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Illicium verum Extracts Anti-Gastro Ulcerogenic Potential on Experimentally Rat Models

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Abstract: Background: The traditional herbal, medicinal plants, drugs are used in treating many diseases. The herbal treatment commonly used in treating most type of ulcers. Different extracts of *Illicium verum* were investigated for their anti-gastro ulcerogenic effect on rats.
Aims: Evaluation of *Illicium verum* extracts as anti-ulcerogenic agent in rat model.
Methods: plant essential oil and extracts (petroleum ether and aqueous alcoholic), were performed and then LD_{50} values were determined (2500 mg/ kg b.wt. for aqueous alcoholic extract and 1250 mg/ kg for petroleum ether extract). Rats were classified into three main groups; negative control administered distilled water, positive controls administered different extracts as well as ulcerogenic sub-group and the last main group is treated animals.
Results: in an *in-vitro* study, aqueous alcoholic extract exhibited the highest antioxidant properties and the lowest effect was recorded for petroleum ether extract. In an *in-vivo* experiment, aqueous alcoholic extract possessed potent anti-ulcerogenic effect as compared to famotin, reference drug. It enhanced the production of reduced glutathione and induced the glutathione reductase activity, superoxide dismutase activity and catalase activity determined in gastric mucosa with marked reduction in lipid peroxides productions in the two ulcerogenic models.
Key words: *Illicium verum*, essential oils, antiulcer, (Alcohol & Aspirin) ulcer, antioxidants.

Introduction

The disordered physiological processes of PUD involve an imbalance between aggressive (acid, pepsin, and Helcobacter pylori) and defensive factors (mucin), prostaglandin, bicarbonate, nitric oxide, and growth factors). Stomach and the first few centimeters of the duodenum are the most common sites for ulcers. Acute peptic ulcers including lesions may be single or multiple and tissues down to the depth of the sub mucosa. The development of ulcers involves severe shock, illness, burns, various postsurgical complications and emotional disturbance. Chronic peptic ulcers sneak through muscle layers of the stomach wall and the epithelial. Peptic ulcers include a lot of complications such as perforation, pyloric stenosis and hemorrhage. Poor or difficult digestion and elimination, incorrect metabolism, mental and physical stresses enhance the development of ulcers[1]. There are number of drugs available for the treatment of peptic ulcers, but these drugs have a clinical evaluation of indicates high incidences of retrogression, drug interactions and side effects. Decreasing gastric lumen mucus production with suppression of mucosal blood flow by administration of ethanol encourage ulcer formation that reduces prostaglandins and glutathione concentrations through the depletion in cysteine level that's required for glutathione biosynthesis with increasing gastric vascular permeability and inducting

leukotreines and free radicals production. These deleterious effects contribute in loss of mucosal maintenance reproduce ulceration. Free radicals strongly participate in the two ulcer types, acute and chronic cases. Therefore, agents can scavenged radicals are considered in treating gastric ulcers[2]. Also administration of aspirin over than recommended dose induces oxyradicals formation and leads to erosive gastritis[3]. Medicinal plants considered as an alternative strategies in drug discovery and a valuable source of new molecules. In traditional medicine there is large plants number known to possess antiulcer properties that may provide a new ulcer drugs or improve existed anti-ulcer drugs specially after chemical manipulation[4] Peptic ulcer disease (PUD), including gastric and duodenal ulcers, is the most prevalent gastrointestinal disorder that requires a well-targeted therapeutic strategy. These are prospective aggressive effects support belief for the development of new antiulcer drugs and search for natural products. Medicinal plants such as *Ocimum sanctum*, *Azadir achtaindica*, *Asparagus racemosus, Musa sapientum, Centella asiatica, Bacopamonnieri* and *Bidenspilosa* may offer better protection and decreasing expected relapse [5]. Gastric disorder is classified in Ayurveda ancient system of Indian medicine, as *sula, parinamsula* and *amlapitta* which match with functional dyspepsia and clinical condition of peptic ulcers [6]. Carbenoxolone, was the first drug effective against gastric ulcer discovered as a result for research on a commonly used indigenous plant, *Glycyrrhiza glabra* (Leguminosceae) [7]. Plant drugs, show the antiulcer activity, constitutes the active ingredients such as flavonoid, tannins and terpenoids [8]. Different classes of drugs are used in the treatment of peptic ulcer but most of these drugs exhibit serious side effects like arrhythmias, gynaecomastia, arthralgia, hypergastrinemia and haemopoeitic changes. Alternative approach in recent years is the research for medicaments from ayurvedic or traditional medicinal system. The use of phytoconstituents as drug therapy to treat major ailments has proved to be clinically effective and less relatively toxic than the existing drugs and also reduces the aggressive factors serving as a tool in the prevention of peptic ulcer. In this time, 75-80% of the world populations still use herbal medicine mainly in developing countries for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. *Illicium verum* Hook. f. (Austrobaileyales: Schisandraceae), a fruit ordinarily known as star anise, is mainly distributed in the tropical and is native to southwest China and Vietnam and subtropical areas of Asia. *Illicium verum* was considered as one of the things "both food and medicine" by the Ministry of Health of the People's Republic of China (2002), implying its low or non-toxicity to humans. Now, the research focus on *I.verum* has been mainly on food and medical fields [9]. The essential oil of *I. verum* can be used as a flavoring and the fruits are commonly used as an ingredient of the traditional "five-spice" powder of Chinese cooking. The extraction from *I. verum* used as diuretic, stomachic, stimulant, and properties, and carminative, is used as a pharmaceutical supplement [10]. The herb is reported to be antifungal, antibacterial and antioxidant. It is able to increase production of milk new mother. The use to facilitate birth and to increase the libido, as well as to relieve menopausal discomforts; oil is used in rheumatism as recommended by some folk remedies[11]. The attributed medicinal properties are carminative, stomachic, stimulant, expectorant and diuretic. In east it is used to combat colic and rheumatism. It is a common flavoring for medicinal tea, cough mixture and pastilles. In traditional system of medicines, *Illicium verum* fruit is used having both culinary and medicinal uses. Its seed oil is used worldwide as medicine. Due to the potential side effects of synthetic antioxidants, essential oil derived from natural products can be served as an alternative source for the further improvement of synthetic antioxidant.

This work focused on evaluation of *Illicium verum* 1) extracts as anti-ulcerogenic herbal medicine in rat model. 2) *invitro* antioxidants activity. 3) chemical composition of essential oil and finally 4) examine histopathologically the stomach of rats treated with *illicium* extracts compared to reference drug.

Materials and Methods:

Plant Material:

Star anise seeds were obtained from local market and identified by Pro. Dr. Lotfy Boulis. Seeds were grounded to small granules and then subjected to sequential soxhlet extraction with petroleum ether and aqueous ethanol (70%). The obtained petroleum ether and ethanol extracts were dried under reduced pressure to be free from any solvent residues. The remained concentrated petroleum ether and aqueous alcoholic extracts were used in biological study.

Two methods were carried out to extract essential oil:

1. Modified (Hydrodistillation) Clevenger trap[12] was used for essential oil extraction.

2. Water and Steam: it is such as hydro-distillation but during this process, the water remains below the plant material, which has been placed on a grate while the steam is introduced from outside the main still (indirect steam). Extraction times were performed (1, 2 and 3 hour) with three replicates for both extraction methods.

