Austin Journal of Pharmacology and Therapeutics

Austin Publishing Group

Open Access Full Text Article

Research Article

Gastroprotective Effects of Standardized Ginkgo Biloba Extract (Egb761), Aniseed, Quercetin and Trans-Anethole on Ethanol-Induced Ulcers in Rats

Mona F Mahmoud*

Department of Pharmacology and Toxicology, Zagazig University, Egypt

***Corresponding author:** Mona F Mahmoud, Department of Pharmacology and Toxicology, Zagazig University, Zagazig 44519, Egypt

Received: June 09, 2014; **Accepted**: Aug 04, 2014; **Published**: Aug 06, 2014

Abstract

The aim of this study is to investigate the anti-ulcer effect of standardized Ginkgo biloba extract (EGb761), aniseed and their active constituents, quercetin and trans-anethole, on the ethanol- induced gastric ulcers. EGb761 (500 mg/kg), aniseed (250 mg/kg), quercetin (100 mg/kg) and trans-anethole (100 mg/kg) were given 30 min before ulcer induction by ethanol using omeprazole as a standard. Four hours later, ulcer index, serum total antioxidant capacity (TAC), gastric nitric oxide (NOx) activity, gastric myeloperoxidase (MPO) activity, tumor necrosis factor- alpha (TNF-α) level, and DNA fragmentation were assessed. Ethanol has been shown to significantly decrease the serum TAC and to considerably increase the gastric NO- activity, gastric MPO activity, gastric TNF-α level and also induced the DNA fragmentation as compared to the control group. It was found that prophylactic administration of EGb761, aniseed, quercetin and trans-anethole reversed all the pathological changes induced by ethanol. This may suggest the implication of these active components in the gastro-protective effect exhibited by EGb761 and aniseed. Trans-anethole and quercetin may be responsible for the gastro protective effect of EGb761 and aniseed. They may represent new promising alternatives for clinical management of the gastric ulcer diseases.

Keywords: EGb761; Ethanol; Omeprazole; Trans –anethole; Quercetin

Introduction

Pharmaceutical products have been shown to decrease mortality and morbidity from gastro duodenal ulcers and peptic diseases. However, they can produce adverse effects or recurrence. Moreover, they are relatively expensive [1], have been concluded that within one year of treatment discontinuation, the recurrence of ulcer was between 40% and 80% in most of the conducted studies with H_2 antagonists and with the proton pump inhibitor, omeprazole. As the gastric ulcer affects about 5% of the global population, the treatment of this painful disease and its prevention has become one of the challenges today. Due to the lack of side effects compared to the synthetic drugs, approximately 60% of the world's population relies almost entirely on plants for medication. Ginkgo biloba L., is a member of the family Ginkgoaceae, it is the world's oldest living species Extracts from its leaves have been used as a food supplement or healthy food without any restriction in Japan as well as in the United States [2]. The main active constituents of the ginkgo leaves include flavonoids mainly quercetin, kaempferol, and isorhamnetine. It is used today as a standardized dry extract of Ginkgo biloba leaves, (EGb 761) which consists of 24% ginkgo flavone glycosides and 6% terpenoids, which considered as the most important active ingredients in the extract [3] Ginkgo biloba extract decreases the gastric injury caused by ethanol or cold-restraint stress. It also improves the mucosal healing of duodenal ulcers [4].

Anise, *Pimpinella anisum* L. is a member of family Umbelliferae. It is indigenous to Turkey, Iran, India, Egypt, Greece and many other warm regions throughout the world [5]. It contains mainly volatile oils such as trans-anethole, flavonoids like, quercetin, [6]. Trans-anethole constitutes about 70–90% of the total oils extracted from aniseed. It is chiefly responsible for the characteristic taste and smell. Tran's anethole shows antioxidant, gastro protective, anti-inflammatory and anticancer actions [7]. Trans anethole acts as anti-oxidants, it inhibits lipid-per oxidation, and hydroxyl radical scavengers. Due to its antioxidant property, it interferes with TNF-α signaling, suppressing TNF-α induced both lipid per oxidation and ROS. It inhibits TNF-α induced cellular responses, which may explain its role in the suppression of inflammation and carcinogenesis [8].

Quercetin is a component of both anise and Ginkgo biloba and displays a variety of biological actions such as antioxidant, antiviral, antiulcer, ant allergic [9], antihypertensive [10], hepatoprotective [11], and helps men with chronic prostatitis [12].

The aim of the present study was to investigate the effects of EGb761, aniseed and their active components, quercetin and trans-anethole on gastric ulcer induced by ethanol. Their effects were compared with omeprazole which is used as a reference standard.

