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ORIGINAL ARTICLE **OPEN ACCESS**

Efficacy of Some Medicinal Plant oils in Indomethacin Toxicated Rats

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ABSTRACT
Gastric ulcer remains one of the dangerous diseases in the world. Even though there are various drugs for its treatment but most of them produce various adverse reactions. This study was conducted to compare the protective effects of pretreatment with some medicinal plants oil (MPO) as ginger oil, thyme oil, anise oil and ranitidine, a reference drug, at a dose of 50 mg/kg b.wt two weeks prior to indomethacin. The rats were randomly divided into six groups. GP (1) (negative control) received only distilled water; GP (2) (ulcerated control) was orally gavages with indomethacin only. GP (3) to GP (6) were pretreated for 2 weeks with ranitidine (drug control) and ginger oil, anise oil and thyme oil respectively. Whole blood and serum were collected six hours later for estimation of some hematological, biochemical, antioxidant and oxidative stress parameters. In addition, gross and histopathological changes were recorded and ulcer index was calculated. Pretreatment with MPO produced significant reduction in gastric mucosal lesions and improved results of hematological, biochemical, antioxidant and oxidative stress assays nearly as ranitidin in different degrees. Ginger oil has more potent anti-ulcer activities than thyme and anise oils against indomethacin-induced gastric lesions, presumably via their antioxidant and anti-inflammatory properties.
Key words: *Indomethacin, Ranitidine, Ginger oil, Thyme oil, Anise oil, Ulcer index Hematological, Biochemical, Antioxidant, Pathology.*

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INTRODUCTION

Gastric ulcer is a vital problem in veterinary medicine especially in small animals [1]. The etiology of peptic ulcer ranged from psychological stress, analgesic and *Helicobacter pylori (H. pylori)* infection [2]. Other gastric non-*H.pylori* like "*Candidatus infection* was colonized the stomach of dogs and cats *and Helicobacter bovis*" was extremely predominant in cattle abomasums. The non-*H. pylori* infections in humans mainly originated from animals[3]. In small animals, gastric ulcer was mainly caused by long-term administration of steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) [4]. The NSAIDs are a group of drugs able to decrease the pain and inflammation, having a potent prostaglandin synthesis inhibitor. Indomethacin is one of the NSAIDs [5, 6].

Moreover, all NSAIDs involved the risk of upper gastrointestinal hemorrhage and peptic ulcer disease [7]. Gastric ulcer developed as a cause of several endogenous factors including the decrease in gastric anti-oxidant enzymes activity and imbalance in the cytoprotective factors [8, 9]. Mild to severe gastritis has been recorded in several animal species as in dogs [4] and horses [10].

Some medicinal plants could achieved the gastroprotective activity, through inhibition of the induced ulcer formation, this could be attributed to their antioxidant properties [8]. The successes were in the usage of famous reachable and more safe medicinal plants oils for gastric ulcer protection in animal model.

Ginger is an important food spice widely used in harmaceutical and cosmetic industries. It contains a unique flavor derived from both volatile and non-volatile oils. The spicy compounds are gingerol and shagaol, while zingiberene is a major constituent of oils [11]. Mohsen *et al.* [12] mentioned that, ginger also useful in gastrointestinal disorders and gastric ulcerogenesis. Ginger also has a valuable anti-emetic

effect in motion sickness, nausea and vomiting related to pregnancy [13]**.** Ginger also reported that it may help in reduction of artherosclerosis and heart attacks through reducing the stickiness of blood platelets [14].

Thyme is enemy of poison, anti spasm, eases flood blowing, invokes sexual activities and promotes consciousness and intelligence as well. It is useful for liver disorder. Meanwhile, it is used in pulmonary infections, catarrh, bronchitis, angina, indigestion, stomach sore and inflation [15]**.** The thyme, other than anti flatulent and anti inflammatory, has other complete, sensitive and semi-sensitive effects on the major cause of gastric ulcer, *helicobacter pylori*, as well as other bacteria like *salmonella, shigella, aerouse staph* and pathogenic E*.coli* [16]**.**

Pimpinella anisum L., a plant belonging to the Umbelliferae family, is one of the oldest medicinal plants [17]**.** Aniseeds contain 1.5–5% essential oil and used as flavoring, digestive, carminative agent as well as helpful in reliefing of gastrointestinal spasms. Anise seeds are used as analgesic in migraine and also as carminative, aromatic, disinfectant, and diuretic in traditional medicine [18]**.**

On the basis of these common uses of the tested oil extracts, this work was therefore aimed to assess the effect of the oil extract of ginger, thyum and anis plants on induced inflammation and ulcer.

MATERIALS AND METHODS

Experimental animals:

Twenty four adult male albino rats (Sprague Dawley) weighing 160-190 grams and were obtained from Helwan Farm of Laboratory Animals, Egypt. Rats were kept under controlled environment, maintained under a 12 hours light:dark cycle, 24^{o}C (± 3^{o}C) and 50-70% humidity. The animals were provided with balanced standard diet and water ad libitum. All rats were reared for one week before the experiment for acclimatization. All animal procedures were performed in accordance with the Ethics Committee of the National Research Centre, Egypt; registration number (09/189) and followed the recommendations of the National Institutes of Health[19]**.**

Medicinal plants oil and drugs:

Indomethacin was purchased from Pharmaceuticals, Egypt. It was suspended in 0.5 % of carboxymethylcellulose (Na salts, Merck, Darmstadt, Germany) in water (6mg/ml).

Rantidin was purchased from Pharco Pharmaceuticals Company, Egypt. It was administrated orally gavages in a dose rate 50mg/kg once daily for 2 weeks.

Medicinal plants oil (MPO)

Isolation of essential oil from Anise

Grind two whole star anise seed pods (approximately 3.5 g) in a mortar and pestle and place the ground material into a 100 mL 2-neck round bottom flask. Add water (40 mL) and begin the distillation. After collecting about 20 mL of cloudy distillate, add more water (20 mL) into the distillation flask from the dropping funnel. Collect a further 20 mL of distillate then stop the distillation. Combine the distillates in a separatory funnel and extract with diethyl ether (2 × 20 mL). Dry the ethereal layer with anhydrous magnesium sulfate and filter the solution. Remove the ether using a rotary evaporator and collect the colourless star anise essential oil [20].