GC–MS analysis of essential oil: samples were supplied into gas chromatography–mass spectrometry instrument at Medicinal and Aromatic Plants Researches Dept., National Research Center with the following specifications, instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp, USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC–MS system was equipped with a TG-WAX MS column(30 m × 0.25 mm i.d., 0.25 µm film thickness). Analyses were carried out using helium as carrier gas at a flow rate of 1.0 ml min−1anda split ratio of 1:10 using the following temperature program: 40°C for 1 min; rising with 4.0°C min−1–160 °C and held for 6 min; rising with 6°C min−1–210◦C and held for 1 min. The injector and detector were held at 210°C. Diluted samples (1:10 hexane, v/v) of 0.2µl of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z40–450. Most of the compounds were identified using two different analytical methods: (a) KI, Kovats indices in reference to alkanes.

***In-vitro* antioxidant properties investigation**

Free radical scavenging effect: The free radical scavenging activity of plant extracts was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH•)[13] DPPH• (0.1 mM) was prepared in methanol and an aliquot (1mL) was added to each extract (3mL) prepared a concentrations of 62.5, 125, 250, 500 and 1000µg/ ml. Mixtures were vigorously shaken and allowed to stand at room temperature for 50 min before recording absorbance at 517 nm (Jasco V630 spectrophotometer). Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH• radical concentration in the reaction medium was calculated from the following equation: DPPH• scavenging effect (%) = 100 − $[(A_0\text{-}A_1)/A_0) \times 100]$, Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample [14]. VC was used as a positive control in all *in-vitro* tests.

Reduction capability: Plant extract reduction capacities were determined as previously reported [15]. Extracts diluted in MeOH to 62.5, 125, 250, 500 and 1000 µg/ml (1ml) were added to phosphate buffer (2.5 ml, 0.2 M, pH 6.6) containing potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The assay mixture was incubated at 50°C for 20 min and the reaction stop with addition of TCA (10%, 2.5 ml) and centrifugation for 10 min at 1000 × *g* (MSE Mistral 2000, UK). The upper layer (ca. 2.5ml) was diluted with methanol (2.5 ml) containing $FeCl_3$ (0.5ml, 0.1%) and the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated greater reducing power.

Superoxide anion scavenging activity: Superoxide anion scavenging activity was based on the method described by [16] in which superoxide radicals are generated in a phenazinemethosulphate (PMS)–nicotinamide adenine dinucleotide (NADH) system by oxidation of NADH and assayed by the reduction of nitrobluetetrazolium (NBT). Superoxide radicals are generated in Tris–HCl buffer (16 mM, pH 8.0, 3 ml) containing NBT (50µM, 1ml), NADH (78µM, 1 ml) and extracts to be assayed (1 ml) at different concentrations.

The reaction was started by adding PMS (phenazinemethosulfate) solution (10 µM, 1ml). The reaction mixture was incubated at 25ºC for 5 min and absorbance reading (560 nm) was performed. Decreasing absorbance indicated increasing superoxide anion scavenging activity. The percentage inhibition of superoxide anion generation was calculated as: percent of inhibition = $[(A_0\text{-}A_1)/A_0] \times 100$; where A_0 was the absorbance of the control (L-ascorbic acid), and A_1 was the absorbance of test extracts.

Metal chelating activity: Ferrous ion chelating was estimated by the method of [17]. 0.05 ml of $FeCl_2$ solution (2 mM), test extracts or V.C. was added and the reaction was initiated by an addition of ferrozine (5 mM, 0.2 ml) with vigorous shaking; after incubating at room temperature for ten minutes, absorbance at 562 nm was recorded. Percentage inhibition of the ferrozine-Fe^{2+}complex was determined based on the equation: inhibition (%) = $[(A_0\text{-}A_1)/ A_0] \times 100$, where A_0 was the absorbance of control while A_1 was the absorbance in the presence of test sample and standard.

Inhibition of lipid peroxidation: The potential of plant extracts at different concentration was determined according to the method of [18] to inhibit peroxidation of linoleic acid. L-ascorbic acid was used as the reference

compound. A pre-emulsion was prepared by mixing 175µg Tween 20, 155µL linoleic acid, and 0.04M potassium phosphate buffer (pH 7.0). 1mL of sample at different concentrations in 99.5% ethanol was mixed with 4.1mL linoleic emulsion, 0.02M phosphate buffer (pH 7, 8mL) and distilled water (7.9mL). The mixed solutions of all samples (21mL) were incubated in screw cap-tubes under dark conditions at 40˚C at certain time intervals. To 0.1mL of this mixture was pipetted and added with 9.7mL of 75% and 0.1mL of 30% ammonium thiocyanate sequentially. After 3 min, 0.1mL of 0.02M ferrous chloride in 3.5% hydrochloric acid was added to the reaction mixture. The peroxide level was determined by reading daily of the absorbance at 500 nm in a spectrophotometer. All test data was the average of three replicate analyses. The inhibition of lipid peroxidation in percentage was calculated by the following equation; % Inhibition = $[(A_0 - A_1) / A_0] \times 100$ (1). Where A_0 was the absorbance of the control reaction andA_1 was the absorbance in the presence of extracts or standard compounds.

Animals, Housing and Experimental Design: The acute toxicity test for plant extracts was estimated to evaluate any possible toxicity. Female albino mice (n=8) were tested by administering different extracts doses by increasing or decreasing the dose, according to the response of animal [19].The dosing patron was 500, 1000, 1500, 2000, 2500, 3000, 3500 and 4000 mg/kg body weight for alc. extract and pet ether extract, while control group received only the normal saline. All groups were observed for any gross effect or mortality during 48hr. Death of half of examined animals was observed at 2500 and 1250 mg/kg b.wt for alcoholic extract and pet ether extract, respectively.

Anti-ulcer effect of *Illicium verum* extracts on rat administered alcohol induced gastric mucosal injury: The anti-ulcerogenic effect on adult female albino rats, weighing 80-100 g. Animals were obtained from animal house of National Research Centre. Each kedge was contained six female albino rats and they were feed on standard diet. They maintained under standard laboratory conditions; housing temperature was 24°C; relative humidity was 65± 5% with light/ dark cycles **(**12/12 h). The dose was selected on basis of acute toxicity. Plant extracts was employed at oral dose as aqueous suspension using distilled water. Three main groups including negative group administered distilled water (first group), second group (positive controls) to investigate any deleterious effect caused by extract, drug or alcohol administration which include four sub-groups administered petroleum ether extract at 125 mg/ kg b.wt, alc. extract at 250 mg/ kg b.wt, famotin at 20mg/ kg [20] . while the third main group was received 1 ml alcohol / 100g orally only to induce gastric ulcer [21]. The third main group includes three sub-groups firstly rats received 1 ml alcohol with *Illicium* petroleum ether treatment at 125mg/ kg b.wt. one hour before and after administration,[22] second sub-group received 1ml alcohol orally with treatment with *Illicium* EtOH at 250 mg/ kg, one hour before and after administration while famotin was administered as reference drug at 20mg/ kg b.wt. by the same way of extracts. Rats were anesthetized and then sacrificed; their stomachs were excised and opened along the greater curvature. Gastric mucosal tissue was taken from the antral portion of the stomach for biochemical estimations. The gastric mucosa was scrapped with a scrapper, homogenized in ice cold phosphate buffer (pH 7.2) to prepare the mucosal homogenate. Homogenates were centrifuged at 3000 rpm for 10 min and the supernatants were used for further studies.

