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Study the Effect of Basil Oil as Herbal Treatment of Acetylsalicylate Induced Gastric Ulcer in Experimental Rat Model

[1]Haithem A.M.A. Farghali, [2]Shimaa F.A.E. Ghozy and [2]Hanaa F. El-Mehiry

[1]Department of Surgery, Anesthesiology and Radiology,
Faculty of Veterinary Medicine, Cairo University, Egypt
[2]Department of Home Economics,
Faculty of Specific Education, Mansoura University, Egypt

Abstract: The current study was carried out to elucidate the effect of basil oil on gastric ulcer induced by acetylsalicylate in rats. Thirty six adult male albino rats (Sprague Dawley Strain) weight 140±10g were used and divided into 6 groups, each of 6 rats for six weeks. The first group was used as a negative control and fed on the basal diet only. Other groups had given acetylsalicylate orally (400 mg/kg B. Wt.). One of these groups left as positive control (Ulcerated rats without treatment) and other ulcerated rats groups treated with either ranitidine hydrochloride or different concentrations of the drug, 1ml, 3ml and 5ml basil oil groups. The results revealed that oral administration of basil oil at different doses showed significant increase in final weight, weight gain percentage, food intake and food efficiency ratio, gastric juice pH, gastric prostaglandin E2, gastric cytochrome P_{450} reductase, blood hemoglobin (Hb), glutathione peroxidase (GPX) and superoxid dismutase (SOD), compared with their corresponding +ve control (Ulcerated rats) except 5ml group which showed a decrease in cytochrome P_{450} reductase activity. On the other side, the gastric ulcer length and volume of gastric juice, gastric nitric oxide, serum interleukin-1, serum tumor necrosis factor-alpha, gastric cyclooxygenase and blood malondialdehyde (MDA) were significantly decreased compared with +ve control (Ulcerated rats). The curative ratio percentage showed insignificant difference between 1 ml and 3 ml basil oil groups compared with ranitidine hydrochloride drug rats group except 5ml rats group. The groups that treated with basil oil at doses 1ml, 3 ml and drug groups when examined with ultrasound showed a significant decrease in gastric wall thickness, longitudinal length and width compared with +ve control (ulcerated rats), except 5 ml group. These obtained (physiological and biochemical) data are confirmed by the histopathological studies and ultrasonographic examinations. From the present study, linalool and eugenol (43.70 % and 13.55 % respectively) which were the major components in the essential oil of basil oil (*O. basilicum)*, would play an important role in the antioxidant activity. The current work suggested that, basil oil could be used for healing acute gastric ulcer disease and implemented for gastric ulcer patients due to its cytoprotective effect coupled with anti-secretory activity.

Key words: Acetylsalicylate · Antioxidants · Basil oil · Gastic ulcer

INTRODUCTION

Anatomically, peptic ulcers are circumscribed breaks (sores) in the surface of gastrointestinal mucosa which included the lining of the esophagus, stomach, or duodenum [1], but in real sense gastric and gastrodudonal ulcerations describe a clinical finding, the cause of which is likely to be multifactorial and to differ from one case to another [2]. Each year peptic ulcer disease (PUD) affects 4 million people around the world [3, 4]. Complications are encountered in 10%-20% of those patients and 2%-14% of the ulcers will perforate [5, 6]. Surgery may be needed for ulcers that bleed, obstruct, perforate or don't heal with other treatments [7]. Peptic ulcers can occur independently or as a complication of many systemic diseases or following administration of various drugs to

Corresponding Author: Hanaa F. El-Mehiry, Department of Home Economics, Faculty of Specific Education, Mansoura University, Egypt.

treat many diseases [1]. Main etiologic factors include use of non-stereoidal anti-inflammatory drugs (NSAIDs), steroids, smoking, Helicobacter pylori (H. pylori) and a diet high in salt [6, 8]. With respect to the association between peptic ulcer and stress (a psychosocial factor (numerous animal experiments performed to date have demonstrated a strong association of stress with the development and recurrence of ulcer disease). However, ulcers are related to stress in only 30-65% of the patients [9]. A major causative factor (60% of gastric and up to 90% of duodenal ulcers) is chronic inflammation due to Helicobacter pylori that colonizes the internal mucosa. The immune system is unable to clear the infection, despite the appearance of antibodies [10].

Ulcers have been reported in dogs, rodents, horses and human secondary to administrations of a variety of non-steroidal anti-inflammatory drugs including piroxicam [11], aspirin and ibuprofen [12], naproxen [13], endomethacine [14] and ketorolac [15]. Steroidal anti inflammatory drugs are also incriminated as cause of gastric ulceration [16], for the treatment of other primary diseases like spinal insult like intervertebral disk disease [17], primary neoplastic condition of the stomach carcinoma and gastric intestinal lymphoma [18]. Gastrinoma and disseminated mast cell disease are also responsible for gastric ulceration [19]. The gastric mucosa protects itself from gastric acid with a layer of mucus, the secretion of which is stimulated by certain prostaglandins. NSAIDs block the function of cyclooxygenase 1 (COX-1) activity, which is essential for the production of these prostaglandins. COX-2 selective anti-inflammatories (such as celecoxib or the since withdrawn rofecoxib) preferentially inhibit COX-2, which is less essential in the gastric mucosa and roughly halve the risk of NSAID-related gastric ulceration [10].

As gastric mass lesions, reasonable sensitivities have been reported on the ultrasound evaluation of gastric peptic ulcer disease with the fluid-filled stomach technique [10, 20]. Sonographic signs of gastric ulcer were classified as gastric wall edema associated with or without ulcer crater. In cases of perforation of gastric ulcers that were gastric wall edema associated with fluid collection. A dish-shaped niche has been reported by some authors in gastric ulcers [10]. Other authors could not detect this finding because of the absence of fluid within the stomach, but they showed a niche like echogenicity extending from the inner echogenic gastric lumen into the thickened gastric wall in about one fourth of their patients [21]. So, ultrasonography proved usefully for detecting benign ulcerations and can be used to supplement follow-up examinations, but it cannot replace endoscopy and contrast radiography. The sensitivity and specificity of B-mode ultrasound imaging (BMUI) was recorded for diagnosis of gastric cancer in rats and they were 96.6% and 78.78%, respectively [22]. Thus, it was a useful method of diagnosis of gastric cancer in rats using B-mode ultrasound images (BMUI) of the stomach of rat which were obtained with an 8 MHz linear array transducer. A study was conducted for ultrasound examination of rat's abdomen [23]. Proper direction, location and method of imaging were described who was showed the preferred position of rat during ultrasound imaging of the stomach. Ultrasound examination was done after anesthetizing and shaving the preferred location in the abdomen of the rat.

There has been much research into the health benefits conferred by the essential oils found in basil (*Ocimum basilicum*). Scientific studies in vitro have established that compounds in basil oil have potent antioxidant, antiviral and antimicrobial properties and potential for use in treating cancer [24-27]. In addition, basil has been shown to decrease the occurrence of platelet aggregation and experimental thrombus in mice [28]. It is traditionally used for supplementary treatment of stress, asthma and diabetes [29]. The composition of essential oils, all characterized by a high content of linalool, included three chemotypes: "linalool," "linalool and methylchavicol" and "linalool and eugenol". Two chemotypes each had their own suite of morphological characters, whereas two groups of cultivars, with different morphological parameters belonged to the same chemotype [30].

The main objective of the present study is to evaluate effect of some levels from basil oil as curative for gastric ulcer in rats.