Materials and Methods

Drugs and chemicals

All drugs were administered orally as single daily doses. All chemicals and reagents were of analytical grade. All drugs and reagents were freshly prepared before use. Standardized Ginkgo biloba extract (EGb761) was used in a dose of 500 mg/kg (Mepaco, Egypt) and suspended in 1% carboxymethyl cellulose (CMC) (w/w) [13]. Anise (seeds of Pimpinella anisum) was used in a dose of 250 mg/ kg (Sekem

Citation: Mahmoud MF. Gastroprotective Effects of Standardized Ginkgo Biloba Extract (Egb761), Aniseed, Quercetin and Trans-Anethole on Ethanol-Induced Ulcers in Rats. Austin J Pharmacol Ther. 2014; 2 (5).7

Co., Egypt) and suspended in distilled water [14]. Quercetin was used in a dose of 100 mg/kg (Tocris Bioscience, UK) and suspended in 2% gum acacia (Suzuki et al., 1998). Trans-anethole oil was used in a dose of 100 mg/kg (Sigma-Aldrich Co., St. Louis, MO, USA) and emulsified in 1% CMC [15], 95 % Ethanol (Biodiagnostic, Egypt) was given in a dose of 5 ml/kg in distilled water (v/v) [16].

Animals

Adult male albino rats weighing (150-200 g) were kept under constant environmental and nutritional conditions throughout the period of investigation. Rats were maintained in clean polypropylene cages (5 rats/ each cage) and fed with regular rat chow and water ad labium. After 2 weeks adaptation period, all rats were deprived of food for 18 hrs before subjecting to ulcerogen in wire mesh bottom cages to prevent coprophagy, but allowed free access to water and were allocated to different experimental groups each consisting of 8 rats. The study was approved by the institutional ethical committee, which follows the guidelines of CPSCEA (Committee for the Purpose of Control and Supervision of Experimental on Animals), which complies with international norms of INSA. Every effort was made to minimize the number of animals and their suffering.

Experimental design

Fifty six adult male albino rats were used in the present study. At the day of the experiment, their weights were measured and randomly assigned into 7 subgroups, each subgroup consists of 8 rats, as follows, Control group, represent normal rats and received the vehicle 1% CMC in distilled water (5 ml/kg). Ethanol group**,** in which acute gastric ulcers were induced by oral administration of 95% ethanol (5 ml/kg) [16]. Omeprazole-treated group, received omeprazole (100 mg/kg, ig.) in water vehicle [17], before receiving ethanol used as a reference drug. EGb761-treated group, received EGb761 (500 mg/kg) suspended in 1% CMC in distilled water 30 min before receiving ethanol.

Aniseed-treated group, received aniseed at a dose of (250 mg/kg) suspended in water vehicle. Quercetin-treated group, received quercetin at a dose of (100 mg/kg) suspended in 2% gum acacia. Trans anethole-treated group, received trans-anethole at a dose of (100 mg/kg) emulsified in 1% CMC–distilled water [15]. All drugs used in this study were administered orally using smooth stainless steel tube connected to an ordinary 3 ml syringe 30 min before receiving ethanol. The tube was introduced into the esophagus during administration to ensure adequate drug delivery and avoid regurgitation.

Induction of gastric ulcer by ethanol

95% ethanol was orally administrated at a dose of 5 ml/kg given 30 min after treatment with the drugs under investigation [18]. Four hours later, rats were sacrificed by cervical dislocation under ether anesthesia according to the method of [19].

Blood sampling, plasma and serum preparation

At the end of the study period, blood samples were taken from the retro-orbital venous plexus of rats using micro capillary tubes according to method of [20], then collected in heparinized (to prevent blood clot) and non heparinized dry glass centrifuge tubes to separate plasma and serum from blood samples, centrifuged at 3000 rpm for 20 minutes at room temperature and the supernatants were obtained. The plasma and the serum were separated, frozen and stored at -80°C for determination of different parameters.

Determination of ulcer index (UI)

Digital pictures of the mucosal surface of each stomach are taken for macroscopically examination, The ulcers were scored according to the method of valcavi et al., 1982 and assessed on the basis of their dimensions as follow , Deep circular ulcers more than 8 mm = 10, 7–8 mm = 8, 6–7 mm = 7, 5–6 mm = 6, 4–5 mm = 5, 3–4 mm = 4, 2–3 mm = 3, 1–2 mm = 2 and 0–1 mm = 1. The deep linear ulcers more than 10 mm in length = 6 and linear ulcer less than 10 mm in length = 3. The score for each single lesion were then summed up for the determination of ulcer index. The protective ratio (%) was calculated according to the following formula,

Preventive ratio (%) = (a-b)/a 100 [4], a, the ulcer index of the ulcerated group b, the ulcer index of the experimental group.

Biochemical analysis

After the measurement of gastric lesions, the glandular segments of stomach were removed and a 10% homogenate was prepared and subjected to biochemical analysis.

The tissue lipid per oxidation was assessed by measuring malondialdehyde (MDA) level which was estimated by the thiobarbituric acid method [21]. Determination of prostaglandin E2 (PGE2) level was measured by enzyme-linked immunosorbent assays (ELISA) [22]. Determination of NO^{-}_{2} and MPO activities was performed by colorimetric method [23]. DNA fragmentation as an index of apoptosis was studied by gel electrophoresis technique [24]**.** In the serum, C-reactive protein (CRP) level was estimated by enzyme-linked immunosorbent assay (ELISA) [25], and total antioxidant capacity (TAC) was determined calorimetrically. TNF-α was determined by RT-PCR method [26]**.** In brief, RNA was extracted, reverse transcribed into cDNA, and amplified by PCR. The isolation of intact RNA requires four essential steps, effective disruption of cells or tissue, denaturation of nucleoprotein complexes, inactivation of endogenous rib nuclease (RNase) activity and removal of contaminating DNA and proteins. At the end of the amplification process, the DNA product was detected using agarose gel electrophoresis. The oligonucleotide primers sequence, TNF alpha gene,

Forward primer, 5-CAATATACAGATGTTCGCTCAAGG-3,

Reverse primer, 5-GTCAAGACAAAGCTGGGCTC-3. Annealing temperature was 60°C. β-actin gene,

Forward primer, 5-CCAGGCTGGATTGCAGTT- 3,

Reverse primer, 5-GATCACGAGGTCAGGAGATG-3. Annealing temperature was 60°C.