Anti-ulcer Effect *of Illicium verum* Extracts on rat injured with Aspirin Induced Gastric Mucosal Injury: The animals were fasted for 48hr [23] prior to experiment while water was permitted. Group 1 received saline, group II received *Illicum* pet. ether extract at 125 mg/ kg b.wt. for seven days, group III received *Illicum* EtOH extract at 250mg/kg b.wt. for seven days, group IV received aspirin at 400 mg/ kg suspended in 0.5% carboxy methyl cellulose, group V received aspirin with pretreatment of *Illicium* pet. ether ext. for seven days group VI received aspirin with pretreatment of *Illicium* EtOH extract for seven days, group VII received aspirin and then treated with *Illicium* pet. ether extract for ten days, therapeutic group. However, group VIII received aspirin and then treated with EtOH extract, therapeutic groups for ten days after aspirin administration. After the experimental period rats were scarified after anesthesia and their stomachs were opened along the greater curvature after four hours of aspirin administration. The gastric tissue was fixed in buffered formalin for histopathological study while gastric mucosal tissues were taken and prepared as mentioned above for biochemical estimations. Reduced glutathione level in the stomach tissue was determined according to the method of [24]. Gastric superoxide dismutase (SOD) activity was estimated by the method of [25]. Catalase (CAT) activity was measured by following decomposition of H_2O_2 according to the method of [26]. Glutathione reductase activity was measured spectrophotometrically at 340nm [27] and the amount of the enzyme reducing 1µmol GSSG per min per mg protein was regarded one activity unit as elsewhere described. The TBARS level, an index of malondialdehyde (MDA), production was determined by the method of [28]. The protein content was determined by the method of Bradford [29].

Histopathological Study: Specimens of stomach from all animals were dissected immediately after death, then opened along the greater curvature and washed thoroughly with distilled water to remove their contents to avoid digestion of upper layers of gastric mucosa by digestive enzymes. All the specimens were fixed in 10% neutral-buffered formal saline for 72 hours at least, washed in distilled water and then dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin wax. Serial sections of 6µm thick were cut and stained with Haematoxylin and eosin [30] for histopathological investigation.

Statistical Analysis: The data of biochemical assessments are expressed as mean ± SD and ANOVA followed by post hoc test (LSD) to compare the groups. The statistical analysis was carried out by the version 9.01 of the SPSS program. Values were considered statistically significant; $P < 0.05$.

Results

Illicium antioxidant properties

There are large number of diseases related to excessive stress of free radicals in human body hemorrhagic shock, Alzheimer and Parkinson's disease, tumor promotion and carcinogenesis, AIDS and gastrointestinal dysfunctions [31]. Therefore, antioxidant properties of *Illicium* pet. ether extract and aqueous alcoholic extract were evaluated. In all tests, the aqueous alcoholic extract showed to have the highest recorded values followed with pet. ether extract. Concerning radical scavenging effect, DPPH radical .

The scavenging effect was increased gradually with increasing the concentration of tested extracts. Aqueous alc. extract and VC, reference compound, reached 90% inhibition percentage at 1000 µg/ mL while petroleum ether produced weak effect as DPPH radical scavenger at all concentrations (**Figure, 1**).

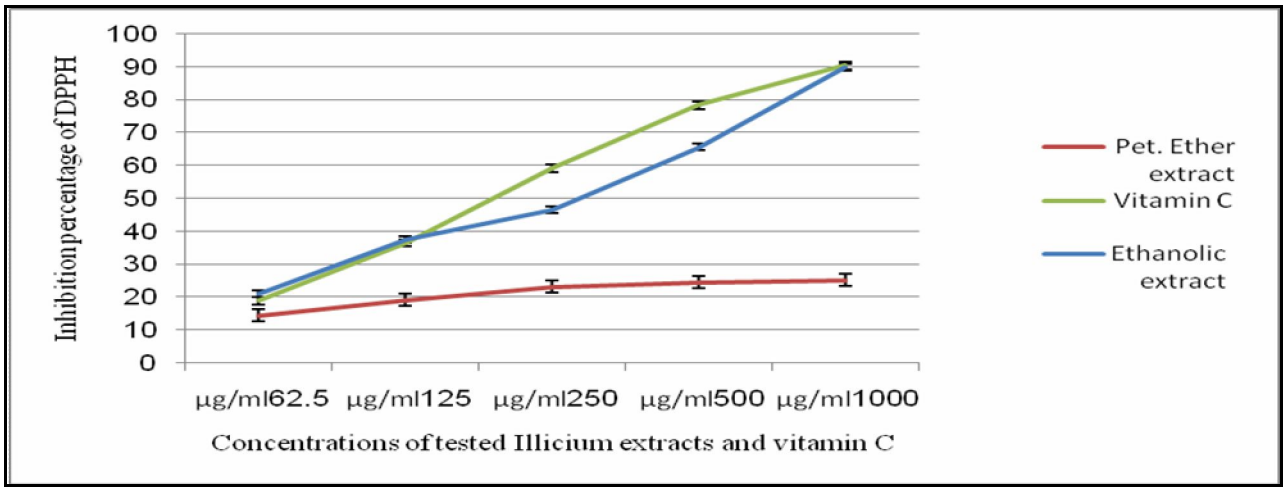

Fig. 1. Free radical scavenging effect of *illicium* verum extracts at different concentration. All data are mean of triplicates. Data were analyzed by ANOVA one way and presented as mean± SD, $P< 0.05$.

The reduction capability of alcoholic *Illicium* verum extract occurred to be higher than pet. ether extract. It represented the highest reduction capability at the four tested concentrations. This capability was enhanced by increasing the concentration but they remained much lower than VC (**Figure, 2**).

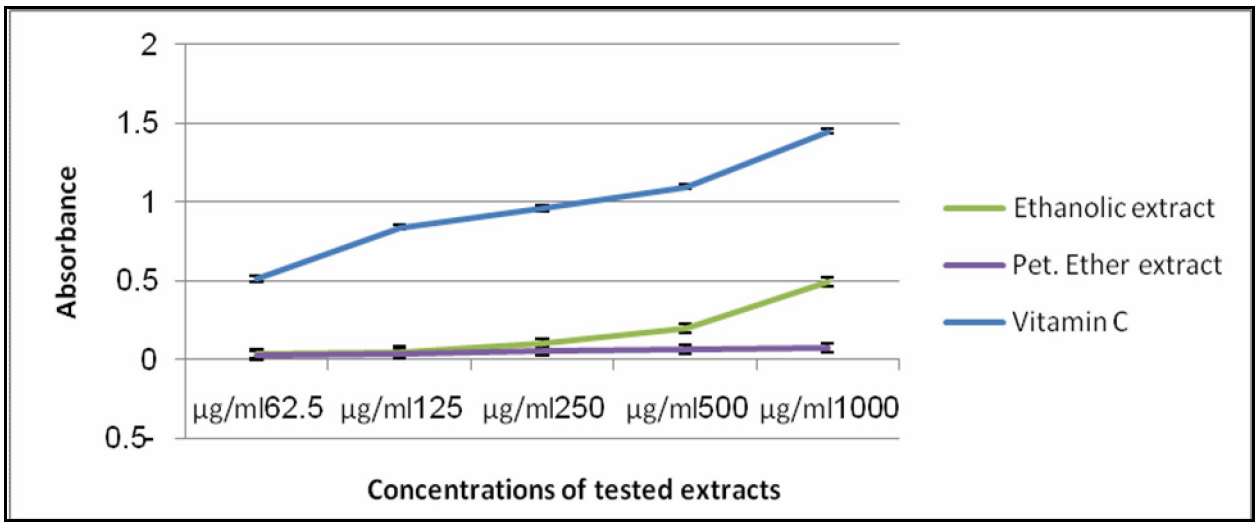

Fig. 2. Reduction capability of *Illicium verum* extracts at different concentration. All data are mean of triplicates. Data were analyzed by ANOVA one way and presented as mean± SD, $P< 0.05$.