MATERIALS AND METHODS

Materials: Thirty male albino rats of Sprague Dawley strain (from Laboratory Animal Colonies, Helwan, Egypt) were used in the current study. The average weight was 145 ± 10g. One gram vial of DL-Lysine Acetylsalicylate (Aspegic©-Ameriya Company for Pharmaceutical and Chemical Industries, Cairo, Egypt) was dissolved in 10 ml distilled water and administered orally as a single dose of freshly prepared aspirin solution in dose rate 400 mg /kg body weight of rats to induce acute gastric ulcer according to Main and Whittle [31]. Ranitidine hydrochloride (Ranitak© 150 mg tablets-SEDICO Pharmaceutical Company, Giza, Egypt)-which used to

inhibit gastric ulcer in control group- was dissolved in distilled water in dose 30 mg/kg of rat using a stomach tube. The basal diet was performed according to NRC [32]. Basil oil (*Ocimum basilicum*) was obtained from Agriculture Research Center, Giza, Egypt. Basil oil was administered daily at doses 1ml, 3 ml and 5ml/kg body weight of rats orally by stomach tube.

Methods: Gas chromatography-Mass Spectrometry (GC-MS): The volatile constituents of basil leaf oils were analyzed using a Hewlett Packard model HP6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with an HP-5MS (5% phenyl-polymethylsiloxane) capillary column (30 m × 0.25 mm i.d., film thickness 0.25μm; Agilent Technologies, USA) interfaced to an HP model 5973 mass-selective detector. The oven temperature was initially held at 100°C and then increased at 2°C min-1 to 220°C. The injector and detector temperatures were 250 and 280°C, respectively. Purified helium was used as the carrier gas at a flow rate of 1 $mlmin^{-1}$. The EI mass spectra were collected at an ionization voltage of 70 eV over the m/z range 29-300. The electron multiplier voltage was 1150 V. The ion source and quadrupole temperatures were set at 230 and 150°C, respectively. Identification of volatile components was performed by comparison of their Kovαts retention indices, relative to C8-C22 n-alkanes and comparison of the mass spectra of individual components with the reference mass spectra in the Wiley 275 and NIST 98 databases and with the corresponding data for components in basil [33-36].

Grouping Design and Feeding of Rats: The rats were fed on the basal diet for five days before starting the experiment for adaptation then the rats were classified into six equal groups (Each contains on 6 rats). Negative control (-Ve) rats group fed on the basal diet only while the other five animals groups were administered orally of freshly prepared aspegic solution to induce gastric ulcer then classified into positive control (+Ve = ulcerated rats) and treated groups that were drug group, 1 ml, 3 ml & 5 ml basil oil treatment groups. Daily food intake and weekly body weight gain were calculated. Food efficiency ratio (FER) was determined according to the method of Chapman *et al.* [37]. At the end of the experiment period (6 weeks), rats were sacrificed after overnight fasting under ether anesthesia. Blood samples were taken from hepatic portal vein. Two batches of blood samples (with or without heparin as anticoagulant) were collected from each rat. One part of blood samples were collected from each animal in clean dry test tube and serum was harvested and kept at -20°C until the determination of biochemical parameters. Another part of blood samples was collected in heparinzed test tube for estimation of haemoglobin content which carried out as soon as possible.

Ultrasonographic Examination: It has been performed at the Surgery, Anesthesiology and Radiology Department, Faculty of Veterinary Medicine, Cairo University, on all groups, using Eickemeyer Digital Ultrasonic Diagnostic Imaging System, Model Magic 2200 (Veterinary use only) and Toshiba Diagnostic Ultrasound Equipment, Model SSA-320A with liner transducers of multi frequency 5-10 MHz, through three methods of applications:

- Direct contact between the transducer and the examined rat using contact jell.
- Through water path when the abdominal region of the animal was submerged in the water and the transducer was toughing the surface of the water.
- Through water path (as method 2) in addition to injection of the examined rat with 3 ml normal saline intera-peritoneum.
- All imaging were performed in fundamental brightness mode (B-mode).
- The examined rat would be prepared for examination by fasting for 12 hours and drinking distilled water (3 ml) using stomach tube five to ten minutes before ultrasound scanning according to Lehman [23].

Measurement the Length of Gastric Ulcer: At the last day of experimental period, all rats were fasted for 12-14hrs and only allowed for drinking water. In the morning of the next day, all rats were sacrificed and their stomachs were tied around both openings (cardiac & pyloric sphincters) and injected by distilled water (3 ml). The gastric juice was then collected in sterilized tube. The stomachs were opened longitudinally, washed with saline and examined under dissecting microscope for ulcer. The length of gastric ulcer was measured and expressed as mean + SE for each group. The curative ratio was then calculated for each treated group according to the method described by Akhtar and Ahmed [38] by using the following equation:

Curative ratio (CR) = (LC - LT/LC) x 100.

where:
LC = Length of ulcer in control positive group.
LT = Length of ulcer in treated group.

Measurement the Volume of Gastric Juice: Gastric juice was collected according to the methods of Niida *et al.* [39] (abdomen was incised and both the stomach and duodenum was exposed and a fistula made by a poly ethane tube inserted into the stomach from a small incision made in the duodenum and held in place by a ligature around pylorus also esophagus was clamped to prevent reflux and loss of the gastric mucosa) in tubes and centrifuged at 500 rpm for 5 minutes. The volume of gastric juice was measured by graduated cylinder and expressed as ml. The gastric juice decrease percentage was calculated each treated group according to the method described by Parmar and Desai [40] by using the following equation:

Decrease ratio (DR) = (VC - VT/VC) x 100.

where:
VC = Volume of gastric juice in control positive group.
VT = Volume of gastric juice in treated group

Measurement of Serum Parameters: Serum levels of total nitric oxide (NO), interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF-α) were determined according to Griess *et al.* [41], Grassi *et al.* [42] and Beutler *et al.* [43], respectively.

Measurement of Blood Parameters: Blood samples were collected for estimation of hemoglobin (HG), glutathione peroxidase (GSP), superoxide dismutase (SOD) and malondialdehyde (MDA) according to Vankampen and Ziglstra [44], Tapple [45], Winterbourn *et al.* [46] and Yagi [47], respectively.

Measurement of Gastric Mucosa: Gastric mucosal of cytochrome P_{450} reductase (Cyto P) activity, cyclooxygenase (Cox-$_2$) activity, prostaglandin E_2 (PGE_2) concentration and were determined according to Mc-Lean and Day [48], Hemler and Lands [49] and Hamberg and Samuelsson [50], respectively.

Histopathological Studies: Gastric tissue samples were taken from different rats in each group immediately after scarification. The tissues were washed with the normal saline solution to remove blood, fixed in 10% neutral formalin as fixative and sent to Cancer Institute for histopathological examination according to Bancroft *et al.* [51].

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 [52].