Statistical analysis

All data were expressed as mean ± standard error of the mean (S.E.M.). Statistical analysis was performed using the SPSS statistical software program, version 16.0. One-way analysis of variance (ANOVA) followed by LSD *post hoc* test were used to compare the mean values of quantitative variables among the groups. The p value

Table 1: Gastroprotective effect of single oral pretreatments of Omeprazole (100mg/kg), Ginkgo biloba extract (EGb 761, 500mg/kg), aqueous anise suspension (Aniseed, 250mg/kg), Quercetin (100mg/Kg) and Trans anethole (100mg/Kg) on the gastric ulcer index in 95% ethanol treated rats.

Treatments	Ulcer index (mm)	Preventive (%)
Control	0	100
Ethanol Control	35* ± 4.52	-
Omeprazole + Ethanol	1.17@ ± 0.48	96.67
EGb761+ Ethanol	1.83 @ ± 0.98	94.76
Aniseed + Ethanol	2.50 @± 1.33	92.86
Quercetin + Ethanol	8.50 @ ± 4.62	75.71
Trans anethole + Ethanol	6.67 @ ± 2.43	80.95

Results are expressed as mean ± SE. (*) significant difference compared to normal control group (@) significant difference compared to Ethanol treated group at $p < 0.05$. n = 6; by One Way ANOVA and Tukey post hoc test.

less than 0.05 were considered statistically significant.

Results

Effect on ulcer index

Oral administration of 95% Ethanol produced marked gastric ulcers which were found mainly in the glandular part of rat stomach. It caused significant ulceration compared to the control group, expressed as increased ulcer index. Oral administration of omeprazole 30 min before ethanol, administration significantly reduced the ulcer index and significantly prevented the incidence of ulceration by 96.67 % compared to ethanol group. Oral administration of (EGb761) 30 min before ethanol, was found to significantly reduce the ulcer index and also significantly prevent the incidence of ulceration by 94.76 % compared with ethanol group. Oral administration of aniseed 30 min before ethanol significantly reduced the ulcer index and significantly prevented the incidence of ulceration by 92.86 % compared with ethanol group ($P< 0.05$).

Oral administration of quercetin 30 min before ethanol considerably reduced the ulcer index and significantly prevented the incidence of ulceration by 75.71 % compared with ethanol group. Oral administration of trans-anethole 30 min before ethanol significantly reduced the ulcer index as shown in Table 1 and notably prevented the incidence of ulceration by 80.95 % compared with ethanol group ($P<0.05$) as shown in Figure 1.

Effect on serum TAC, gastric NO2- activity, gastric MPO activity and gastric TNF-α

Ethanol administration induced a significant reduction of serum TAC and a significant increase in gastric content of $NO2^-$, TNF-α and gastric MPO activity ($P<0.05$) as compared with the control group. Omeprazole showed a significant increase in the serum TAC by 55.27%, and significant decrease in gastric NO2-, MPO activity and TNF-α by 43.64, 41.93 and 59.39% compared to the ethanol group ($P<0.05$).

Administration of EGb761 showed a significant increase in the serum TAC by 30.19%, and significant decrease in gastric NO_2^- activity, MPO activity and TNF-α by 65.51, 21.77 and 57.89% as compared with the ethanol group. Aniseed also showed a significant elevation in the serum TAC by 58.66%, and significant decrease in gastric NO_2^-, MPO activity and TNF-α level by 52.05, 51.07 and 55.64% compared to the ethanol group.

Furthermore, quercetin increased the serum TAC by 42.51%, and induced a significant decrease in gastric NO_2^- activity, MPO activity and TNF-α by 57.15, 39.24 and 64.66% in comparison to the ethanol group. Administration of trans-anethole also showed a significant increase in the serum TAC by 39.16%, and significant decrease in gastric NO_2^- activity, gastric MPO activity and gastric TNF-α by 26.86, 49.46 and 68.42% as compared with the ethanol group (Table 2).

Effect on DNA fragmentation

Control group showed absence of DNA fragmentation in gastric mucosa, (Lanes 1). In Lane 2, 95% ethanol (5 ml/kg) induced gastric epithelial cell damage with numerous cells sloughed off into the gastric lumen due to cell death, which was associated with DNA fragmentation and was reflected in a significantly high rate of apoptosis in gastric mucosa of ethanol control group.

However, in Lane 3,4,5,6 and 7, ethanol-induced DNA fragmentation was markedly reduced by pretreatment with quercetin, aniseed, EGb761, omeprazole and trans-anethole. All pretreatments provided significant protection against DNA damage. This protection was slightly higher in EGb761, omeprazole and trans-anethole than quercetin and aniseed, suggesting the antiapoptotic role of all pretreatments in preventing cell death during ulceration (Figure 2).