The presence of metal ions in tissue increase the oxidative stress by catalyzing cell oxidation reactions [32]. The two tested extracts showed to have languorous chelating effect at the four tested concentrations. The maximum chelation percentage was 26% for alcoholic extract at 1000µg/ mL (**Figure, 3**). .

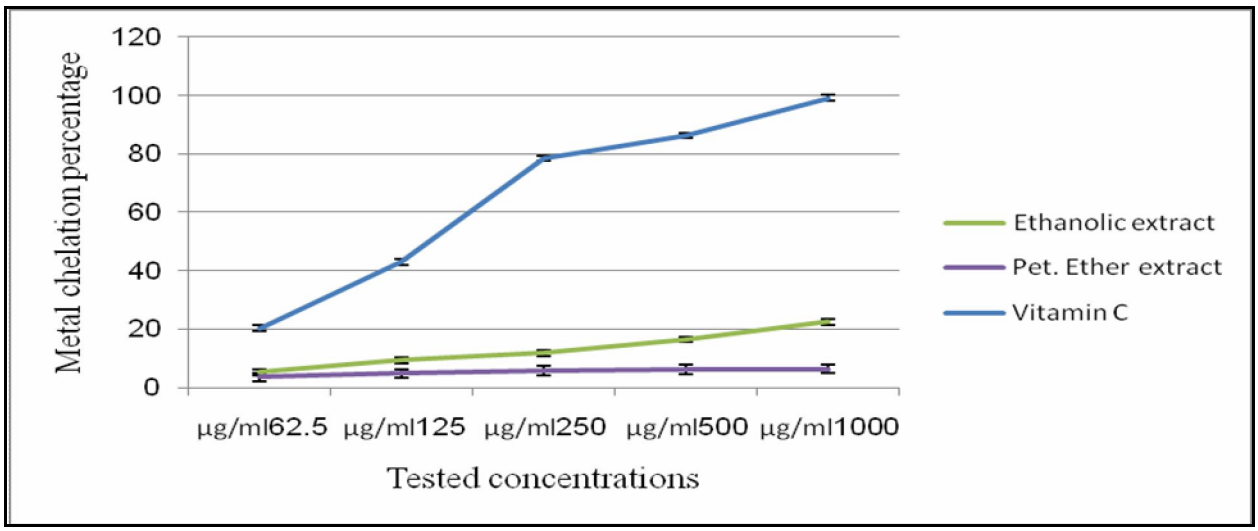

Fig. 3. Metal chelating effect of *Illicium* extracts at different concentration. All data are mean of triplicates. Data were analyzed by ANOVA one way and presented as mean± SD, $P< 0.05$.

The alcoholic extract of *Illicium* possesed strong superoxide radical scavenging produced by phenazine. It represented 94% scavenging effect at 1000µg/ mL while pet. ether extract remained the feeble one in scavenging super radicals (**Figure, 4)**.

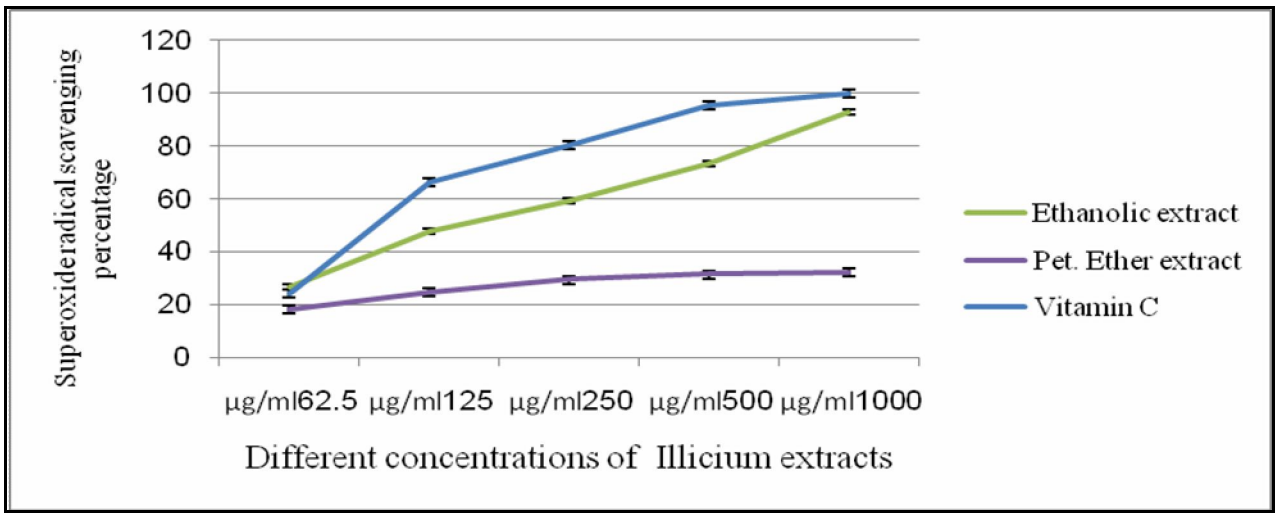

Fig. 4. Superoxide radical scavenging effect of *Illicium* extracts at different concentration. All data are mean of triplicates. Data were analyzed by ANOVA one way and presented as mean± SD, $P< 0.05$.

The antioxidant effect of extracts reflected on their properties as inhibitors for lipid peroxidation when they were tested by linoleic assay. *Illicium* alcoholic extract remained the best effector to inhibit lipid peroxide production, inhibition percentage reached 100% at 1000µg/ mL. The inhibition was increased in a concentration dependent manner with two extracts. Alcoholic extract inhibition percentage of lipid peroxidation ranged from 35 to 100% while pet. ether extract ranged from 32 to 80% (**Figure, 5**).