RESULTS AND DISCUSSION

Data presented in Table 1 illustrated that Individually, basil leaf oil yielded 80 identified constituents representing 91.11 % with dominant components consisting of linalool (43.70%) followed by eugenol (13.55 %), 1,8-cineole (10.18 %), α-epi-cadinol (5.76 %), γ-cadinene (1.99 %), γ-terpineol (1.75 %) and γ-muurolene (1.35 %), respectively. As can be observed, eight components (linalool oxide, (E)-sabinene hydrate, (Z)-myroxide, δ-terpineol, γ-terpineol, exo-2-hydroxycineole acetate, β-copaene and (E)-sesquisabinene hydrate) were detected only in the essential oil of O. basilicum. These results are in a good agreement with those of most published studies on the chemical composition of basil essential oil in which linalool was found to be the predominant constituent: [53] (69%), [54] (45.7%) and [55] (41.2%). In other studies, methyl chavicol (estragole) was represented as the major constituent in the basil leaf oils as can be seen in the study of Telci *et al.* [33], Chalchat *et al.* [35] and Khatri *et al.* [56]. Additionally, methyl eugenol was detected as the main component in basil leaf oil in Thailand by Suppakul *et al.* [36]. These differences indicate that the chemical composition of the essential oils of basil varieties may be correlated with different environmental and ecological conditions, as well as by genetic factors.

Data recorded in Table 2 illustrated that the positive control rats group showed a significant decrease in final weight, weight gain %, food intake and food efficiency ratio (FER) compared to their corresponding normal control animal group. These results may be attributed to lose of appetite, disturbance in the gastric enzymes secretions, changes in the pH of gastric secretion or/and alteration in the level of neuropeptide-Y hormone (NPY). These data are confirmed with Helmy [57] and El-Metwally [58]. The treated groups with basil oil (1ml, 3ml and 5 ml) and drug group showed significant increase in final weight, weight gain %, food intake and FER compared to positive control group (ulcerative rats). These results are in parallel with those obtained by Stajkovic *et al.* [59]. These data may be due to the biochemical properties of oil which acts as antioxidant agent and its pharmedynamics and pharmakinetics actions on the healing of gastric ulcer. These results are in harmony with those obtained by Chiang *et al.* [24], Muller *et al.* [60] and Makri and Kintzios [61].

Table 1: Structural assignment and relative peak area percent of the volatile components of the essential oil obtained from of *Ocimum basilicum* leaves.

Component	*LTPRI*[a]	Relative peak area, % *O. basilicum*	Component	*LTPRI*[a]	Relative peak area, % *O. basilicum*
(*E*)-2-Hexenol	849	0.06	Methyl chavicol	1196	0.21
α -Thujene	924	0.02	Geraniol	1253	0.03
α -Pinene	932	0.19	Chavicol	1259	0.07
Camphene	949	0.02	*iso*-Bornyl acetate	1283	0.65
Sabinene	971	0.33	Carquejol acetate	1295	0.05
Amyl vinyl carbinol	974	0.04	Eugenol	1355	13.55
β-Pinene	978	0.58	*exo*-2-Hydroxycineole acetate	1358	0.05
β-Myrcene	988	0.75	α-Ylangene	1373	0.08
Dehydro-1,8-cineole	991	0.02	β-Bourbonene	1380	0.10
α -Terpinene	1017	0.02	*iso*-Longifolene	1385	0.06
ortho-Cymene	1024	0.01	β-Elemene	1387	0.57
Limonene	1028	0.23	Cyperene	1391	0.05
1,8-Cineole	1033	10.18	Methyl eugenol	1400	0.09
β- (*E*)-Ocimene	1044	0.57	α -Cedrene	1410	0.09
γ-Terpinene	1056	0.06	(*E*)-Caryophyllene	1416	0.08
(*Z*)-Sabinene hydrate	1071	0.24	β-Cedrene	1420	0.04
Caprylyl acetate	1078	0.08	α -(*E*)-Bergamotene	1431	0.10
Terpinolene	1084	0.07	β-(*Z*)-Farnesene	1438	0.05
Linalool oxide	1087	0.07	(*Z*)-Muurola-3,5-diene	1442	0.05
Linalool	1099	43.70	α -Humulene	1451	0.36
(*E*)-Sabinene hydrate	1099	0.17	β-(*E*)-Farnesene	1454	0.20
(*Z*)-Myroxide	1138	0.01	(*Z*)-Muurola-4(14),5-diene	1458	0.35
Camphor	1146	0.28	β-Acoradiene	1462	0.04
iso-Menthone	1162	0.03	γ-Gurjunene	1471	0.06
δ-Terpineol	1170	0.29	γ-Muurolene	1477	1.35
Borneol	1172	0.37	γ-Himachalene	1481	0.47
Terpinen-4-ol	1179	0.18	β-Selinene	1485	0.04
γ-Terpineol	1195	1.75	Bicyclogermacrene	1491	0.04
β-(*E*)-Guaiene	1498	0.59	1,10-Di-epi-cubenol	1611	0.76
γ-Patchoulene	1503	0.51	β-Acorenol	1629	0.05
β-Bisabolene	1505	0.09	α -Epi-cadinol	1640	5.76
γ-Cadinene	1509	1.99	β-Eudesmol	1650	0.11
(*E*)-Calamenene	1516	0.35	α -Cadinol	1652	0.32
(*Z*)-Nerolidol	1521	0.30	α -Epi-bisabolol	1683	0.06
α -Cadinene	1533	0.03	α-Bisabolol	1685	0.10
Longicamphenylone	1560	0.08	Zierone	1698	0.03
Spathulenol	1573	0.25	β-(*Z*)-Santalol	1713	0.12
(*E*)-Sesquisabinene hydrate	1579	0.01	(*E*)-3-Tetradecen-5-yne	1889	0.24
β-(*Z*)-Elemenone	1580	0.06	--	--	--

[a] Linear temperature program retention index; [b] trace amount, < 0.005 %

Table 2: Effect of basil oil treatment on body weight gain %, food intake and FER against aspirin- induced gastric ulcers in rats

		Gastric ulcer				
Groups				Basil oil		
Variables	Control (-ve)	Control (+ve)	Drug	1 ml/kg	3 ml/kg	5 ml/kg
Initial weight(g)	149.33±2.05a	150.03±1.63a	147.50±1.69a	145.12±2.94a	146.33±4.03a	149.67±1.25a
Final weight (g)	243.00±2.45 a	204.67±3.68d	221.33±2.62c	216.33±8.73c	220.33±1.25c	231.67±4.64b
Weight gain %	62.76±3.86 a	36.48±3.94c	50.58±2.23b	49.17±4.57b	50.66±4.40b	54.82±4.41b
Food intake (g/d)	15.32±2.14 a	12.85±2.55b	15.45±2.42a	15.40±2.33a	15.71±2.71a	15.81±2.14a
FER	0.125±0.01a	0.082±0.03b	0.111±0.02a	0.115±0.04a	0.112±0.05a	0.113±0.01a

Values with the same letters indicate insignificant difference and vice versa.

As shown in Table 3, the treated groups with basil oil at doses 1 ml, 3 ml and drug group showed a significant decrease in gastric wall thickness, longitudinal length and width compared to positive control group, except 5ml group which showed enhancement in these parameters but was insignificant difference compared to positive control group. Our study corroborates the findings of Dhuley [62] reported an increase in gastric wall mucus thickness (barrier mucus) in rats treated with rhinax, a herbal formulation consisting water extracts of *Withania somnifera* L. (root), *Asparagus racemosus* Willd. (root), *Mucuna prurience* (root), *Phyllanthus emblica* Gaertn. (fruit), *Myristica fragrance* Houtt. (seed), *Glycyrrhiza glabra* L. (root), against physical and chemical factors-induced gastric and duodenal ulcers in rats. Increased mucus secretion by the gastric mucosal cells can prevent gastric ulceration by several mechanisms, including lessening of stomach wall friction during peristalsis and gastric contractions, improving the buffering of acid in gastric juice and by acting as an effective barrier to back diffusion of H^+ ion [63].