Isolation of essential oil from Thymus vulgaris

The steam distillation was done using a Neoclevenger system: In 5 L round bottom glass flask were added 300 g of milled Thymi herba and 3 L of distilled water (vegetal material/ extraction solvent rate = 1/10 (m/v). The mixture was left under reflux for 5 hours. The yellow and intense aromatic volatile oil (2.7mL) was collected and stored at 4°C [21].

Isolation of essential oil from ginger

The ground fresh ginger (200g) and dry ginger (50g) rhizomes were hydro distilled for 5 hrs in a Clevenger type apparatus. The oils were dried over anhydrous sodium sulphate [22].

Experimental design:

Rats were randomly divided into six groups (4×6). The experimental design was as the following:

(GP1) (negative control) received only distilled water.

(GP2) (ulcerated control) was orally gavages with indomethacin only.

(GP3) was orally supplemented with rantidine + indomethacin.

(GP4) was orally supplemented with thyme oil + indomethacin.

(GP5) was orally supplemented with ginger oil + indomethacin.

(GP6) was orally supplemented with Anise oil + indomethacin.

Ranitidine was administered at a dose 50mg/kg b.wt according to Rodríguez *et al***.** [23], serve as drug control. Oils of ginger, anise and thyme were administered the at a dose of 0.1 ml/kg b.wt, respectively. The MPO and ranitidine were orally gavages once daily for 2 weeks [24]. Then, the rats were deprived of

food for 24 hours prior to ulcer induction with indomethacin (50 mg/kg b.wt once orally) [25] for all groups except (GP1).

Samples collection and preparation:

Blood sampling: the blood samples were taken from the retro orbital plexus and were immediately divided into two separate tubes. One of them was plain centrifuge tube for serum separation. The clear serum was stored in epindorf tubes at -20°C for subsequent biochemical analysis. The other blood sample was collected on EDTA (0.5mg/ ml blood) for hematological examination.

A. All rats were sacrificed by cervical dislocation 6 hours after ulcer induction, blood samples were taken and humanely midline incision was done in the abdomen and the stomachs were incised lengthways the greater curvatures. The stomach was washed with normal saline and stomach macroscopic examination and assessment of the mean ulcer index (MUI) in addition to the % inhibition of ulcer were done.

B. Degrees of ulceration:

Stomach sample was washed carefully with normal saline and was pinned on a board. The ulcers were scored using glass magnifier and graded as the method of Sabiu *et al*.**,** [26] with few modification. In details, the ulcers were graded to 0, 1, 2, 3, 4, 5 and 6 as, almost normal mucosa, vascular congestions, one or two lesions, 3 or 4 lesion, severe lesions, very severe lesions and mucosa full of lesions respectively. The mean ulcer index (MUI) and the percentage of inhibition were calculated for each group according to the method:

MUI = total ulcer score / number of rats ulcerated

Percentage (%) inhibition = (MUI of the indomethacin group - MUI of MPO or ranitidine groups)*100/ MUI of the indomethacin group.

C.Samples for tissue extract: after macroscopic examination, one half of the glandular stomach mucosa was put on iced cold surface soon. It was cut, weighed and homogenized by means of ultrasonic homogenizer (Cole-Parmer Instrument Company, USA) in ice cold saline formed 10 % homogenate. Then centrifuged (4000 rpm for 15 minutes at 4°C) and the supernatant were preserved at -80 °C until used.

D. Tissue specimens for histopathology were collected from different parts of stomach, in addition to, liver, kidneys, cecum and heart and fixed in 10% formalin for histopathological examination.

Hematological study

The complete blood samples were used for determination of the erythrogram (RBC count, PCV and Hb), blood indices calculation, total leukocyte count (TLC) [27] and differential leukocyte count DLC) [28].

Serum biochemical analysis:

Frozen serum samples were used for determination of AST (Randox Co., UK), ALT (Randox Co., UK), ALP, total protein, albumin, cholesterol (Biodiagnostic, Egypt), creatinine, urea (Diamond, Egypt). The globulin and A/G ratio were calculated according to Kaneko *et al*. [29].

Estimation of malondialdehyde **(**MDA) was based on reaction of malondialdehyde with thiobarbituric acid (1:2 molecules respectively) in acidic medium, according to Satoh [30]**.**

Estimation of catalase was based on its reaction with specific H2O2 quantity and the reaction had stopped by catalase inhibitor after specific time [31].

Nitric oxide (NO) was estimated according to nitrite method. In the presence of nitrite (in acidic media), the resultant substance was coupled with N-(1-naphthyl) ethylenediamine. The azo dye had a bright reddish purple color and could be measured [32]**.**

All antioxidant and oxidative stress kits (readymade diagnostic) were obtained from Bio-diagnostic, Egypt, and estimated by enzymatic colorimetric method using semiautomatic spectrophotometer (BM-Germany 5010).

Histopathological examination

Specimens were routinely processed, fixed embedded in paraffin wax. Paraffin sections of 5 µm thickness were cut and picked up on uncoated slides, dried, deparafinized, rehydrated with graded alcohol, washed and stained with H&E according to Bancroft and Gamble [33].

Statistical analysis

All data were statistical analysis using SAS 9. 2 for windows, (SAS Institute Inc., U.S.A). One-way analysis of variance (ANOVA) and Duncan's multiple-range tests were done for different hematological and serum biochemical parameters. The data were expressed as mean ± SD($P<0.05$).

RESULTS

Hematological results

Rats in (GP2) had significantly reduced RBCs count, Hb and Hct value ($p < 0.05$) when compared with rats in (GP1). Meanwhile, (GP3) to (GP6) showed significantly increased Hb and Hct value when compared with (GP2). MCV and MCHC levels were insignificantly changed among all groups (Table 1).