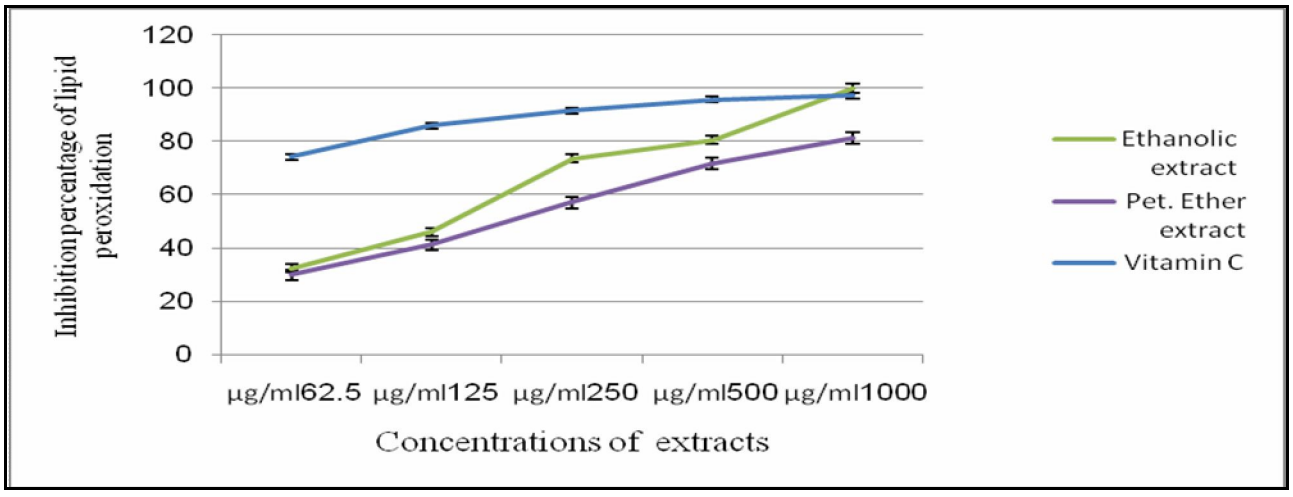

Fig. 5. Lipid peroxide inhibition effect of *Illicium* extracts at different concentration. All data are mean of triplicates. Data were analyzed by ANOVA one way and presented as mean± SD, $P< 0.05$.

The recorded antioxidant properties in the *in-vitro* tests support the probability of *Illicium verum* extracts as anti-ulcerogenic agent. Animals were treated with ethanol to reproduce ulcer and the two *Illicium* extracts, pet. ether and aqueous alcoholic extracts, were tested as protective materials against gastric ulcers. Administration of **Table 1** revealed that *Illicium* extracts didn't produce any deleterious effect as observed in investigated bio-parameters. Ethanol adminstration significantly reduced the glutathione concentration in stomach wherase aqueous alcoholic extract administration significantly induce the glutatione production in tissue to record value more than –ve control, administered distelled water, (19.08 and 15.46 mg/g tissue, respectively). The aqueous extract enhanced GSH production to reach 15.18 mg/g tissue. Likewise, glutathione reductase activity had the same trend of reduced glutathione production. The activity was induced by aqueous alcoholic extract administration (28.32µmol/ min/ mg protein) more than pet. ether extract (24.32µmol/ min/ mg protein) to ameliorate the status of injured tissue (11.16µmol/ min/ mg protein, for ethanol induced group). As well, administration of *Illicium* aqueous alcoholic extract possessed a plausible ameliorative effect on superoxide

dismutase and catalase activities, they were enhanced by aqueous alcoholic extract administration to record 36.73 and 18.30 U/ min/ mg protein for SOD and CAT, respectively) while pet. ether extract induced them to be 33.33 and 16U/ min/ mg protein, respectively. It is clear from these determined antioxidant parameters that the two extracts can reduce the production of lipid peroxidation through their effect as antioxidant agent. Ethanol duplicated the lipid peroxide production in injured tissue (27.09µmol/g tissue) however the two extracts decreased lipid peroxide concentration (14.53 and 15.73µmol/g tissue for aqueous alcoholic extract and pet. ether extract, respectively). Enhancing the antioxidant status of tissue reflectred on decreasing lipid peroxide production to reach the lowest recorded values as compared to ¯ve control (17.14µmol/g tissue).

Table 1: Impact of *Illicium verum* extracts on gastric mucosal antioxidant parameters in ethanol induced gastric mucosal injury model.

Parameters Groups		Glutathione concentration mg/g tissue	Glutathione reductase µmol/min/mg protein	Superoxide dismutase U/ min/ mg protein	Catalase U/min/ mg protein	Lipid peroxide concentration µmol/g tissue
Normal group (¯ve control)		15.46 ±0.52^{a}	35.2 ±2.04^{d}	39.16 ±1.087^{f}	28.35 ±2.12^{g}	17.14 ±1.44^{m}
+ve control groups	Ethanol vehicle	9.89 ±0.75	11.16 ±1.28	15.29 ±1.11	6.85 ±0.83	27.09 ±2.31
	Illicium aqueous alcoholic extract 250 mg/kg)	17.73±0.49^{c}	39.44±1.64	47.57±2.37	32.07±1.29	14.82±2.00^{k}
	Illicium pet. ether extract (125mg/ kg)	14.45 ±0.84$^{a\,b}$	34.24 ±2.10^{d}	41.58±1.95^{f}	27.57±1.43^{g}	15.73 ±1.95^{k}
Treated groups	Ethanol+ famotin (20mg/ kg)	13.77 ±0.64^{b}	19.73±1.00^{e}	24.19 ±2.45	11.89 ±1.84	19.79 ±2.74^{m}
	Ethanol+ *Illicium* aqueous alcoholic extract (250mg/ kg)	19.08 ±0.82^{c}	28.32 ±0.99	36.73±1.87	18.30±1.92^{h}	14.53±1.67^{k}
	Ethanol + *Illicium* pet. ether (250mg/kg)	15.18 ±0.96^{a}	24.32±1.02^{e}	33.33±1.84	16 .00±1.00^{h}	15.37±1.35^{k}

Data are presented as mean triplicates ± S.D. Statistically significant $P< 0.05$
Groups have the same letter in each parameter indicates statistically insignificant difference between them

Table 2 revealed that aspirin induces the production of oxidized metabolites in animal model which strongly participate in mucosal injury [33]. Administration of aspirin significantly induced the production of lipid peroxides (38.62µmol/ g tissue) with significant decrease in GSH concentration in tissue (8.87mg/g tissue) accompanied with decreasing in glutathione reductase activity (11.67µmol/ min/mg), reduction of superoxide dismutase activity (14.07U/mg protein) and Catalase activity (6.85U/ mg protein). In an opposite manner, the aqueous alcoholic extract in the protective experiment exhibited potent effect as antioxidant inducer. It amplified the GSH concentration to 24.56 mg/ g tissue, glutathione reductase activity (20.80µmol/ min/ mg protein), SOD activity (42.64U/ min/ mg protein), CAT activity (31.13U/ min/ mg protein) with reduction in lipid peroxide production (15.35µmol/g tissue). Furthermore, the same trend was occurred with pet. ether extract but with lower effect than aqueous alcoholic extract. In this concern, famotin remained occur the lowest effects on determined parameter than the other treatments.

In the therapeutic experiment, treating animals with aqueous alcoholic extract improved determined antioxidant parameters than the petroleum ether extract. It showed marked enhancement in GSH concentration (20.51 mg /g tissue), glutathione reductase activity (16.54 µmol/min/mg protein), SOD activity (51.77U/min/mg

protein) and CAT activity (32.77U/min/mg protein) with decreasing lipid peroxide production (14.24µmol/ g tissue). However, the pet. ether extract produced lower effect than aqueous alcoholic extract. It increased GSH concentrations (14.48 mg/ g tissue) with increasing the activity of G. reductase (12.24µmol/min/mg protein), SOD (48.27 U/min/mg protein) and CAT (29.03U/min/mg protein) with reduction in lipid peroxide production (16.73µmol/ g tissue) .

It is clear from the mentioned data in **Table 2** that petroleum ether extract of *Illicium verum* exhibited protective effect equals to its therapeutic effect in most cases whereas protective effect of aqueous alcoholic extract was better than its therapeutic effect.