The results in Table 4 indicated that the gastric ulcer length was significantly decreased in all treated rat groups compared with control (+ve) group. The groups treated with basil oil at doses 1 ml and 3ml showed non insignificant difference compared to drug group in curative ratio percentage except 5 ml group which showed significant decrease. These results were in agreement with those obtained by Suppakul *et al.* [36] and Farnsworth and Bunyapraphatsara [64]. The last authors reported that basil essential oil and their principal constituents were found to exhibit antimicrobial activity against aspirin; it may also help relieve intestinal gas. The major active ingredients of basil essential oil are terpenoids, such as eugenol, thymol and estragole. These ingredients contribute the potential health benefits of basil. The volume of gastric juice was significantly increased in (+ve) group control compared to (-ve) control group, while all treated gastric ulcer rat groups showed significant decrease compared with (+ve) control group. The PH of gastric juice was significantly decreased in control (+ve) group compared with (-ve) control group. The groups administered with basil oil 1ml, 3ml and 5ml showed increased insignificant difference compared with (+ve) control group. The results are in agreement with those obtained by Singh and Majumdar [65] which conclude that the fixed oil of *Ocimum sanctum* L. was found to possess significant antiulcer activity against aspirin-, indomethacin-, alcohol-, histamine-, reserpine-, serotonin and stress-induced ulceration in experimental animal models. Significant inhibition was also observed in gastric secretion and aspirin-induced gastric ulceration in pylorus legated rats. Basil oil may be considered to be a drug of natural origin which possesses both anti-inflammatory and antiulcer activity.

As shown in Table 5, the positive control group showed significant increase in gastric tissues total nitric oxide level, serum interleukin-1 and serum tumor necrosis factor-alpha compared to (-ve) control group. All of the treaded groups and drug group showed significant decrease in these parameters compared to positive control group. The excessive release of TNO from gastric epithelial cells induced by acetylsalicylate has been reported to exert detrimental effects [66, 67]. Inhibition of the increase in INOS expression induced by aspirin in the gastric mucosa leads to a reduction in gastric mucosal damage [68]. In the present study, the results of the groups administered by basil oil are in agreement with those reported by Mari *et al.* [69]. They explained that anti-inflammatory compounds of flavonoids that present in basil inhibited INOS expression.

The statistical data in Table 6 presented that, (+ve) control rat group showed significant increase cyclooxygenase (COX2) activity, while showed significant decrease cytochrome P_{450} reductase activity and prostaglandin E2 concentration and compared to (-ve) control group. The groups treated with basil oil (1 ml, 3ml and 5 ml) and drug group showed significant decrease in cyclooxygenase (COX2), while showed a significant increase prostaglandin E2 compared to (+ve) control group. Treatment with drug and basil oil showed also significant increase cytochrome P_{450} reductase at doses 1 ml and 3 ml except 5 ml basil oil showed insignificant different compared to (+ve) control group. A compound called eugenol found in the oils of basil can block the activity of an enzyme in the body called cyclooxygenase 2 (COX 2). Many over the counter NSAID's (Non Steroidal Anti-inflammatory Drug's), such as Ibuprofen and Diclofenac work by inhibiting this enzyme. Basil can help relieve musculoskeletal as well as bowel pain and inflammation [70]. Another study by Vats *et al.* [71], who reported that the basil also has an inflammatory-cascade normalizing action that promotes the healthy metabolism and activity of arachadonic acid, prostaglandins, leukotrienes and platelets. Basil is a natural COX-2 enzyme modulator, by stopping the cascading effect caused by COX-2 enzyme. It is also an antioxidant that helps to support the body's functions and maintain them in a normal range by neutralizing free radicals.

Table 3: Effect of basil oil treatment of ultrasound findings in gastric wall thickness, longitudinal length and width of rat stomach against aspirin-induced gastric ulcers in rats

	Gastric ulcer					
Groups				Basil oil		
Variables	Control (-ve)	Control (+ve)	Drug	1 ml/kg	3 ml/kg	5 ml/kg
Gastric wall thickness (mm)	2.63±0.18c	7.42±0.03a	4.47±0.03bc	2.75±0.32bc	5.43±0.52c	5.25± 0.03ab
Longitudinal length (mm)	10.4 ±0.05 c	18.8 ±0.14a	12.5±0.03c	9.46±0.08c	12.3±0.07c	21.01± 0.07ab
Longitudinal width (mm)	5.03±1.06 c	13.2 ±1.34a	12.5 ±2.69ab	7.13± 3.65c	12.4±2.72ab	12.7± 5.18ab

Values with the same letters indicate insignificant difference and vice versa.

Table 4: Effect of basil oil treatment of on the length of gastric ulcer, the volume of gastric juice, the PH of gastric juice collected from stomachs against aspirin-induced gastric ulcers in rats

	Gastric ulcer					
Groups				Basil oil		
Variables	Control (-ve)	Control (+ve)	Drug	1 ml/kg	3 ml/kg	5 ml/kg
Gastric ulcer length (mm.)	0.00±0.00	7.46±0.98a	2.70±0.18c	2.34±0.53c	2.58±0.46c	3.65±1.50b
CR (%) Curative ratio %	-	-	76.11±4.16a	74.64±6.89a	73.45±2.47a	50.03±10.64b
Volume of gastric juice (1ml)	0.64±0.08 e	6.05±0.16a	2.21±0.16d	3.27±0.25c	3.65±0.14c	4.34±0.10b
DR (%)	-	-	57.93±1.09a	52.82±2.38b	50.68±1.92b	37.92±1.88c
PH of gastric juice (mEq/L)	4.62±0.53 a	2.06±0.41c	3.00±0.17b	3.56±0.39b	3.50±0.24b	3.25±0.42b

Values with the same letters indicate insignificant difference and vice versa. CR: Curative Ratio. DR: Decrease Ratio

Table 5: Effect of basil oil treatment of gastric tissues total nitric oxide, Interleukin-1 and tumor necrosis factor-alpha against aspirin- induced gastric ulcers in rats

	Gastric ulcer					
Groups				Basil oil		
Variables	Control (-ve)	Control (+ve)	Drug	1 ml/kg	3 ml/kg	5 ml/kg
NO (pg/mg)	37.06±2.94d	68.12±6.76a	44.67±5.29cd	41.46±3.37cd	47.27±6.67bc	55.35±4.83b
IL-1 (pg/mg)	13.46±1.06c	49.19±1.34a	26.64±2.69b	22.34±8.18b	27.97±2.72b	29.40±6.65b
TNF-α (pg/mg)	3.20±0.81d	13.75±1.94a	6.06±0.66c	4.29±0.48d	6.18±0.63c	8.17±0.31b

Values with the same letters indicate insignificant difference and vice versa.