Discussion

Peptic ulcer is one of the most common diseases that affect about

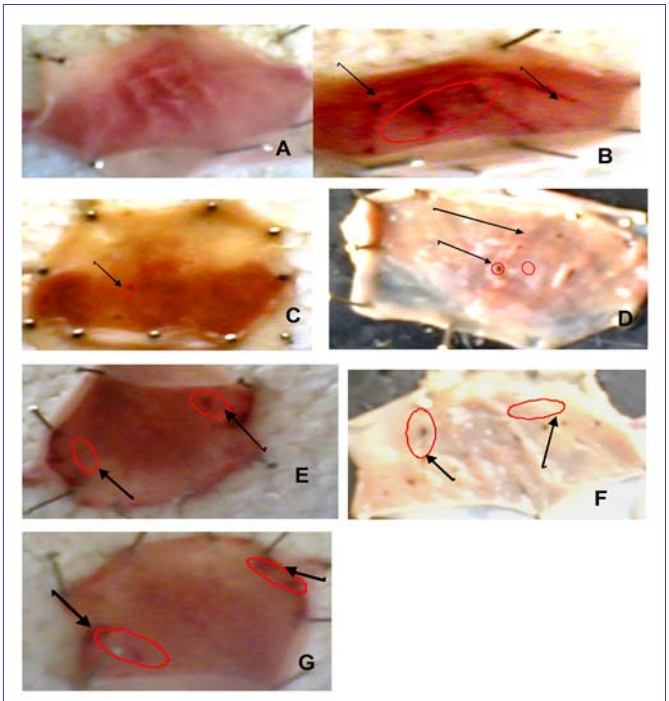

Figure 1: Photomacrographs of rat stomachs cut along the greater curvature obtained from, control group (A), ethanol control group (B) and groups pretreated with omeprazole (C), EGb761 (D), Aniseed (E), Quercetin (F) and Trans anethole (G) showing a remarkable presence of many circular and linear gastric ulcers in ethanol control group, which are markedly decreased in groups pretreated with omeprazole > EGb761 > Aniseed > Trans- anethole > Quercetin.

Table 2: Effect of single oral pretreatments of Omeprazole (100mg/kg), Ginkgo biloba extract (EGb 761, 500mg/kg), aqueous anise suspension (Aniseed, 250mg/kg), Quercetin (100mg/Kg) and Trans anethole (100mg/Kg) on serum total antioxidant capacity (TAC), gastric mucosal nitrite (NO2 -) level, gastric mucosal myeloperoxidase (MPO) activity and gastric mucosal expression of mRNA for tumor necrosis factor alpha (TNF-a) by ratio of TNF-a mRNA over β-actin mRNA in 95% ethanol treated rats.

Parameters	Control	Ethanol	Omeprazole	EGb761	Aniseed	Quercetin	Trans-anethole
TAC (µmol/L)	292.89 ± 22	194.39* ± 14.69	301.83@ ± 22.75	253.08@ ± 24.75	308.43@ ± 16.88	277.04@ ± 14.95	270.52@ ± 21.85
NO2- (µmol/g)	12.48 ± 1.22	20.21 * ± 1.06	11.39@ ± 1.03	6.97@ ± 0.63	9.69@ ± 0.7	8.66@ ± 0.84	14.78@ ± 1.24
MPO (mU/g)	1.51 ± 0.14	3.72 * ± 0.15	2.16@ ± 0.19	2.91@ ± 0.1	1.82@ ± 0.16	2.26@ ± 0.2	1.88@ ± 0.16
TNF-α	0.14 ± 0.01	1.33 * ± 0.13	0.54@ ± 0.03	0.56@ ± 0.05	0.59@ ± 0.05	0.47@ ± 0.04	0.42@ ± 0.04

Results are expressed as mean ± SE. (*) significant difference compared to normal control group (@) significant difference compared to Ethanol treated group at $p < 0.05$. n = 6; by One Way ANOVA and Tukey post hoc test.

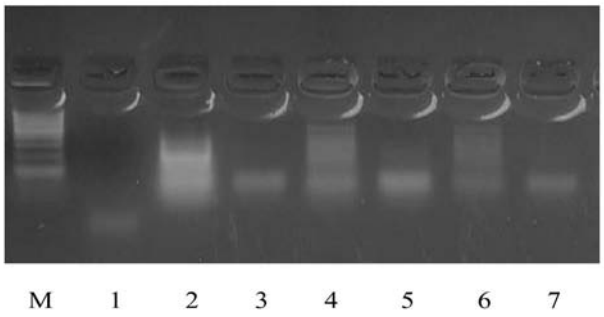

Figure 2: Effect of 95% ethanol (5ml/kg, i.g.) with or without single oral pretreatments of omeprazole (100mg/kg, score= 2), EGb 761 (500mg/kg), Aniseed (250mg/kg), quercetin (100mg/kg) and trans-anethole (100 mg/kg) on DNA fragmentation as an index of apoptosis. The DNA strand break assay in gastric mucosa of control and experimental rats was performed using agarose gel electrophoresis and photographed under UV light. Lane M, DNA ladder with 100bp, Lane 1, Control group (score= 0), Lane 2, ethanol group (score= 6), Lane 3, (Quercetin + ethanol) group (score= 3), Lane 4, (Aniseed + ethanol) group (score= 3) Lane 5, (EGb 761 + ethanol) group (score= 2), Lane 6, (Omeprazole + ethanol) group (score= 2), Lane 7, (Trans anethole + ethanol) group (score= 2), 0 score ◊ no fragmentation, 6 score ◊ severe fragmentation, 1-3 score ◊ mild fragmentation, 4-5 score ◊ moderate fragmentation, and n = 6 in each group.