Table 2: Protective and therapeutic potential of *illicium verum* extracts on the gastric antioxidant parameters in aspirin ulcer model

Parameter / Group		Glutathione concentration mg/g tissue	Glutathione reductase µmol/min/mg protein	Superoxide dismutase U/ min/ mg protein	Catalase U/min/ mg protein	Lipid peroxide µmol/g tissue
⁻ve Control		14.95±1.43 ^{d}	35.55±1.37	38.61±2.19	28.35±2.43^{k}	17.14±1.23 ^{p}
⁺ve control groups	*Illicium* aqueous alcoholic extract (250 mg/ kg)	39.17±2.63	24.90±1.96	53.97±2.89 ^{e}	56.47±2.51	14.75±1.09 ^{t}
	Illicium pet. ether extract (125mg/ kg)	30.99±1.82	21.47±2.16 ^{b}	44.73±1.97 ^{f}	49.30±2.61	15.99±1.25 ^{t}
	Aspirin (400 mg/kg)	11.67±0.95	8.87±0.98 ^{a}	14.07±1.42	6.85±1.00	38.62±2.36
	Aspirin+ famotin (20mg/ kg)	15.19±1.17 ^{h}	10.89±1.64 ^{a}	28.88±1.94	13.89±1.13	14.94±1.38 ^{t}
Protective treatments	Aspirin + *Illicium* aqueous alcoholic extract (250mg/ kg)	24.56±1.48	20.80±1.85 ^{b}	42.64±2.38 ^{f}	31.13±1.45^{m}	15.35±1.39 ^{t}
	Aspirin + *Illicium* pet. ether extract (125mg/ kg)	20.31±1.31 ^{g}	13.07±1.07 ^{c}	34.29±2.41	27.40±2.14^{k}	17.71±1.41 ^{p}
Therapeutic treatments	Aspirin + *Illicium* aqueous alcoholic extract (250 mg/ kg)	20.51±1.43 ^{g}	16.54±1.11^{d}	51.77±1.99 ^{e}	32.77±2.47^{m}	14.24±1.27 ^{t}
	Aspirin + *Illicium* pet. ether extract (125 mg/ kg)	14.48±1.11 ^{h}	12.24±1.36 ^{c}	48.27±2.18	29.03±1.29^{k}	16.73±1.19 ^{p}

Data are presented as mean triplicates ± S.D. Statistically significant $P< 0.05$
Groups have the same letter in each parameter indicates insignificant difference between them

Analysis of *Illicium verum* essential oil

Table 3 summarized the effect of both extraction methods and the boiling time on essential oil constituents. Thirty five components were identified in the essential oil of *A. cerefolium* L. underwent at different treatments that represented 99.83–99.92% of the oils. The major components were anethol (67.53 % - 73.19%),

anisaldehyde (1.72% - 13.19%), D-limonene (2.98% - 6.21%) and L-linalool (1.78% - 2.66%). In this respect [34]reported that trans-anethole is the major component in star anise oil, and ranged between 86.66% and 94.21%. [35]found that the main component of star anise essential oil is trans-Aanethole, which accounts for 80-90 %. The results depicted in **Table 3** show that water steam distillation caused the maximum relative percentage of anethol which recorded 73.19,82.14 and86.14% vice versa 71.17, 67.53 and 72.7% with hydro-distillation method for 1, 2 and three hours after water boiling, respectively. On the contrary, the highest relative percentage of anisealdehyde which recorded 13.08, 13.19 and 7.59% under hydro-distillation method for 1,2 and 3 hours after boiling water while the lowest percentage (1.72%) under water steam method for 3 h after boiling water. D-limonene reached to its maximal percentage (6.21%) as a result of water steam distillation for 1 hour after boiling followed by hydro-distillation for 1 h after boiling water which recorded 4.39%.Water steam distillation for 2 hours after boiling water gave the highest relative percentage of L-linalool (2.66%) followed by the same extraction methods for 1 h after boiling water which recorded (2.48%).

Table 3. Essential oil constituents of star anise plant as affected by essential oil extraction methods

Constituents	**RT**	**1**	**2**	**3**	**4**	**5**	**6**
α-Pinene	3.89	0.29	0.30	0.37	1.13	0.26	0.16
β-Pinene	5.26	0.04	-----	----	0.10	------	0.04
Delta3-carene	6.14	0.20	0.28	0.42	0.52	0.28	0.10
α-Phellandrene	6.53	----	-----	0.06	0.09	0.07	-----
β-Myrcene	6.63	0.07	0.12	0.16	0.19	0.14	0.07
α-Terpinene	6.88	0.05	0.07	0.08	0.12	0.07	----
D- Limonene	7.34	4.39	4.34	5.67	6.21	3.32	2.98
Eucalyptol	7.63	1.87	2.32	1.24	2.83	1.50	0.85
γ-Terpinene	8.58	0.07	0.18	0.14	0.20	0.18	0.10
o-Cymene	9.35	0.35	0.51	0.49	0.78	0.44	0.16
α- Humulene	9.59	0.04	----	0.08	0.08	0.06	----
p-Menthone	15.29	-----	0.13	0.08	0.12	0.10	0.08
Isomenthone	16.19	----	0.08	0.05	0.08	0.06	0.05
L-linalool	18.18	2.23	2.45	1.72	2.48	2.66	1.78
α-Farnesene	18.76	----	----	0.07	0.89	1.10	0.05
Teroinene-4-ol	19.68	0.68	0.76	0.64	------	------	0.66
α-Terpinyl acetate	20.60	0.04	-----	-----	-----	-----	-----
Pulegone	20.87	0.07	0.27	0.18	0.30	0.28	0.27
Estragol	21.64	1.93	3.65	3.74	2.83	1.50	1.52
Sabina Ketone	21.81	0.12	0.12	0.11	0.13	0.15	0.06
B-Fenchyl Alcohol	22.57	0.76	0.90	0.96	0.92	0.80	0.60
Carvol	23.59	0.07	0.07	0.06	0.07	--------	0.06
Trans-Anethole	24.18	0.96	1.04	2.04	1.39	1.27	0.95
Anethol	26.29	71.17	67.53	72.70	73.19	82.14	86.14
Grraniol	26.81	0.08	0.11	0.15	0.10	0.13	0.07
p-Cymene-8-ol	26.98	0.06	0.08	----	-----	-----	-----
Methyleugenol	30.61	----	------	------	-----	-----	0.63
Anisaldehyde	31.88	13.08	13.19	7.59	4.32	2.24	1.72
Methyl anisate	33.72	0.09	0.09	0.08	0.06	0.07	0.06
Cinnamyl acetate	35.74	0.05	0.08	0.06	----	----	0.05
Anise Ketone	36.29	0.61	0.57	0.28	0.16	0.10	0.08
Anisylacetone	37.31	----	0.06	-----	-----	0.06	0.05
1-Propanone,1-(4-methoxyphenyl)	38.29	0.26	0.29	0.24	0.17	0.17	0.13
Chavicol	39.62	0.24	0.25	0.44	0.40	0.68	0.45
Total identified		99.87	99.84	99.90	99.86	99.83	99.92

1- **Hydrodistillation for1h** **4- Water Steam for 1 h**
2- **Hydrodistillation for2h** **5- Water Steam for 2 h**
3- **Hydrodistillation for3h** **6- Water Steam for 3 h**

Histopathological results:

Microscopically, examined stomach of normal control, untreated rats, rats treated with petroleum ether extract and rats treated with ethanolic extract revealed no histopathological changes (Figs. 6a ,b & c). Meanwhile, sections from rats treated with famotin showed inflammatory cells infiltration in lamina propria (Fig. 6d). Improvement in the histopathological picture was noticed in stomach of rats treated with ethanol vehicle, as examined stomach revealed submucosal oedema with inflammatory cells infiltration (Fig. 6e). Moreover, stomach of rat treated with famotin as protective agent showed submucosal oedema associated with few inflammatory cells infiltration (Fig. 6f). However, marked improved stomach picture was noticed in rats from the groups of protective petroleum ether ethanol ulcer and the group of protective ethanolic extract in treating ulcer with ethanol, as the stomach from those rats revealed apparent normal gastric mucosa (Figs. 6g & h).

