8).* (C) Indomethacin induced ulcer group, treated with fenugreek oil *(G9).* (D) Indomethacin induced ulcer group, treated with ginger oil *(G10).* (E) Indomethacin induced ulcer group, treated with pepp



(A) Mean ulcer score



(B) Ulcer index



(c) Preventive index

Fig (5): Effect of oral administration of fenugreek, ginger and peppermint oils during 3 day ulcer induction by indomethacin in rats

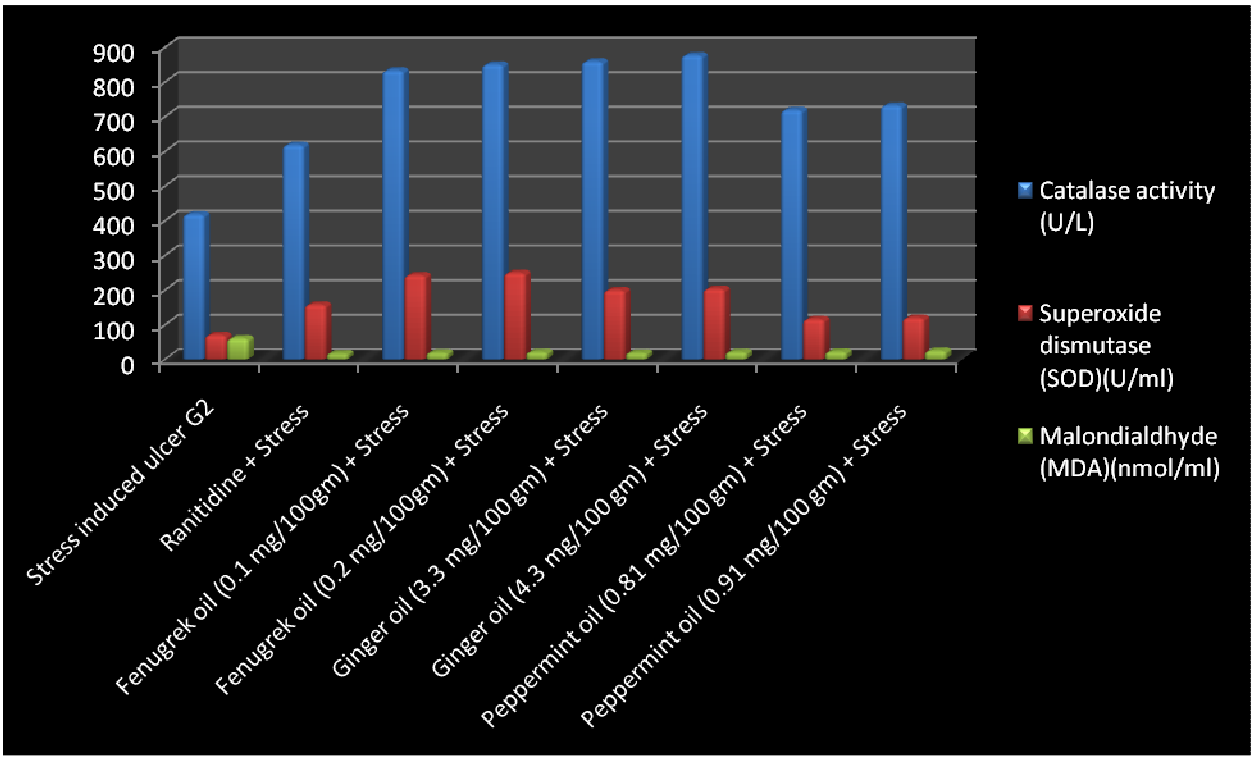


Fig (6): Effect of oral administration of fenugreek, ginger and peppermint oils during 3 day ulcer induction by indomethacin in rats