5% of the global populations. Alcohol intake, several environmental agents, drugs and the stressful lifestyle may contribute to the development of the peptic ulcer. Gingko and aniseed are known to possess antiulcer effects. Whether this effect is related to the total extract or to certain active constituent is not known. The present study was undertaken to investigate the anti-ulcer effect of standardized Ginkgo biloba extract (EGb761), aniseed and whether this effect is attributed to their active constituents, quercetin and trans-anethole, on ethanol- induced gastric ulcers in comparison with omeprazole.

In this study, ethanol was used to induce peptic ulcer. Acute administration of 95% ethanol produced marked gastric ulcers which are found mainly in the glandular part of rat stomach as indicated by the remarkable increase in ulcer index. It has been reported previously that 95% ethanol induced a significant ulceration in the gastric mucosa of rats [27]. Ethanol- induced peptic ulcer involved an increase in oxidative stress in stomach tissue as evidenced by the reduction of serum total antioxidant capacity (TAC). The present study also reported that ethanol produced a significant increase in gastric NO content. Nitrosative stress may contribute to ethanol induced ulcers. Nitric oxide reacts with superoxide anions leading to formation of peroxynitrite, a potent oxidizing agent and other NO radicals. Those radicals in turn can lead to Cytotoxicity and ulcer formation [28]. Ethanol may induce an inflammatory process in the stomach which may precede the development of ulcer [29]. This was manifested in the current study by elevation of the gastric myeloperoxidase (MPO) activity, which is an enzyme released from leukocytes after administration of ethanol. Ethanol initiated the migration of activated leukocytes, then as a response of the inflammatory reaction, leukocytes produced H_2O_2 and radicals that caused injury deeper in the mucosa [30]. Both the migrated leukocytes and the gastric mucosa may produce pro inflammatory cytokines like TNF-α, which plays a central role in the formation of gastric ulcers by initiating the early inflammatory process. TNF-α also decreased gastric blood flow and up regulated gastric mucosal gene expression for gastrin, cyclooxygenases, vascular endothelial growth factor [31]. Data of the current work revealed that ethanol produced a significant increase in gastric TNF-α content. This increase in TNF-α level was associated with appearance of acute gastric mucosal lesions [32]. The inflammatory process developed in the stomach may lead to death of the mucosal cells via apoptosis. This is confirmed in the present study by increasing DNA fragmentation in ethanol group. Ethanol induced a severe DNA damage and increased DNA content of gastric mucosa indicating increased cell shedding and decreased life span of cells [33].

It was observed in the current investigation that EGb761, aniseed, quercetin and trans-anethole produced a significant decrease in ulcer index. Only EGb761 and aniseed were similar to omeprazole in potency. However, both quercetin and trans-anethole were less potent than omeprazole. EGb761 provided a dose-dependent protection against the ethanol-induced gastric ulcers in rats [4]. Furthermore, aqueous suspension of aniseed protected rats against chemically-induced gastric ulcers as necrotizing agents mainly containing ethanol [14]. For further investigation of the mechanisms of actions of EGb761and aniseed, their effects on serum TAC, gastric NO content, gastric MPO activity, gastric TNF-α content and gastric DNA fragmentation as an indicator of apoptosis was studied. The present study revealed that both EGb761 and aniseed showed a potent antioxidant effect as they restored the serum TAC. Moreover, EGb761 antioxidant properties may result from its ability to scavenge free radicals and to neutralize ferryl ion induced per oxidation [34,35]. Aniseed also showed a strong total antioxidant activity, reducing power, and superoxide anion and hydrogen peroxide scavenging, and metal chelating activities [5]. Our results were also confirmed by the ability of EGb761 and aniseed to elevate SOD and reduce catalase activities in indomethacin treated rats (unpublished results). Moreover, EGb761 produced a significant reduction of gastric NO content. EGb761 decreased nitrogen species and had also NO-scavenging properties in a cellular system. Moreover, this effect may be also related to EGb761