TNO: total nitric oxide IL-1: Interleukin-1 TNF-α: tumor necrosis factor-alpha

Table 6: Effect of basil oil treatment of gastric tissues cytochrome P450 reductase activity, cyclooxygenase activity and prostaglandin E2 concentration against aspirin- induced gastric ulcers in rats

	Gastric ulcer					
Groups				Basil oil		
Variables	Control (-ve)	Control (+ve)	Drug	1 ml/kg	3 ml/kg	5 ml/kg
Cyto P_{450} Reductase (g/mg)	2.03±0.32a	0.71±0.07c	1.55±0.23ab	1.89±0.48a	1.49±0.58ab	1.13±0.04c
Cox-$_2$ (pg/mg)	5.24±0.45d	17.92±1.23a	6.66±0.35c	6.25±0.53cd	7.60±0.90bc	8.69±0.50b
PGE_2 (pg/mg)	536.07±45.31a	305.37±15.54c	471.37±21.92ab	480.53±33.60ab	452.23±58.68b	415.90± 11.62b

Values with the same letters indicate insignificant difference and vice versa

Cyto P_{450}: cytochrome P_{450} reductase COX2: cyclooxygenase activity PGE_2: prostaglandin E_2

Table 7: Effect of basil oil treatment of blood hemoglobin, glutathione-peroxidase, superoxide dismutase and malondialdehyde against aspirin- induced gastric ulcers in rats

Groups	Gastric ulcer					
				Basil oil		
Variables	Control (-ve)	Control (+ve)	Drug	1 ml/kg	3 ml/kg	5 ml/kg
HB(g/dl)	13.76±0.32a	9.81±0.62b	13.42±0.74a	12.67±0.95a	13.10±0.50a	13.72±1.64a
GSP (mmol/l)	8.35±1.37a	4.34±0.44d	6.91±1.46b	8.31±1.32a	7.13±1.16ab	5.11±0.53c
SOD (mmol/l)	25.26±7.01a	13.41±1.21c	21.71±3.21ab	23.37±2.11ab	22.71±3.61ab	16.38±2.61b
MDA (mmol/l)	3.22±0.25b	9.71±1.41a	4.71±0.68b	4.01±0.61b	4.36±0.77b	7.21±1.10a

Values with the same letters indicate insignificant difference and vice versa. HB: hemoglobin
GSP: Glutathione-peroxidase. SOD: Superoxide dismutase. MDA: Malondialdehyde

As shown in Table 7, the positive control group showed a significant decrease in blood hemoglobin (Hb), glutathione peroxidase (GPX), superoxid dismutase (SOD), while a significant increase in blood malondialdehyde (MDA) compared to (-ve) control group. Treatment with drug and basil oil showed also significant increase hemoglobin (Hb), glutathione peroxidase (GPX), superoxid dismutase (SOD), at doses 1ml, 3ml and 5ml basil oil. While, decrease insignificant in blood malondialdehyde (MDA) compared to (+ve) control group. The antioxidative effect is mainly due to the phenolic acids, flavonoids and anthocyanins present in basil. Free radical scavenging activity is one of the important antioxidant properties because of the deleterious role of free radicals [72]. These data may be due to the effect is exacerbated by the fact that aspirin irritates the mucous membrane lining the stomach. Aspirin taken with a meal also decreases the absorption of iron, vitamin C and folic acid (one of the B vitamins). Therefore, a person taking aspirin may suffer a deficiency unless there is a compensatory increase in the intake of these nutrients [73]. The effects of basil are not limited to its antimicrobial properties because evidence indicates that it also can lower oxidative damage in animal models. Feeding mice 200 and 400 mg/kg body weight with a hydroalcoholic extract of basil leaves for 15 days markedly increased GPX(1.22-1.4 fold), glutathione (GSH) reductase (1.16-1.28 fold), catalase (1.56-1.58 fold) and superoxide dismutase [74].

Regarding ultrasonographic examination, it was found that longitudinal scan using direct contact between the transducer and the examined rat (method one of scanning) at the left para-lumber region of apparent healthy rat (control -ve group) illustrated the echogenic patterns and relationships between left kidney (L.K.), spleen (Sp.) and stomach (St.). With advance, oblique scan at the level of left para-costal region showed the hyper-echoic gastric wall with internal folding of the gastric mucosa and the reverberation artifact indicating gas content of the stomach. Transverse scan at the left para-costal region showed the normal rosette shape of the pyloric region (P.). Transverse scan at the level of right para-costal region showed normal echo-pattern of liver, anechoic sac indicating gall bladder (G.B.) and stomach (St.) (Photo 1 to 13). Method two of scanning using water path when the abdominal region of the animal was submerged in the water and the transducer was toughing the surface of the water put the stomach in the focal zone of the transducer and enhanced the measurement of the diminutions. Method three of scanning using water path (as in method two) in addition to injection of the examined rat with 3ml normal saline intera-peritoneum gave the best results to assist the echogenesity and measurements of the gastric different diminutions.

From the obtained data, ultrasound examination of the stomach in the rate as experimental model and follow up of the pathological changes in the gastric diminutions can be used as a noninvasive method for diagnosis. The data of biochemical investigation and ultrasonographic examination are confirmed by the histopathological studies. Stomach of (- ve) control group showed normal histological gastric mucosa (Photo 14). While, stomach of (+ve) control rat group showed focal necrosis of gastric mucosa associated with mucosal and submucosal eosinophilic cells infiltration (Photo 15). Stomachs of treatment groups with during 1ml and 3 ml basil oil showed no histopathological changes (Photo16-18). Moreover, stomach of 5ml basil oil group showed congestion of mucosal blood vessel and submucosal edema (Photo 19). Histopathological studies on the gastric mucosa revealed that aspirin administration induced a mucosal ulceration, associated with significant increase in lipid peroxidation. This was manifested as lamina epithelial necrosis, blood vessels congestion and leukocytic infiltration [75, 76]. This effect on histological derangement was in accordance with our results. While, both 1ml and 3ml basil oil treatment groups had protective