Oral administration of Indomethacin to rats significantly increased TLC and neutrophil counts in (GP2) compared with rats in (GP1). Rats in (GP5) and (GP6) showed significant increase in TLC, in contrast to (GP3) and (GP4), when compared with (GP1) (Table 2).

Biochemical results

The activities of liver aminotransferases (ALT & AST) and alkaline phosphatase (ALP) were significantly increased in (GP2) when compared with (GP1). Both aminotransferases were corrected in other groups (Table 3).

Total cholesterol and triglyceride levels were significantly increased in (GP2) when compared with (GP1). However, their levels in (GP5) and (GP6) were significantly reduced when compared with (GP2) (Table 3).

Albumin level fell much lower in (GP2) than in (GP1). Then returned nearly to its normal level in (GP3) to (GP6). Meanwhile, levels of total protein were insignificantly changed (Table 3).

Significant elevation in both serum urea and creatinine levels was shown in (GP2) when compared with (GP1) (Table 3). In treatment groups (GP3) to (GP6), significant improvement in serum urea and creatinine levels was observed.

Rats in (GP2) showed significant increase in gastric and serum MDA level ($p < 0.05$) (Table 4) when compared with (GP1). In contrast, gastric, serum NO and CAT levels were significantly down-regulated in (GP2) when compared with (GP1). Most of these alterations were ameliorated in treatment groups (GP3) to (GP6) when compared with (GP2) except of serum NO levels.

Mean ulcer index (MUI) and (%) inhibition of ulcer

The present study showed that MPO significantly inhibited gastric mucosal ulceration induced by indomethacin in (GP2) comparing to rantidine treated group (GP3)(Table 5) (Fig.1).

Gross and microscopic examination of stomach

Gross and microscopic examination of rat stomach from (GP1) to (GP6) were shown in (Figs.2&3). Non-glandular and glandular gastric mucosae of groups (GP1), (GP5) and (GP6) showed normal appearance. Multiple ulcerations with raised edges in non-glandular mucosa and erosions in glandular mucosa were observed in (GP2). Few erosive areas were seen in non-glandular portion from (GP3). Meanwhile, mild congestion only was noticed in non-glandular mucosa of (GP4).

Microscopic examination of stomach revealed normal non-glandular and glandular gastric mucosae in (GP1) and (GP5), multiple ulcerations with raised edges in non-glandular mucosa and erosions in glandular mucosa of (GP2), normal non-glandular stomach with ulceration in glandular mucosa from (GP3), congested blood vessels in submucosa of non- glandular portion with focal necrosis in mucosa, edema and leukocytic cells infiltration in submucosa of glandular portion of (GP4), normal non-glandular mucosa, necrosis of tip of glands in glandular mucosa (GP6).

Microscopic examination of other organs

Microscopic examination of liver from (GP1) to (GP6) was presented in (Fig.4). Liver of (GP1) showed normal histological picture, vacuolar degeneration in (GP2), cellular swelling with individual cell death in (GP3), large vacuoles in cytoplasm of hepatocytes, with moderate leukocytic cells infiltration in portal tract, cloudy swelling of hepatocytes with more eosinophilic cytoplasm in (GP4), minute vacuoles in cytoplasm of hepatocytes and mild leukocytic cells infiltration in portal tract in (GP5), individualized hepatocytes and dilated hepatic sinusoids in (GP6).

Microscopic examination of kidneys showed normal histological picture in (GP1), hyaline droplet degeneration in tubular epithelium in (GP2), vacuolar degeneration in tubular epithelium in (GP3), interstitial leukocytic cells infiltration in (GP4), mild perivascular leukocytic cells infiltration in (GP5), tubular dilation with the presence of desquamated epithelial cells (GP6) (Fig.5).

Microscopic examination of cecum showed normal histological picture in (GP1) and (GP5), loss of superficial epithelium, congested blood vessels and edema in submucosa from (GP2), reduced thickness of mucosa in (GP3), dilated cecal crypts with eosinophilic material in thinner mucosa and moderate edema in submucosa from (GP4), loss of superficial epithelium and necrosis in mucosa, congested blood vessels, leukocytic cells infiltration and marked edema in submucosa from (GP6) (Fig. 6).

Microscopic examination of heart show normal histological picture in (GP1) and (GP5), disorganized carciac myocytes due to hyalinization and edema in (GP2), mild hyalinization, edema and congestion in (GP3), mild hyaline degeneration in cardiac myocytes from (GP4), mild hyalinization and congested blood vessels in (GP6) (Fig. 7).

Table (1): Erythrogram (Mean ± S.D) of rats treated with some medicinal plants oils for two weeks.

Groups	RBC 10^6/µL	Hb g/dL	PCV %	MCV fL	MCH pg	MCHC %
GP1	8.18±0.40^{a}	12.05±0.52^{b}	38.36±1.63^{a}	46.92±1.99^{b}	14.74±0.63^{b}	31.42±0.03 ^{a}
GP2	6.09±0.31^{b}	9.45±0.43^{c}	31.81±2.07^{b}	52.19±0.74^{b}	15.57±1.30^{ab}	29.86±2.83 ^{c}
GP3	7.10±0.72^{ab}	11.93±0.55^{b}	41.91±3.26^{a}	60.07±10.95^{ab}	17.06±2.57^{ab}	28.54±0.87 ^{d}
GP4	6.45±1.13^{b}	11.84±0.60^{b}	41.67±4.46^{a}	67.57±16.89^{a}	18.99±3.56^{a}	28.68±2.66 ^{d}
GP5	8.24±0.77^{a}	12.36±0.37^{b}	40.48±1.85^{a}	49.31±2.29^{b}	15.09±1.05^{b}	30.57±0.75 ^{b}
GP6	7.89±1.20^{a}	13.19.±0.53^{a}	42.86±2.80^{a}	55.82±10.81^{ab}	17.21±3.22^{ab}	30.89±1.93 ^{b}

Different small superscript letters are significantly different at p≤0.05.

Table (2): Leukogram (Mean ± S.D) of rats treated with some medicinal plants oils for two weeks.