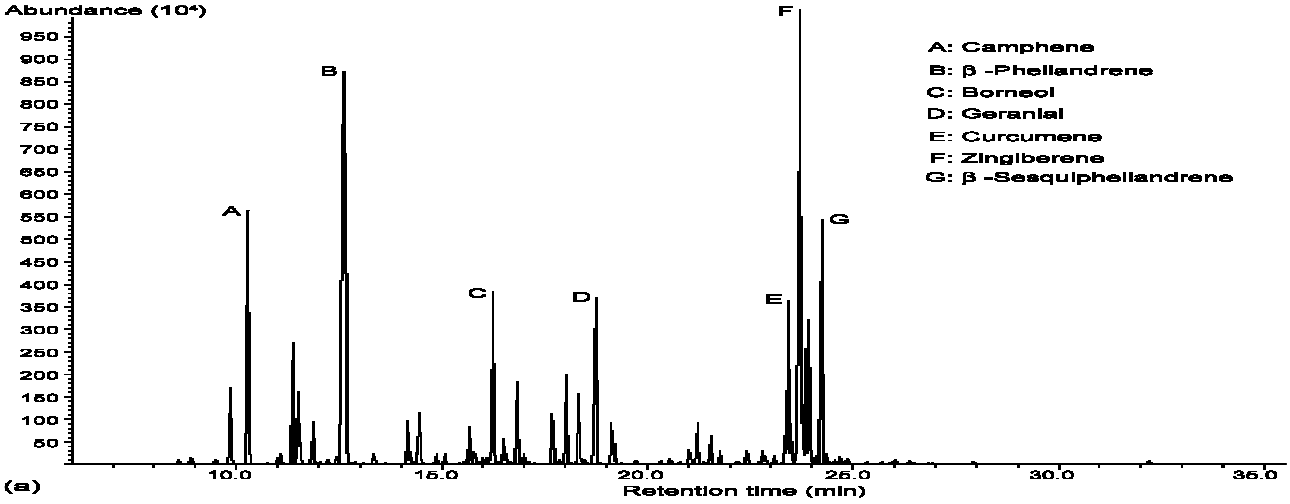


Fig (7): The GC–MS of ginger oil

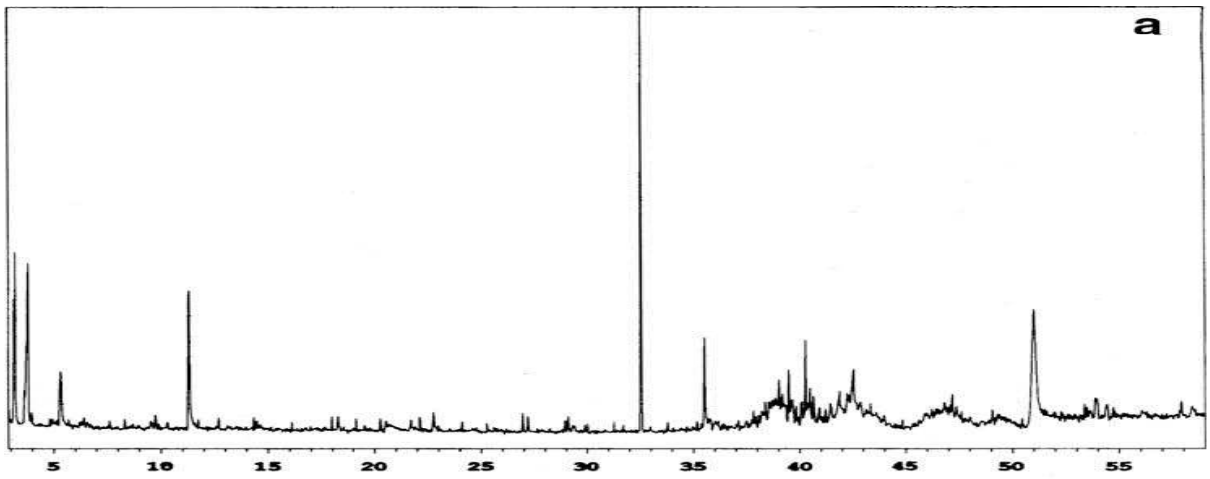


Fig (8): The GC–MS of fenugrek oil

CONCLUSION

From our results, we can conclude that ginger oil and fenugreek oil have beneficial effects as antiulcer agents. This may be attributed to their great antioxidant properties due to presence of polyphenolic compounds and due to having other chemical compounds as previously indicated that they are very important in treatment of gastric ulcer like Linalool , Zingiberene and Geraniol and also presence of Rosmarinic acid in fenugreek oil but peppermint oil doesn't have the potent capacity against gastric ulcer and also we recommend using of fenugreek oil with dose 0.1 mg/100gm and using ginger oil with dose 3.3mg/100gm as they gave the best results than the other tested doses in ameliorating the induced gastric ulcers .

Acknowledgment
Special thanks go to Prof. Dr. El-Sayed Ahmed Abdul-Aziz and Prof. Dr. Sameh Mohamed Mostafa El-Nabtity, Professors of Pharmacology Department, Fac., of Vet., Med., for their assistance in this study.

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