induced inhibition of iNOS [36]. Furthermore, EGb761 and aniseed decreased gastric MPO activity in ethanol treated rats. This effect may be attributed to the ability of these agents to reduce neutrophil infiltration and its ability to decrease pro-inflammatory cytokines such as TNF-α and IL-1β [13]. Inhibition of the synthesis, production and release or inhibition of the biological activity of these cytokines might explain the ability of EGb761 to reduce inflammation. This is confirmed in the present study by the reduction of TNF-α production. The present work also showed that EGb761 and aniseed markedly decreased ethanol-induced DNA fragmentation, an indicator of apoptosis. EGb761 significantly inhibited ethanol-induced gastric lesions in rats via blockade of ethanol-induced apoptosis [5]. Recent studies indicated that EGb761 had antioxidant properties due to the presence of flavonoids [37]. In the same way, the gastro-protective effects of aniseed may be attributed to various compounds present in the plant, volatile oils, flavonoids and coumarins among others as the major compounds. Aniseed and its compounds have been identified as active oxygen scavengers [5]. Therefore, in the present study, the major flavonoid, quercetin found in both plants and trans-anethole (the major volatile oil) found in aniseed were chosen for further investigation of the mechanisms of actions of EGb761and aniseed. Both quercetin and trans-anethole reduced the ulcer index in ethanol-treated rats. Quercetin and trans-anethole produced a gastric cytoprotective and gastric ulcer healing actions against ethanol induced mucosal injury [15,38]. Their protective effect may be mediated by their effect on both oxidative and Nitrosative stress, inflammation and apoptosis. They have antioxidant properties as observed in the current study as it increased the serum TAC. This is due to the ability of flavonoids to scavenge free radicals, to chelate metal ions and to act synergistically with other antioxidants [39]. Antioxidant or free radical scavenging activity has been related to the number and position of free hydroxyl groups, which could act by their hydrogen donating capability [40]. Quercetin and its related compounds protect lipids and proteins from otherwise-lethal doses of gamma radiation, largely through their antioxidant properties [41]. Quercetin also reduced gastric NO content. It inhibits iNOS expression through the inhibition of the NF-kB pathway [42]. On the other hand, it was reported that the most abundant constituent of the aromatic plant Pimpinella anisoides species that most effectively inhibited NO production released from macrophages was trans-anethole [43]. The present study revealed that quercetin and trans-anethole counteracted the inflammatory process in gastric mucosa as they reduced gastric MPO activity which is associated with neutrophil infiltration. Quercetin might possess anti-inflammatory action by suppressing the neutrophil/cytokine cascade at the inflammatory sites [44]. A rapid inhibition of free radical generation could contribute to a lower level of neutrophil infiltration into the inflamed tissue [45]. Quercetin in EGb761 reduced neutrophil infiltration in inflamed tissue and reduced MPO activity [13]. The present study revealed that quercetin and trans-anethole showed a significant reduction in gastric TNF-α content. Neutrophil and monocytes were thought to be the targets of the anti-inflammatory effects of quercetin, since they are known to produce the inflammatory mediators. Quercetin suppressed the production and release of inflammatory mediators including TNF-α as it inhibited the function of inflammatory cells that produced these inflammatory mediators [46]. It inhibits cytokine production through inhibition of the NF-kB pathway [42]. On the other hand, trans-anethole inhibits neutrophil infiltration and TNF α induced cellular responses by inhibiting H2O2-induced NF- B activation through antioxidant activity [8]. Among all the cytokines, TNF-α is one of the most potent inducers of apoptosis and that trans-anethole abrogated TNF-α induced apoptosis [8]. Our results showed that quercetin and trans-anethole markedly decreased ethanol-induced DNA fragmentation, an indicator of apoptosis. Previous studies reported that quercetin protected against DNA damage and cell death [30]. Taken together all the studied drugs had a powerful anti-ulcer effect compared to omeprazole. The gastro protective effect of EGb761 and aniseed may be attributed to their content of quercetin and trans-anethole.

Conclusion

The observed results showed that all drugs under investigation were able to reverse the pathological changes caused by ethanol. Moreover, it was found that all investigated drugs were as potent as omeprazole in the reduction of the pathological changes caused by ethanol. EGb761 was found to be more potent than omeprazole in decreasing the Nitrosative stress indicating high antiulcer effects exhibited by these drugs. The novelty of this study is that quercetin and trans-anethole exhibited potent anti-ulcer effects, so they may be responsible for the anti-ulcer effect of aniseed and EGb761 respectively. Trans-anethole and quercetin might be new alternatives for management of gastric ulcer diseases and further studies are required on their anti-ulcerogen process.

References

1. Toma W, Trigo JR, de Paula ACB, Brito ARMS. Modulation of gastrin and epidermal growth factor by pyrrolizidine alkaloids obtained from Senecio brasiliensis in acute and chronic induced gastric ulcers. Can J Physiol Pharmacol. 2004; 82: 319–325.

2. Suzuki R, Kohno H, Sugie S, Sasaki K, Yoshimura T, Wada K, et al. Preventive effects of extract of leaves of ginkgo (Ginkgo biloba) and its component bilobalide on azoxymethane-induced colonic aberrant crypt foci in rats. See comment in PubMed Commons below Cancer Lett. 2004; 210: 159-169.

3. Trumbeckaite S, Bernatoniene J, Majiene D, Jakstas V, Savickas A, Toleikis A. Effect of Ginkgo biloba extract on the rat heart mitochondrial function. See comment in PubMed Commons below J Ethnopharmacol. 2007; 111: 512-516.

4. Chen SH, Liang YC, Chao JCJ, Tsai LH, Chang CC, Wang CC, et al. Protective effects of Ginkgo biloba extract on the ethanol-induced gastric ulcer in rats. World J Gastroenterol. 2005; 11: 3746-3750.