Groups	TLC 10^3/µL	Neutrophil 10^3/µL	Lymphocyte 10^3/µL	Monocyte 10^3/µL	Eosinophil 10^3/µL	Basophil 10^3/µL
GP1	10.48±1.45^{c}	3.02±0.49^{d}	7.03±1.10^{ab}	0.29±0.14^{c}	0.11±0.10^{c}	0.03±0.04
GP2	25.67±7.86^{a}	17.66±5.53^{b}	6.77±2.62^{ab}	0.93±0.67^{ab}	0.3±0.25^{abc}	0.0±0.00
GP3	15.72±1.89^{cb}	6.14±2.39^{cd}	8.58±2.75^{a}	0.48±0.17^{bc}	0.46±0.13^{a}	0.06±0.08
GP4	16.50±2.06^{b}	8.83±1.83^{c}	7.06±0.34^{ab}	0.40±0.13^{c}	0.21±0.06^{bc}	0.00±0.00
GP5	26.88±0.57^{a}	21.96±0.80^{a}	3.85±0.25^{b}	0.99±0.15^{a}	0.09±0.12^{c}	0.00±0.00
GP6	26.12±2.42^{a}	17.55±2.60^{b}	7.33±3.12^{a}	0.78±0.24^{abc}	0.35±0.14^{ab}	0.09±0.13

Different small superscript letters are significantly different at p≤0.05.

Table (3): Some serum biochemical (Mean ± S.D) of rats treated with some medicinal plants oils for two weeks.

Groups	AST (µ/L)	ALT (µ/L)	ALP	T.P gm/dl	Albumin gm/dl	Globulin	A/G ratio	Cholesterol (mg/dl)	triglycerid mg/dl	Urea mg/dl	Creatinin mg/dl
GP1	33.67± 5.79b^{c}	9.60± 1.45^{b}	222.17± 59.54^{b}	5.65± 0.49^{a}	4.29± 0.13^{a}	1.36± 0.56^{b}	2.04± 0.52^{ab}	71.67± 8.73^{b}	74.67± 8.96^{b}	30.28± 2.66^{d}	0.72± 0.09^{d}
GP2	58.00± 5.89^{a}	96.14± 51.53^{a}	388.67± 23.04^{a}	5.29± 0.53^{a}	3.01± 0.35^{b}	2.28± 0.87^{b}	1.70± 1.01^{ab}	148.33± 10.27^{a}	358.00± 115.15^{a}	52.33± 8.83^{a}	18.37± 3.65^{a}
GP3	28.20± 8.63b^{c}	16.91± 2.23^{b}	275.00± 11.23^{b}	5.99± 0.76^{a}	4.16± 0.22^{a}	1.83± 0.96^{b}	1.93± 1.33^{ab}	96.67± 7.93^{b}	92.67± 2.49^{b}	38.26± 5.93^{c}	0.71± 0.29^{d}
GP4	52.40± 6.79^{a}	15.55± 2.53^{b}	103.58± 11.08^{c}	6.05± 0.75^{a}	4.19± 0.29^{a}	1.86± 0.53^{b}	2.40± 0.53^{ab}	69.67± 40.55^{b}	126.67± 25.62^{b}	37.33± 5.75^{c}	0.71± 0.22^{b}
GP5	38.60± 6.73^{b}	22.90± 9.88^{b}	225.50± 25.30^{b}	5.73± 0.51^{a}	3.84± 0.67^{a}	1.89± 1.09^{b}	2.90± 1.55^{a}	60.33± 35.46^{b}	102.33± 28.89^{b}	45.78± 6.67^{b}	1.72± 0.86^{b}
GP6	26.00± 9.07^{c}	21.15± 5.73^{b}	142.08± 50.07^{c}	7.58± 0.59^{a}	3.98± 0.16^{a}	3.60± 0.62^{a}	1.02± 0.19^{b}	103.67± 33.48^{b}	81.67± 38.58^{b}	45.29± 4.30^{b}	0.82± 0.38^{c}

Different small superscript letters are significantly different at p≤0.05.

Table (4): Some antioxidant and oxidative stress parameters (Mean ± S.D) of rats treated with some medicinal plants oils for two weeks.

Groups	Nitric oxide (µ mol/L)		Catalase (nmol/ml)		MDA (nmol/ml)	
	Serum	Stomach Homogenate	Serum	Stomach Homogenate	Serum	Stomach Homogenate
GP1	4.17±0.63^{ab}	3.80±0.72^{c}	6.13±0.76^{ab}	13.42±1.39^{b}	8.46±1.52^{b}	7.09±1.92^{c}
GP2	0.61±0.35^{d}	1.37±0.14^{d}	3.05±0.29^{c}	8.23±0.20^{c}	18.73±3.52^{a}	30.74±1.42^{a}
GP3	5.14±2.13^{a}	6.02±0.68^{ab}	6.30±2.01^{ab}	12.94±1.71^{b}	11.34±2.29^{b}	6.26±3.98^{c}
GP4	4.27±3.08^{ab}	7.64±83^{a}	5.53±1.80^{bc}	14.43±3.06^{ab}	9.76±1.39^{b}	13.68±4.81^{b}
GP5	2.36±2.07^{c}	4.49±2.11^{bc}	5.39±1.37^{bc}	13.64±2.28^{b}	11.80±1.87^{b}	9.68±4.59^{bc}
GP6	1.99±0.99^{c}	5.55±1.43^{bc}	6.52±2.44^{a}	17.29±3.03^{a}	10.38±2.55^{b}	11.19±3.63^{bc}

Different small superscript letters are significantly different at p≤0.05.

Table (5): the mean ulcer index (Mean ± S.D) of rats treated with some medicinal plants oils for two weeks.

Groups	MUI [Score 0–6]
GP1	-
GP2	4.75+-
GP3	1.50+-
GP4	1.88
GP5	2.31
GP6	2.50

MUI : Mean Ulcer index
Different small superscript letters are significantly different at p≤0.05.