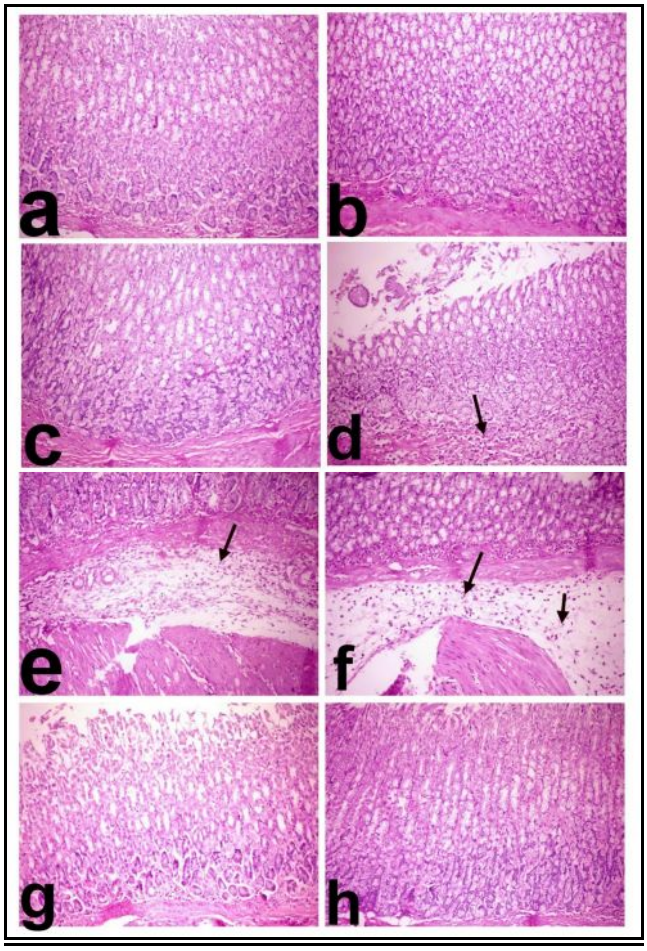

Figure 6. Photomicrograph of Stomach sections of rat from: (a) Normal control, untreated group showing no histopathological changes. (b) +ve petroleum ether extract group showing no histopathological changes. (c) +ve ethanolic extract group showing no histopathological changes. (d) +ve famotin group showing inflammatory cells infiltration in lamina propria (arrow). (e) ethanol vehicle group showing submucosal oedema with inflammatory cells infiltration. (f) group treated with famotin as protective agent showing submucosal oedema (small arrow) associated with few inflammatory cells infiltration (large arrow). (g) group protective petroleum ether ethanol ulcer showing apparent normal gastric mucosa. h) from group of protective ethanolic extract in treating ulcer with ethanol showing no histopathological changes (H & E X 100).

Histopathological results of asprin ulcer

Histopathologically, stomach of normal control, untreated rats, rats treated with petroleum ether extract and rats treated with ethanolic extract showed histopathological changes (Figs. 7a,b & c). examined sections from rats treated with famotin showed mild changes described by presence of few inflammatory cells infiltration in submucosa (Fig.7d) . Meanwhile, stomach of rats treated with aspirin 400 mg/kg bw revealed severe histopathological changes confined as focal necrosis of gastric mucosal glands associated with inflammatory cells infiltration (Fig. 7e), submucosal oedema associated with congestion of submucosal blood vessels (Fig. 7f). Using of famotin as protective agent revealed some improvement in the histopathological picture of the stomach, it revealed submucosal oedema associated with few inflammatory cells infiltration (Fig. 8a). Moreover, protection with petroleum ether in treating aspirin ulcer revealed regeneration in the gastric mucosa, examined stomach showed only mucosal and submucosal inflammatory cells infiltration (Fig. 8b) . On the other hand, protection with ethanolic extract in treating aspirin ulcer revealed marked improved picture, the examined stomach from this group revealed apparent normal gastric mucosa (Fig. 8c). However, the therapeutic effect of famotin against aspirin ulcer showed incomplete recovery as the stomach of those rats revealed necrosis of epical gastric mucosa (Fig. 8d) in some examined sections. petroleum ether extract as therapeutic agent against aspirin ulcer also revealed some changes in the stomach as submucosal oedema and submucosal strands of fibroblasts proliferation (Fig. 8e). Moreover, the therapeutic effect of ethanolic extract in treating aspirin ulcer showed regeneration of gastric mucosa, submucosal oedema associated with few inflammatory cells infiltration (Fig. 8f).

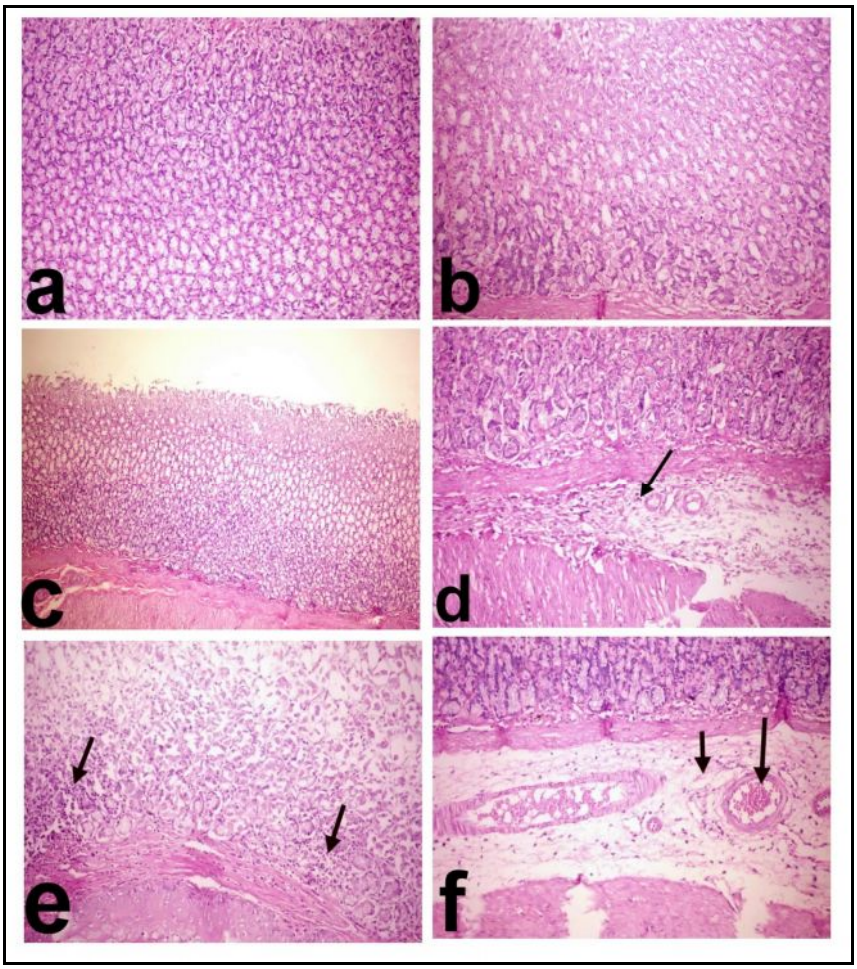

Figure 7. Stomach of rat from: a) normal control, untreated group showing no histopathological changes. b) +ve petroleum ether extract group showing no histopathological changes. c)+ve ethanolic extract group showing no histopathological changes. d) +ve famotin group showing few inflammatory cells infiltration in submucosa (arrow). e) group treated with aspirin 400 mg/kg bw showing focal necrosis of gastric mucosal glands associated with inflammatory cells infiltration (arrows). f) group treated with asprin 400 mg/kg showing submucosal oedema (small arrow) and congestion of submucosal blood vessels (large arrow) (H & E X 200).