5. Gülçn I, Oktay M, Kreçci E, Küfrevolu Ö. Screening of antioxidant and antimicrobial activities of anise (Pimpinella anisum L.) seed extracts. Food Chem. 2003; 83: 371-382.

6. Tirapelli CR, de Andrade CR, Cassano AO, De Souza FA, Ambrosio SR, da Costa FB, et al. Antispasmodic and relaxant effects of the hidroalcoholic extract of Pimpinella anisum (Apiaceae) on rat anococcygeus smooth muscle. See comment in PubMed Commons below J Ethnopharmacol. 2007; 110: 23-29.

7. Freire RS, Morais SM, Catunda-Junior FE, Pinheiro DC. Synthesis and antioxidant, anti-inflammatory and gastroprotector activities of anethole and related compounds. See comment in PubMed Commons below Bioorg Med Chem. 2005; 13: 4353-4358.

8. Chainy GB, Manna SK, Chaturvedi MM, Aggarwal BB. Anethole blocks both early and late cellular responses transduced by tumor necrosis factor: effect on NF-kappaB, AP-, JNK, MAPKK and apoptosis. See comment in PubMed Commons below Oncogene. 2000; 19: 2943-2950.

9. Hsiu SL, Hou YC, Wang YH, Tsao CW, Su SF, Chao PD. Quercetin significantly decreased cyclosporin oral bioavailability in pigs and rats. See comment in PubMed Commons below Life Sci. 2002; 72: 227-235.

10. Edwards RL, Lyon T, Litwin SE, Rabovsky A, Symons JD, Jalili T. Quercetin reduces blood pressure in hypertensive subjects. See comment in PubMed Commons below J Nutr. 2007; 137: 2405-2411.

11. Janbaz KH, Saeed SA, Gilani AH. Studies on the protective effects of caffeic acid and quercetin on chemical-induced hepatotoxicity in rodents. See comment in PubMed Commons below Phytomedicine. 2004; 11: 424-430.

12. Taepongsorat L, Tangpraprutgul P, Kitana N, Malaivijitnond S. Stimulating effects of quercetin on sperm quality and reproductive organs in adult male rats. See comment in PubMed Commons below Asian J Androl. 2008; 10: 249-258.

13. Mustafa A, El-Medany A, Hagar HH, El-Medany G. Ginkgo biloba attenuates mucosal damage in a rat model of ulcerative colitis. See comment in PubMed Commons below Pharmacol Res. 2006; 53: 324-330.

14. Al Mofleh IA, Al Rashed RS. Nonsteroidal, antiinflammatory drug-induced gastrointestinal injuries and related adverse reactions, Epidemiology, pathogenesis and management. Saudi J. Gastroenterol. 2007; 13, 107-113.

15. Tognolini M, Ballabeni V, Bertoni S, Bruni R, Impicciatore M, Barocelli E. Protective effect of Foeniculum vulgare essential oil and anethole in an experimental model of thrombosis. See comment in PubMed Commons below Pharmacol Res. 2007; 56: 254-260.

16. Chaturvedi A, Kumar MM, Bhawani G, Chaturvedi H, Kumar M, Goel RK. Effect of ethanolic extract of Eugenia jambolana seeds on gastric ulceration and secretion in rats. See comment in PubMed Commons below Indian J Physiol Pharmacol. 2007; 51: 131-140.

17. Cavallini ME, Andreollo NA, Metze K, Araújo MR. Omeprazole and misoprostol for preventing gastric mucosa effects caused by indomethacin and celecoxib in rats. See comment in PubMed Commons below Acta Cir Bras. 2006; 21: 168-176.

18. Dias PC, Foglio MA, Possenti A, de Carvalho JE. Antiulcerogenic activity of crude hydroalcoholic extract of Rosmarinus officinalis L. See comment in PubMed Commons below J Ethnopharmacol. 2000; 69: 57-62.

19. Valcheva-Kuzmanova S, Krasnaliev I, Galunska B, Belcheva A. Influence of DL-alpha-tocopherol acetate on indomethacin-induced gastric mucosal injury in rats. See comment in PubMed Commons below Auton Autacoid Pharmacol. 2007; 27: 131-136.

20. Elwakkad ASE, Alazhary DB, Mohamed S, Elzayat SR, Hebishy MA. The enhancement effect of administration of caffeine in combination with Green tea and its component on lipid profile elements in obese rats. N Y Sci J. 2012; 5: 30-37.

21. Mihara M, Uchiyama M. 1978. Determination of malonaldehyde precursor in tissue by thiobarbituric acid test. Anal Biochem. 1978; 86: 271–278.

22. Awad AB, Smith AJ, Fink CS. Plant sterols regulate rat vascular smooth muscle cell growth and prostacyclin release in culture. See comment in PubMed Commons below Prostaglandins Leukot Essent Fatty Acids. 2001; 64: 323-330.

23. Muthuraman, Sood S. Antisecretory, antioxidative and antiapoptotic effects of montelukast on pyloric ligation and water immersion stress induced peptic ulcer in rat. Prostaglandins Leukot. Essent Fatty Acids. 2010; 83: 55-60.

24. Jainu M, Devi CS. Antiulcerogenic and ulcer healing effects of Solanum nigrum (L.) on experimental ulcer models: possible mechanism for the inhibition of acid formation. See comment in PubMed Commons below J Ethnopharmacol. 2006; 104: 156-163.