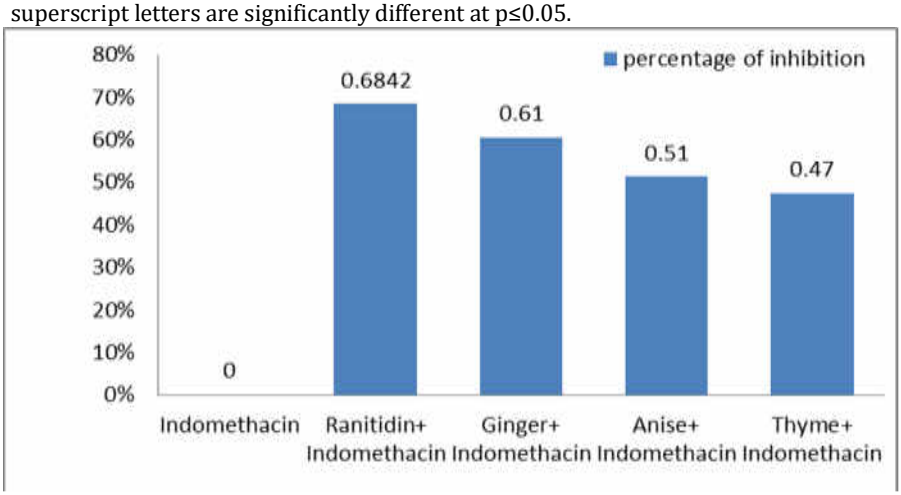

Fig. (1): Percentage of inhibition

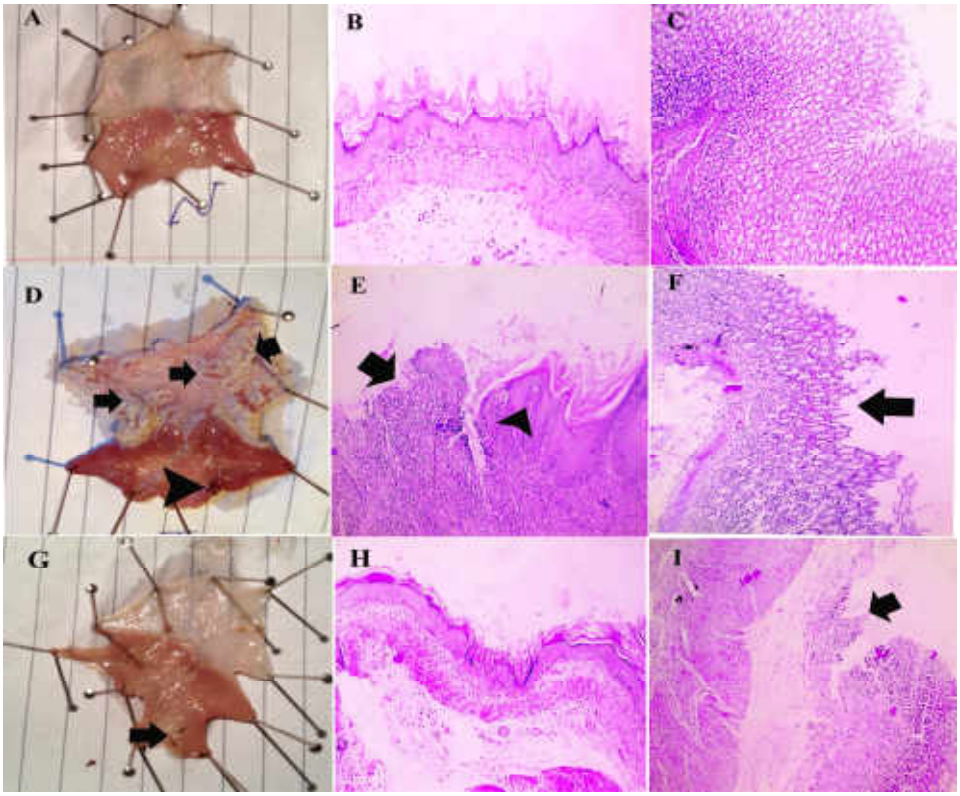

Fig. 2: (A-C): Rat stomach of (GP1) shows normal gross picture **(A)**, normal microscopic pictures of non-glandular **(B)** and glandular gastric mucosae **(C)** (H&E X100). **(D-F):** Rat stomach from (GP2) shows multiple ulcerations with raised edges in non-glandular mucosa (arrows) and erosions in glandular mucosa (arrowhead) **(D).** Microscopic picture shows ulceration (arrow) with hyperplastic edge (arrowhead) **(E)** and erosion in glandular mucosa (arrow) **(F)** (H&E X100). **(G-I):** Rat stomach from (GP3) shows linear ulceration in glandular portion (arrow) **(G)**. Microscopic picture shows normal non-glandular stomach **(H)**, ulceration in glandular mucosa (arrow) **(I)** (H&E X100).