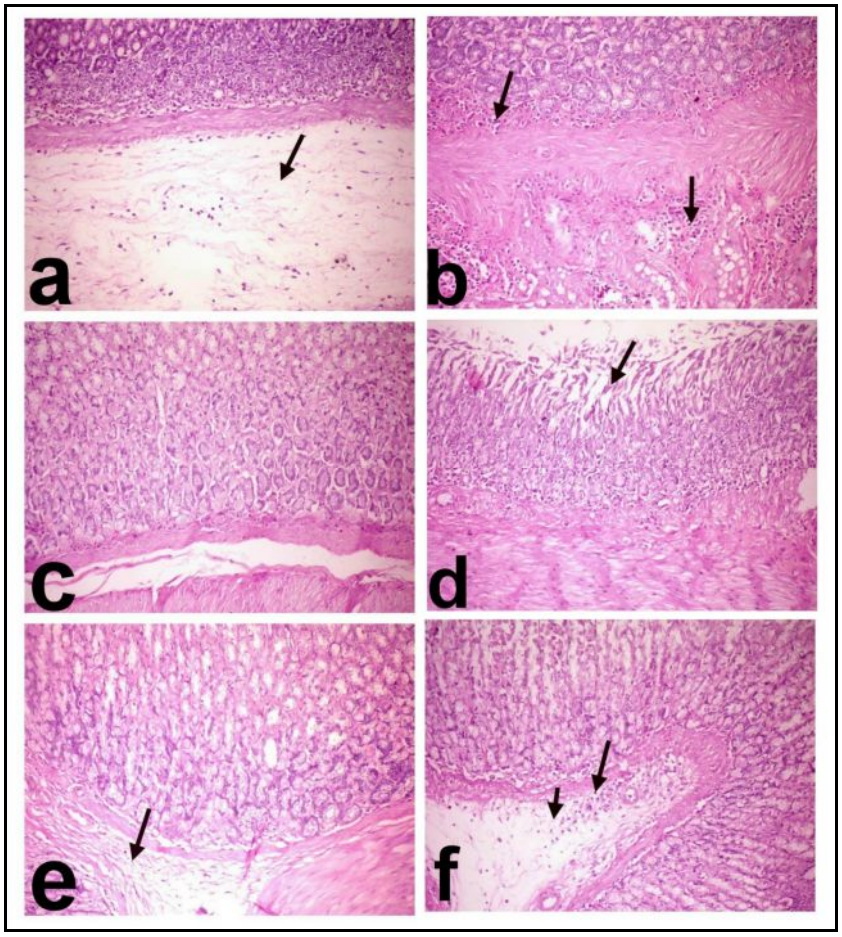

Figure 8. Stomach of rat treated with: a) famotin as protective agent showing submucosal oedema associated with few inflammatory cells infiltration (arrow). b) protective petroleum ether in treating aspirin ulcer showing mucosal and submucosal inflammatory cells infiltration (arrows). c) protective ethanolic extract in treating aspirin ulcer showing apparent normal gastric mucosa. d) therapeutic effect of famotin against aspirin ulcer showing necrosis of apical gastric mucosa (arrow). e) petroleum ether extract as therapeutic agent against aspirin ulcer showing submucosal fibroblasts proliferation (arrow). f) the therapeutic effect of ethanolic extract in treating aspirin ulcer showing submucosal oedema (small arrow) associated with few inflammatory cells infiltration (large arrow) (H & E X 200).

Discussion

The oil is used as substitute for European aniseed in commercial drinks. Chinese star anise considered as one of the flavors used in "china five spices". *I. verum* is a spice frequently used in meat and Chinese cooking. It is stronger than aniseed and it is used in baked goods, confections and liquors. It is effective against Japanese termites and adult German cockroaches, has insecticidal properties there does not see any contraindication for herb or drug interaction. Shikimic acid which extracted from *I. verum* is one of the main ingredients in the antiviral drug Tamiflu used to fight avian influenza [9]. *I.verum* has been reported to possess antioxidant properties[36] as well as significant anticancer potential[37]. Antioxidant properties may be recommended in enhancing shelf life of products such as spices[38]. Natural antioxidants are known to protect cells from damage induced by oxidative stress, which is generally considered to be a cause of ageing, degenerative disease, and cancer [39,40]. Star anise is one of many spices that contain bioactive compounds as well as a number of phenolic and flavonoid compounds, having antioxidant, preservative and antimicrobial properties[41]. The presented results are in accordance with reported effects of *Pimpinella anisum* which is one of the medicinal plants that have been used for different purposes in traditional medicine of Iran. Heretofore, different studies were performed on the essential oil and extracts of *Pimpinella anisum* to identify the chemical compounds and pharmacological properties of this plant, and various properties such as antimicrobial antifungal, antiviral, antioxidant, and insecticidal effects which have been reported of aniseeds. The findings

revealed that aniseeds can cause gastric protection, muscle relaxant, and affect digestive system. These findings are in agreement with *anisum* has hypolipidemic and hypoglycemic effects and reduces lipid peroxidation in diabetic patients. In addition to, aniseeds showed anticonvulsant impact, reduced morphine dependence, and induced conditioned place aversion in mice. Aniseed also has beneficial effects on menopausal and dysmenorrhea hot flashes in women. The recorded activities in this work for *Illicium verum* are attributed to its components. Especially its essential oil content. The plant essential oil is traditionally obtained by hydro-distillation. This technique has been controversial for subsequent determination of the oil chemical composition because of the possible transformation of aroma-active compounds by heat, steam, and pH [42]. Steam distillation process, in which the combination of the relatively high temperature of steam and the hydrolytic influence of water may cause the degradation of essential oil components [43,44]. It was reported that the most important compounds of aniseeds essential oils were estragole, *trans*-anethole, *γ*-hymachalen, *p*anisaldehyde and methyl chavicol. Due to broad spectrum of very few clinical studies and pharmacological effects of this plant, performed on this plant, more clinical trials are recommended to evaluate the beneficial effects of *Pimpinella anisum* in human models and identification of active compounds of plant which can lead to synthesis of new drugs from the active ingredients in future. Our results are in agreement with that anise significantly inhibited gastric mucosal damage induced by necrotizing agents and indomethacin. The antiulcer effect was further confirmed histologically[45].

Conclusions:

Our findings show that star anise fruits could be considered as a good source of natural compounds with significant antioxidant and anti gastric ulcer activities (ethanol and aspirin) which can be attributed to the high percentage of the main constituents (phenolic compounds) or to synergy among the different oil constituents.

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