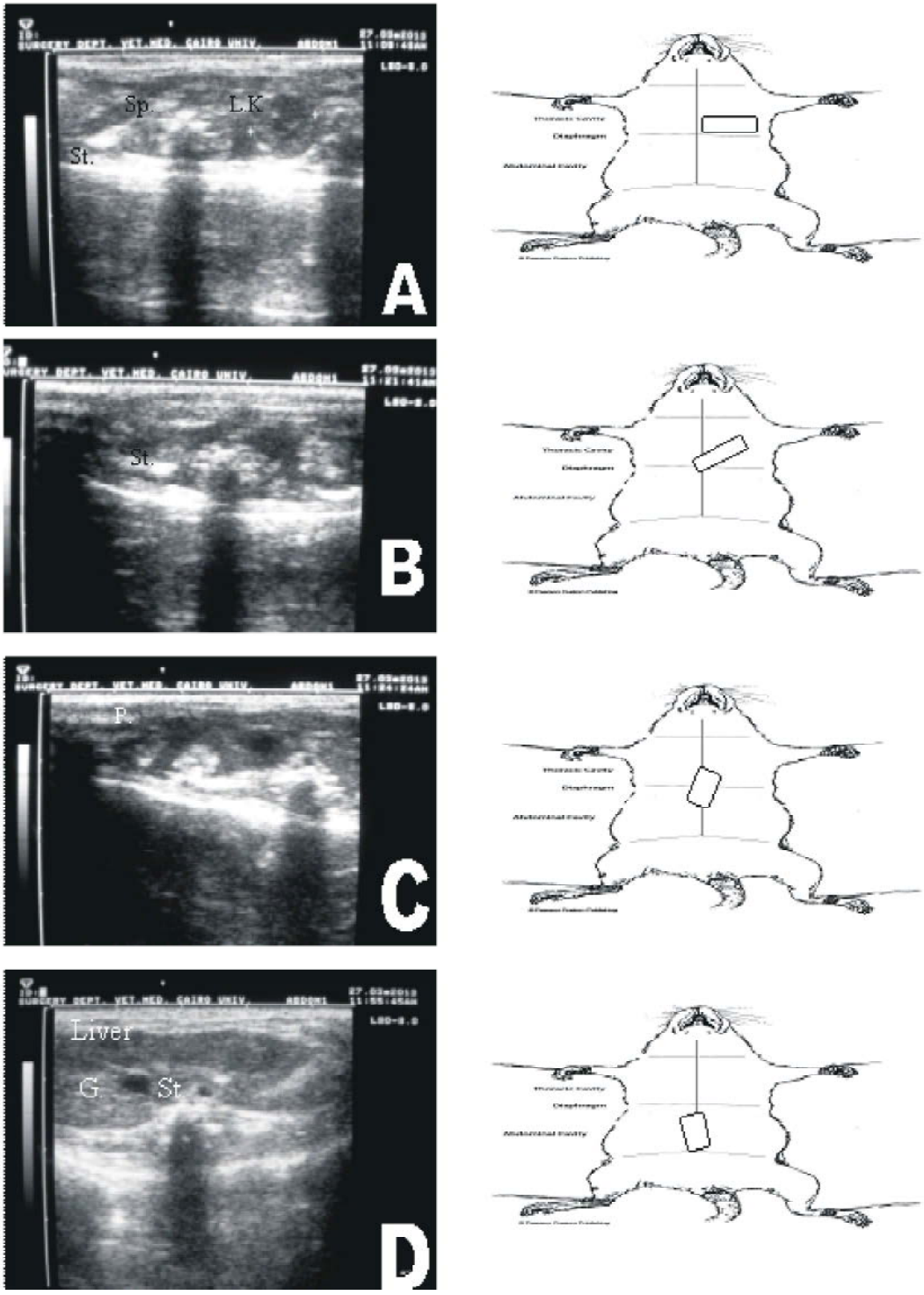

Photo 1: A. Longitudinal scan (Direct contact with the probe) at the left para-lumber region of apparent healthy rat showed left kidney (L.K.), spleen (Sp.) and stomach (St.). B. Oblique scan at the level of left para-costal region showed the hyper echoic gastric wall with internal folding of the gastric mucosa and the reverberation artifact indicating gas content of the stomach. C. Transverse scan at the left para-costal region showed the normal rosette shape of the pyloric region (P.). D. Transverse scan at the level of right para-costal region showed normal echo-pattern of liver, anechoic sac indicating gall bladder (G.B.) and stomach (St.)

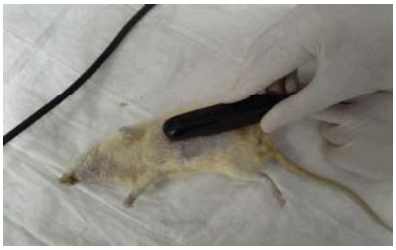

Photo 2: Position of liner transducer for longitudinal scan (direct contact probe) at the left para-lumber region

Photo 3: Position of liner transducer for longitudinal scan (through water path with or without intra-peritoneal injection with saline) at the left para-lumber region

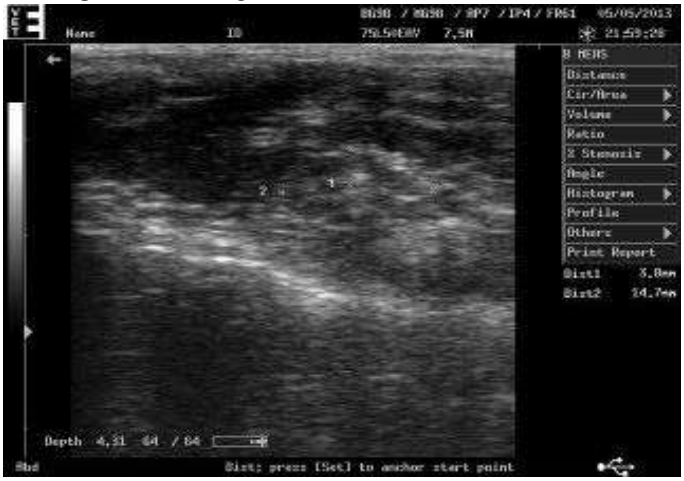

Photo 4: Longitudinal scan of a rat from -ve control group (direct contact probe) showed 3.8 mm gastric wall thickness and 14.7 mm longitudinal length of the stomach

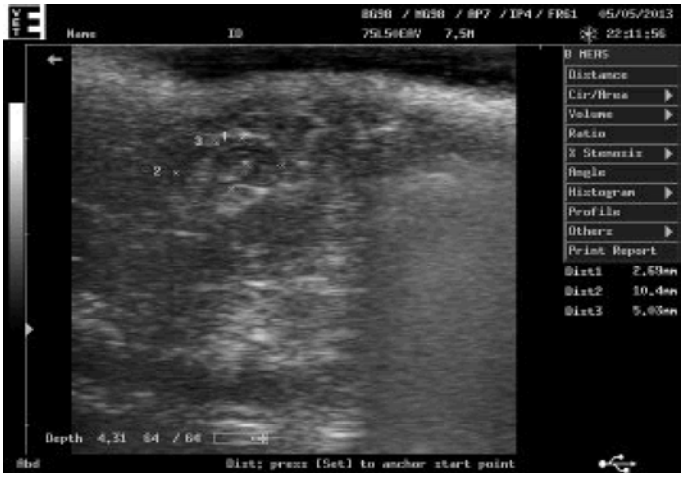

Photo 5: Longitudinal scan of a rat from -ve control group (through water path without intra-peritoneal injection with saline) showed gastric wall thickness 2.63 mm, longitudinal length 10.4 mm and width 5.03 mm

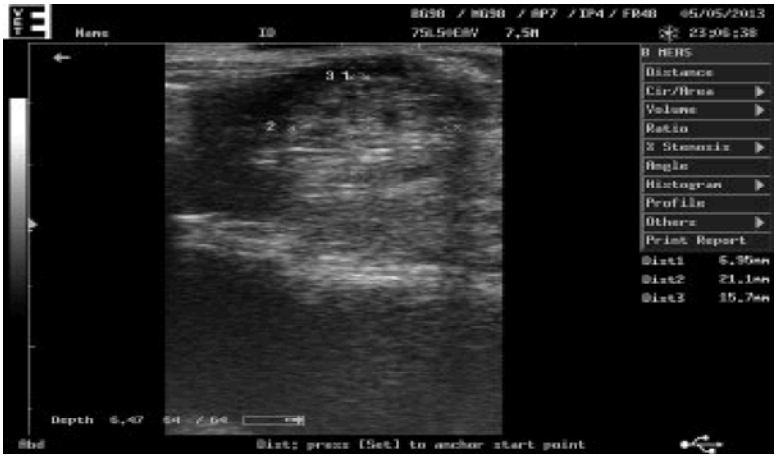

Photo 6: Longitudinal scan of rat from +ve control group (through water path associated with intra-peritoneal injection with saline) showed gastric wall thickness 6.35 mm, longitudinal length 21.1 mm and width 15.7 mm