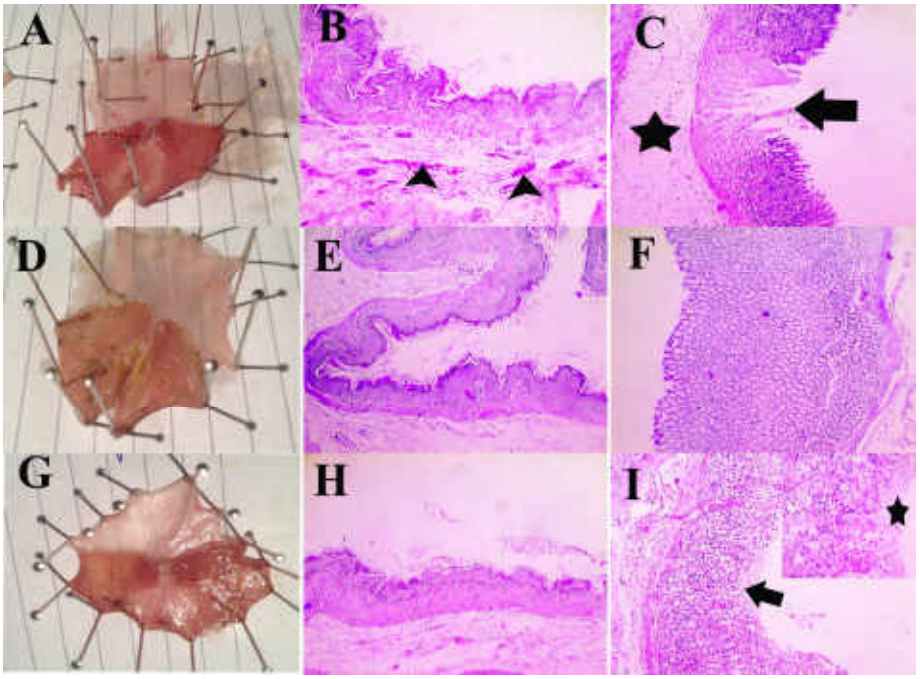

Fig. 3: (A-C): Rat stomach from (GP4) shows mild congestion in non-glandular mucosa **(A)**. Microscopic picture reveals congested blood vessels in submucosa of non- glandular portion (arrowheads) **(B)** focal necrosis in mucosa, edema and leukocytic cells infiltration in submucosa of glandular portion **(C)** (H&E X100). **(D-F):** Rrat stomach from (GP5) shows normal appearance **(D).** Microscopic picture reveals normal glandular **(E)** and non-glandular mucosa **(F)** (H&E X100). **(G-I):** Rat stomach from (GP6) shows normal gross picture **(G)**, normal non-glandular mucosa **(H)**, necrosis of tip of glands in glandular mucosa (arrow) **(I)** (H&E X100), insert is high magnification to show necrotic tips (asterisk) (H&E X200).

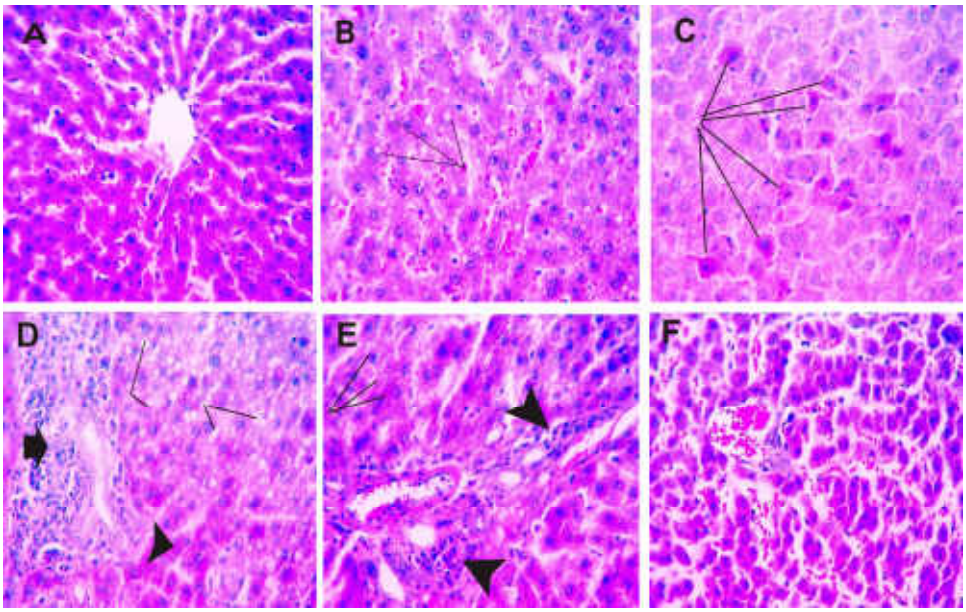

Fig. 4. (A-F): Light micrographs of liver show normal histological picture in (GP1) **(A)**, vacuolar degeneration in (GP2) **(B)**, cellular swelling with individual cell death (black lines) in (GP3) **(C)**, large vacuoles in cytoplasm of hepatocytes (black lines), with moderate leukocytic cells infiltration in portal tract (arrow), cloudy swelling of hepatocytes with more eosinophilic cytoplasm (arrowhead) in (GP4) **(D)**, minute vacuoles in cytoplasm of hepatocytes (black lines) and mild leukocytic cells infiltration in portal tract (arrowheads) in (GP5) **(E)**, individualized hepatocytes and dilated hepatic sinusoids in (GP6) **(F)** (A-F: H&E X200).

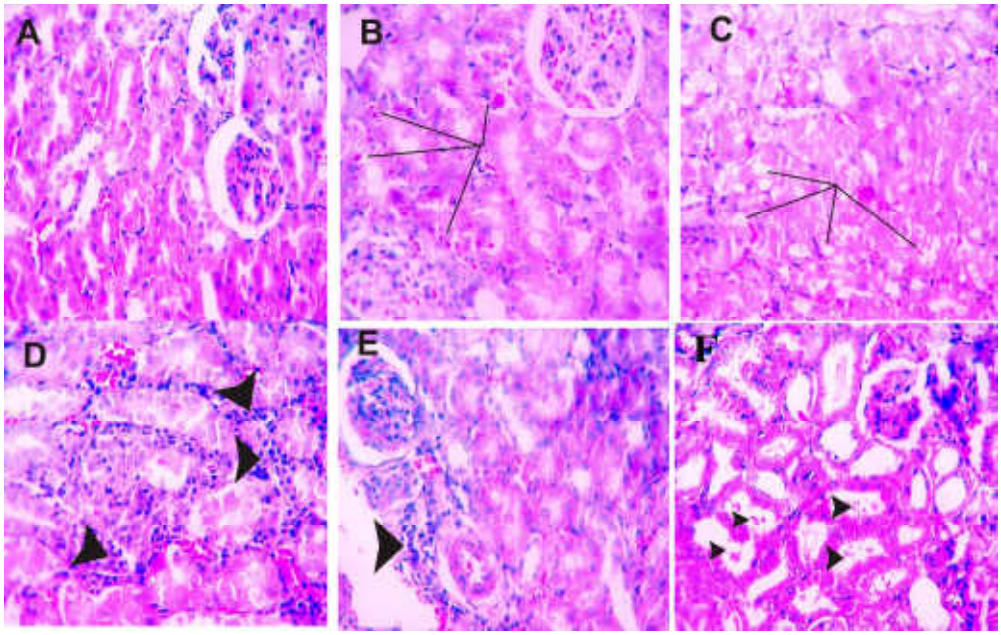

Fig. 5. (A-F): Light micrographs of kidneys show normal histological picture in (GP1) **(A)**, hyaline droplet degeneration in tubular epithelium in (GP2) (black lines) **(B)**, vacuolar degeneration in tubular epithelium in (GP3) (black lines) **(C)**, interstitial leukocytic cells infiltration (arrowheads) in (GP4) **(D)**, mild perivascular leukocytic cells infiltration (arrowhead) in (GP5) **(E)**, tubular dilation with the presence of desquamated epithelial cells (GP6) (arrowheads) **(F)** (A-F: H&E X200).

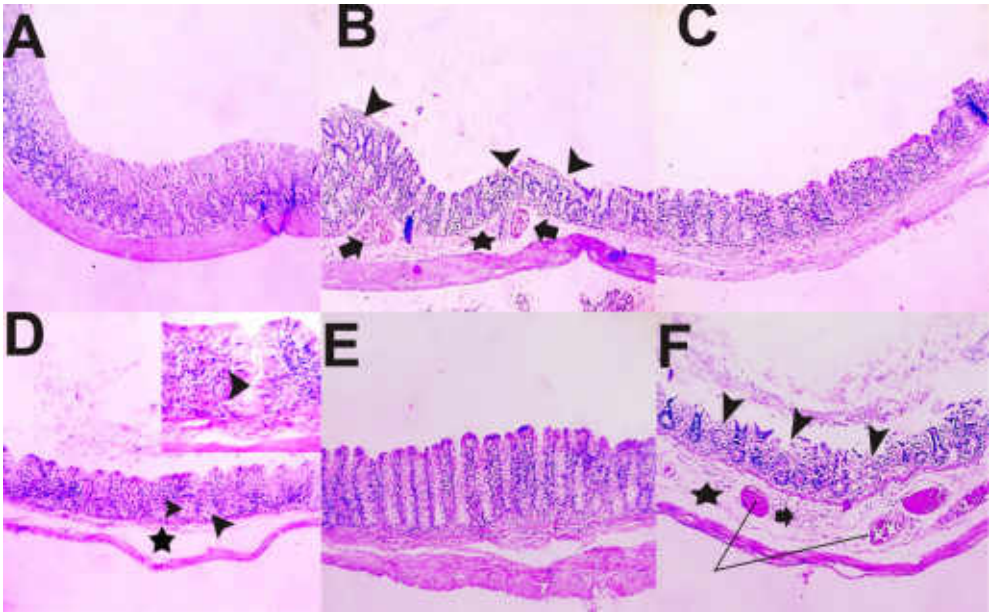

Fig. 6. (A-F): Light micrographs of cecum show normal histological picture in (GP1) **(A)**, loss of superficial epithelium (arrowheads), congested blood vessels (arrows) and edema (asterisk) in submucosa from (GP2) **(B),** reduced thickness of mucosa in (GP3) **(C)**, reduced thickness of mucosa, dilated cecal crypts with eosinophilic material (arrowheads) and moderate edema in submucosa (asterisk) from (GP4) **(D)** insert: high magnification to show dilated cecal crypts with eosinophilic material (arrowhead) (H&E X200), high well-organized cecal crypts in (GP5) **(E)**, loss of superficial epithelium and necrosis in mucosa (arrowheads), congested blood vessels (black lines), leukocytic cells infiltration (arrow) and marked edema (asterisk) in submucosa from (GP6) **(F)** (A-F: H&E X100).

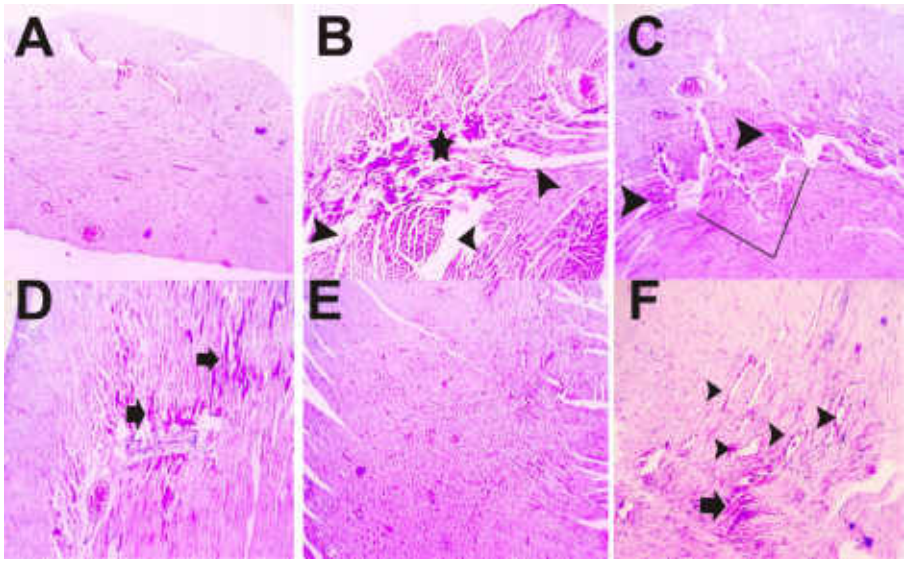

Fig. 7 (A-F): light micrographs of heart show normal histological picture in (GP1) **(A)**, disorganized carciac myocytes (asterisk) due to hyalinization and edema (arrowheads) in (GP2) **(B),** mild hyalinization (arrowheads), edema and congestion (black lines) in (GP3) **(C)**, mild hyaline degeneration in cardiac myocytes (arrows) from (GP4) **(D)**, normal well organized cardiac myocytes in (GP5) **(E)**, mild hyalinization (arrow), congested blood vessels (arrowheads) in (GP6) **(F)** (A-F: H&E X100).