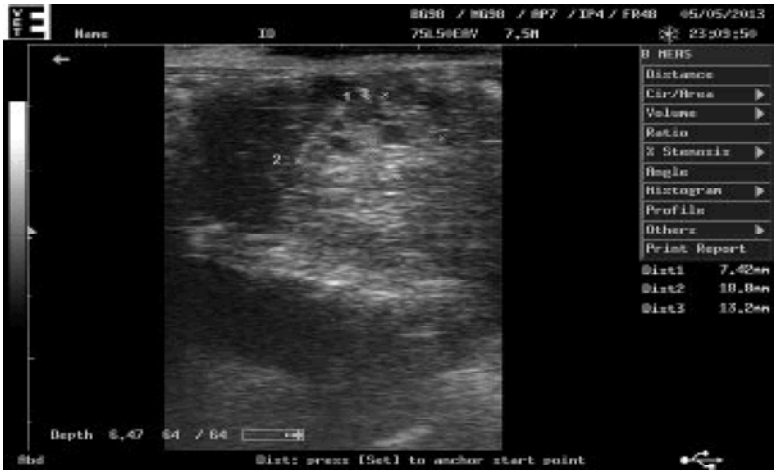

Photo 7: Longitudinal scan of rat from +ve control group (through water path associated with intra-peritoneal injection with saline) showed gastric wall thickness 7.42 mm, longitudinal length 18.8 mm and width 13.2 mm

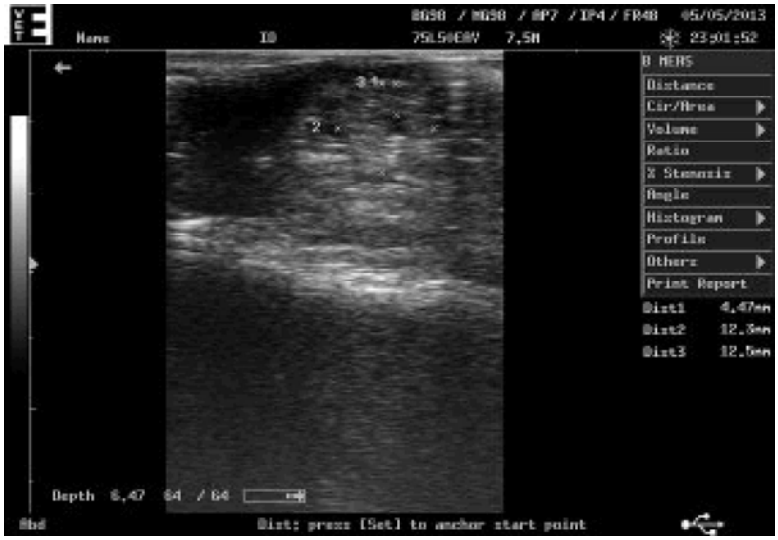

Photo 8: Transverse scan of a rat from drug group (through water path without intra-peritoneal injection with saline) showed gastric wall thickness 4.47 mm, longitudinal length 12.5 mm and width 12.5 mm

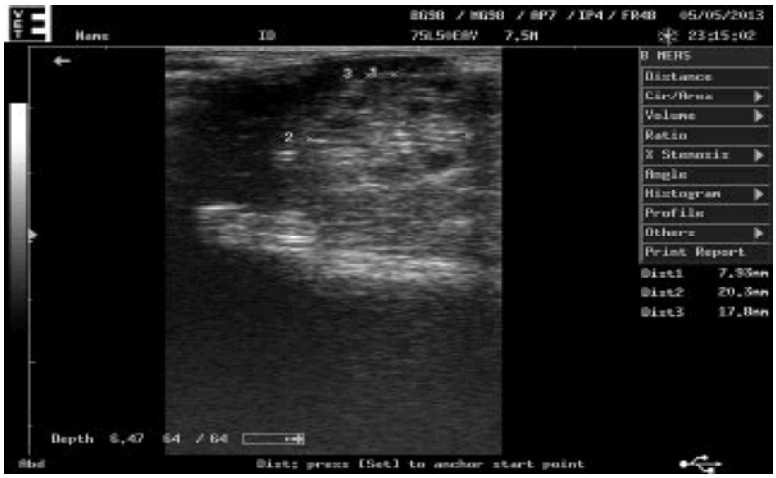

Photo 9: Longitudinal scan of a rat from the +ve control group (through water path associated with intra-peritoneal injection with saline) showed gastric wall thickness 7.33 mm, longitudinal length 20.3 mm and width 17.8 mm

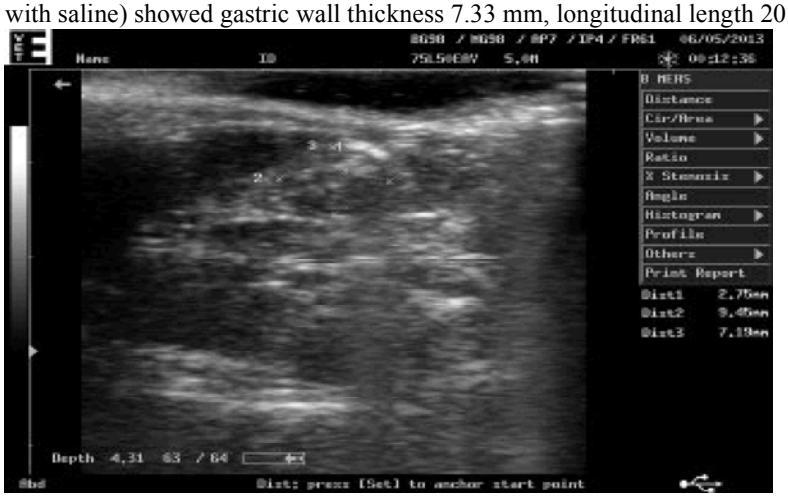

Photo 10: Longitudinal scan of a rat from group 1 ml basil oil (through water path without intra-peritoneal injection with saline) showed gastric wall thickness 2.75 mm, longitudinal length 9.45 mm and width 7.13 mm

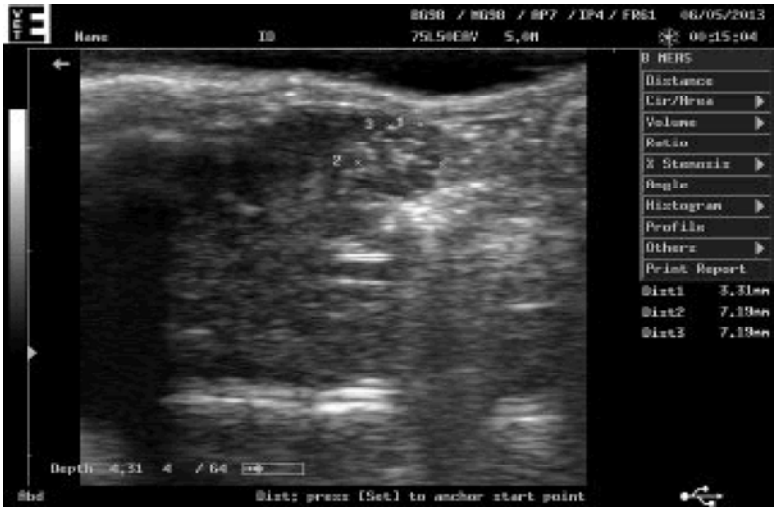

Photo 11: Transverse scan of a rat from group 1 ml basil oil (through water path without intra-peritoneal injection with saline) showed gastric wall thickness 3.31 mm, longitudinal length 7.13 mm and width 7.13 mm