DISCUSSION

Non-steroidal anti-inflammatory drugs (NSAIDs) as ibuprofen, aspirin and indomethacin are widley used as analgesics and anti-inflammatory agents; they produce their curative effects through inhibition of prostaglandin synthesis [34]**.** The studies recorded by Lichtenberger *et al*, [35] reported that NSAIDs have a detergent-like action, which disrupts the membrane integrity of gastrointestinal tract. The mechanisms suggested for the gastric damage caused by NSAIDs are inhibition of prostaglandin synthesis and inhibition of epithelial cell proliferation in the ulcer margin, which is critical for the reepithelization of the ulcer crater [36]. Moreover, microscopic examination of indomethasin exposed group showed lesions not only in stomach but also liver, kidneys, heart and cecum. Smith *et al.,* [37] reported that, administration of indomethacin caused periportal hepatic necrosis, this effect on the liver may be one of the causes of death in treated rats. Indomethacin can cause also intestinal lesions related to the amount of indomethacin excreted into bile [38]**.** In the present study, indomethacin produced significant reduction in RBCs count, Hb, Hct, serum albumin, NO and CAT values. On the other hand, indomethacin induced significant increases of TLC, neutrophil counts, levels of ALP, total serum bilirubin, ALT, AST, total cholesterol, triglyceride, urea, creatinine, gastric and serum MDA. Bush, [39] mentioned that, increases in the serum levels of AST and ALT (especially ALT) are associated with liver damage. The toxicity of some NSAIDs can be directly related to their biliary excretion. Some drugs such as indomethacin, ibuprofen and aspirin known to have effective anti-inflammatory are associated with some side effects such as gastric erosions and abdominal ulcers after prolonged use [40]**.** It has been recorded that, NSAIDs inhibit the cyclooxygenase 1 enzyme which synthesizes prostaglandin (PG) needed for haemostasis and the maintenance of the gastric lining of the stomach [41]**.**

Rantidine is a histamine H2-antagonist used in treatment of gastric and duodenal ulcer and for gastro-esophageal reflux disease [42]**.** Ranitidine inhibits gastric acid secretion, which stimulated by pentagastrin, histamine and normal meals [43]**.**

The concern over the severe side effects of these anti-inflammatory drugs have led to the search for new anti-inflammatory agents from plants and plant products with low toxicity and minimal side effects. Moreover, there has been a considerable interest in finding natural antioxidants from plant materials to replace synthetic ones for effective management of therapeutic drug toxicity such as peptic ulcer [44]**.** Pretreatment with MPO produced significant reduction in gastric mucosal lesions and improved results of hematological, biochemical, antioxidant and oxidative stress assays nearly as ranitidin in different degrees. Pretreatment with ginger oil was more effective than thyme and anise oils because it induced higher percent of ulcer inhibition, reduced gastric lesions and secondary lesions in other organs.

Ginger oil exhibited anti-ulcerogenic effect against indomethacin induced gastric ulcer when compared with ranitidine. The key of using ginger as a potential anti-ulcer agent originated from the finding that two of its active constituents, 6-gingerol and the terpenoid zingiberene, can ameliorate gastric lesion

formation [45]. Surprisingly, some constituents of ginger have been shown to inhibit the synthesis of PGs [46]. It is therefore not understood how crude extracts of ginger can antagonize the effect of NSAIDs compounds [47, 48] reported that, ginger has a beneficial role in curing of gastrointestinal disorders like gastric ulcer. Shen *et al*. [49] also reported the anti-inflammatory effect of ginger roots on osteoarthritic cow through inhibition of COX-2 enzyme, pro-inflammatory cytokines and prostaglandins which are all components of the inflammatory response. Sharma and Srivastava [50] also reported that ginger inhibited paw oedema in an experimentally induced arthritis in rats. Administration of ginger oil was capable of protecting the cells from intracellular oxidative damage *in vivo* by increasing the serum antioxidant status as clearly observed from the data. The current study indicated that ginger oil could inhibit the oxygen radicals as seen from the inhibition of nitric oxide, catalase and MDA in serum and stomach homogenate. The antioxidative potential of ginger oil may be due to the mixture of dozens of compounds of different functional groups, polarity and chemical behavior which produces either synergistic or antagonistic effect on antioxidant activity. Many researchers suggested that phenolic groups play an important role in antioxidant activity of ginger oil [51]. Regarding to the cholesterol level, it found that ginger treated group showed a significant decrease in the cholesterol level when compared to toxicated rats. These results were agree with those of Bhandari *et al*., [52] who recorded that, ginger has anti-hperlipidimic effect an reducing the cholesterol level when administered to rabbits.

The thyme, in addition to its calmative effects of spasmodic cough and digestive discomforts, anti flatulent and anti -inflammation effects, it has remedial effects on the main cause of gastric ulcer. In the same trend, it was found that thyum inhibited nitric oxide, catalase and MDA in serum and stomach homogenate. The antioxidant properties of thyme oil have been reported by Miura *et al*., [53]. This antioxidant activity is mainly due to the presence of phenolic compounds thymol and carvacrol [54].

Anise aqueous suspension was previously proved to have anti-ulcer and anti-secretory effects [55]. The chemical constituents of anise responsible for its anti-ulcer activity are still unknown. However, chemical studies demonstrated that anise contains estrarole [56], anethol [57], eugenol [58], anisaldehyde, methylchanicol [59], coumarins [60] and terpenes as the major compounds. Anise and its compounds have been identified as free radicals or active oxygen scavengers [61]. In addition, the ability of anise suspension to protect gastric mucosa against lesions induced by chemical irritants is likely by maintaining the structural integrity of gastric epithelium and balance of aggressive factors and inherent protective mechanisms [62].

CONCLUSION

All medicinal plants improved the results nearly as ranitidin (treatment for ulcer gastritis) in different grades. According to gross and microscopic examination, ginger oil was more potent than thyme and anise oils as anti-ulcer activities against indomethacin-induced gastric lesions, presumably via their antioxidant and anti-inflammatory properties of oil extract.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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