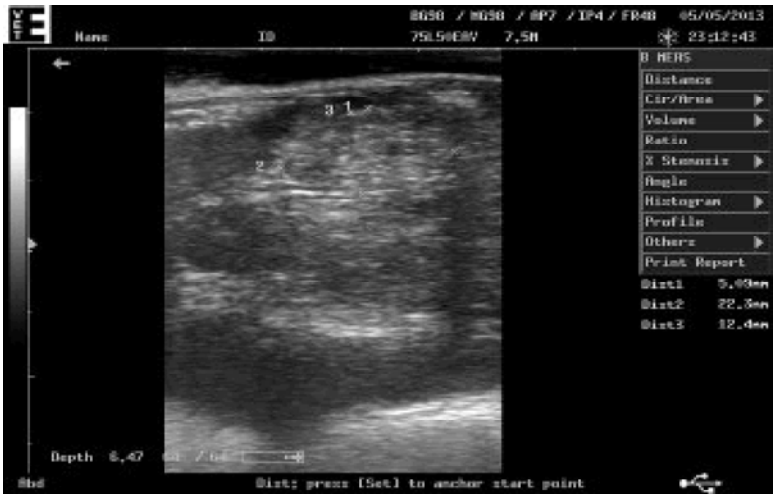

Photo 12: Longitudinal scan of a rat from group 3 ml basil oil (through water path associated with intra-peritoneal injection with saline) showed gastric wall thickness 5.43 mm, longitudinal length 22.3 mm and width 12.4 mm

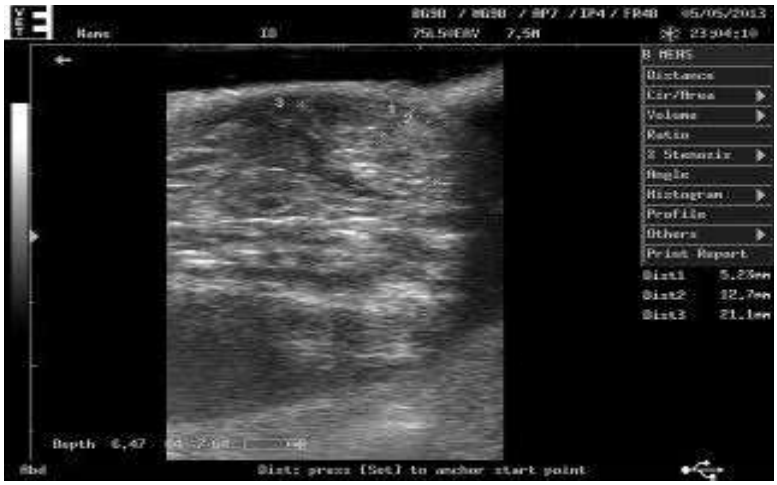

Photo13: Longitudinal scan of a rat from group 5 ml basil oil (through water path associated with intra-peritoneal injection with saline) showed gastric wall thickness 5.25 mm, longitudinal length 21.1 mm and width 12.7 mm

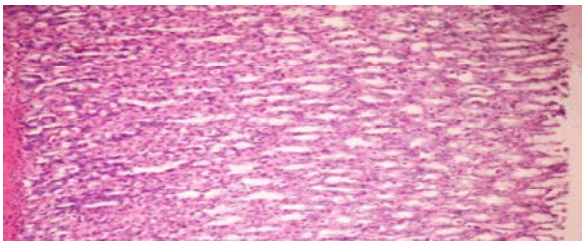

Photo 14: Stomach of rat from control group showing normal gastric mucosa (H and E X 200)

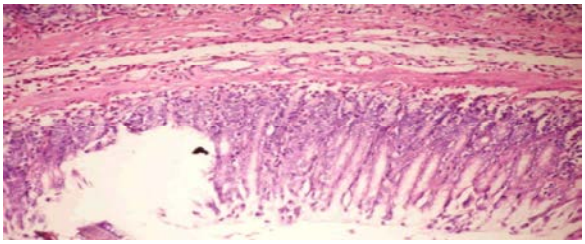

Photo 15: Stomach of rat from positive control group showing focal necrosis of gastric mucosa associated with mucosal and submucosal eosinophilic cells infiltration (H and E X 200)

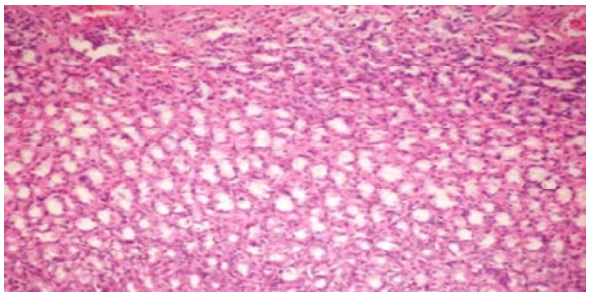

Photo 16: Stomach of rat from drug group showing no histopathological changes (H and E X 200)

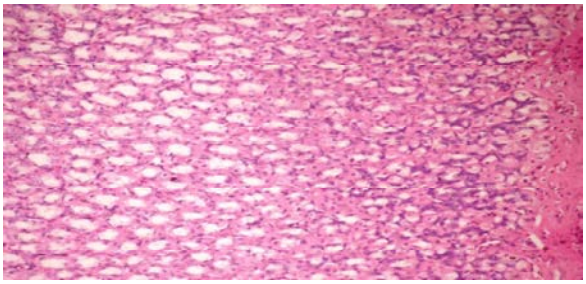

Photo 17: Stomach of rat from group 1ml basil oil showing no histopathological changes (H and E X 200)

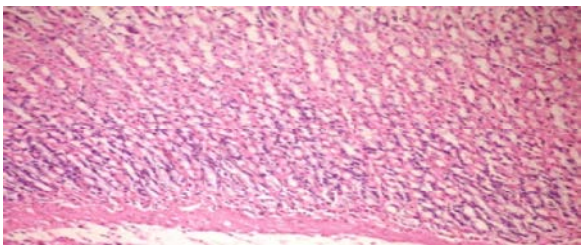

Photo 18: Stomach of rat from group 3 ml basil oil showing no histopathological changes (H and E X 200)

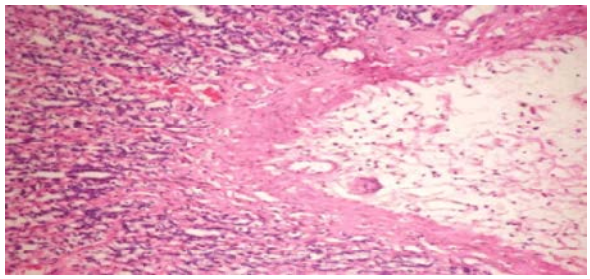

Photo 19: Stomach of rat from 5 ml basil group showing congestion of mucosal blood vessel and submucosal edema (H and E X 200)

effect against aspirin-induced inflammatory infiltration and congestion at the ulcer sites. It prevented gastric mucosal lesions through its flavonoid content that could scavenge free radicals, inhibit lipid peroxidation and increase prostaglandins and mucosal content of the gastric mucosa; showing cytoprotective effects [77].

CONCLUSION

It could be concluded that, the administration of basil oil (especially, at lower concentration 1ml followed 3 ml than 5 ml) improved the histopathological changes in the gastric mucosa as well as gastric acid secretion that may be due to its cytoprotectivity effect coupled with anti-secretory activity. The high amount of essential fatty acids especially linalool and eugenol in *Ocimum basilicum* is likely to be responsible for the higher antioxidant activity of the basil oil. The recommended dose of basil oil is 7 to 21 ml (average 14 ml) per one kilogram body weight for ulcer patients per day (equal 1 to 3ml/kg B.Wt of rats) to be used for treatment of acute gastric ulcer.

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