25. Mary M Kimberly, Hubert W Vesper, Samuel P Caudill, Gerald R Cooper, Nader Rifai, Francesco Dati, et al. Standardization of immunoassays for measurment of high-sensitivity C-reactive protein. phase, evaluation of secondary reference materials. Clin Chem. 2003; 49: 611–616.

26. Heinig J, Wilhelm S, Bittorf T, Müller H, Brock J, Briese V. Semiquantitative determination of IL-1 alpha, TNF-alpha, PDGF-A, PDGF-B, and PDGF-receptor in term human placenta using polymerase chain reaction (PCR). Zentralblatt fur Gynakologie. 1993; 115: 317-322.

27. Tan PV, Dimo T, Dongo E. Effects of methanol, cyclohexane and methylene chloride extracts of Bidens pilosa on various gastric ulcer models in rats. See comment in PubMed Commons below J Ethnopharmacol. 2000; 73: 415-421.

28. Reiter TA. NO* chemistry: a diversity of targets in the cell. See comment in PubMed Commons below Redox Rep. 2006; 11: 194-206.

29. Eamlamnam K, Patumraj S, Visedopas N, Thong-Ngam D. Effects of Aloe vera and sucralfate on gastric microcirculatory changes, cytokine levels and gastric ulcer healing in rats. See comment in PubMed Commons below World J Gastroenterol. 2006; 12: 2034-2039.

30. Hamauzu Y, Forest F, Hiramatsu K, Sugimoto M. Effect of pear (Pyrus communis L.) procyanidins on gastric lesions induced by HCl/ethanol rats. Food Chem. 2007; 100: 255-263.

31. Gao Y, Zhou S, Wen J, Huang M, Xu A. Mechanism of the antiulcerogenic effect of Ganoderma lucidum polysaccharides on indomethacin-induced lesions in the rat. See comment in PubMed Commons below Life Sci. 2002; 72: 731-745.

32. KwiecieÅ S, Brzozowski T, Konturek SJ. Effects of reactive oxygen species action on gastric mucosa in various models of mucosal injury. See comment in PubMed Commons below J Physiol Pharmacol. 2002; 53: 39-50.

33. Sairam K, Rao ChV, Babu MD, Kumar KV, Agrawal VK, K Goel RK. Antiulcerogenic effect of methanolic extract of Emblica officinalis: an experimental study. See comment in PubMed Commons below J Ethnopharmacol. 2002; 82: 1-9.

34. Chao JC, Chu CC. Effects of Ginkgo biloba extract on cell proliferation and cytotoxicity in human hepatocellular carcinoma cells. See comment in PubMed Commons below World J Gastroenterol. 2004; 10: 37-41.

35. Kwon YS, Ann HS, Nabeshima T, Shin EJ, Kim WK, Jhoo JH, et al. Selegiline potentiates the effects of EGb 761 in response to ischemic brain injury. See comment in PubMed Commons below Neurochem Int. 2004; 45: 157-170.

36. Kotakadi VS, Jin Y, Hofseth AB, Ying L, Cui X, Volate S, et al. Ginkgo biloba extract EGb 761 has anti-inflammatory properties and ameliorates colitis in mice by driving effector T cell apoptosis. See comment in PubMed Commons below Carcinogenesis. 2008; 29: 1799-1806.

37. Erdogan H, FadillioÄŸlu E, Kotuk M, Iraz M, Tasdemir S, Oztas Y, et al. Effects of Ginkgo biloba on plasma oxidant injury induced by bleomycin in rats. See comment in PubMed Commons below Toxicol Ind Health. 2006; 22: 47-52.

38. Dekanski D, Janićijević-Hudomal S, Ristić S, Radonjić NV, Petronijević ND, Piperski V, et al. Attenuation of cold restraint stress-induced gastric lesions by an olive leaf extract. See comment in PubMed Commons below Gen Physiol Biophys. 2009; 28 Spec No: 135-142.

39. Santos MR, Rodríguez-Gómez MJ, Justino GC, Charro N, Florencio MH, Mira L. Influence of the metabolic profile on the in vivo antioxidant activity of quercetin under a low dosage oral regimen in rats. See comment in PubMed Commons below Br J Pharmacol. 2008; 153: 1750-1761.

40. Guardia T, Rotelli AE, Juarez AO, Pelzer LE. Anti-inflammatory properties of plant flavonoids. Effects of rutin, quercetin and hesperidin on adjuvant arthritis in rat. See comment in PubMed Commons below Farmaco. 2001; 56: 683-687.

41. Chawla R, Arora R, Sagar RK, Singh S, Puri SC, Kumar R, et al. 3-O-beta-D-Galactopyranoside of quercetin as an active principle from high altitude Podophyllum hexandrum and evaluation of its radioprotective properties. See comment in PubMed Commons below Z Naturforsch C. 2005; 60: 728-738.

42. Comalada M, Camuesco D, Sierra S, Ballester I, Xaus J, Gálvez J, et al. In vivo quercitrin anti-inflammatory effect involves release of quercetin, which inhibits inflammation through down-regulation of the NF-kappaB pathway. See comment in PubMed Commons below Eur J Immunol. 2005; 35: 584-592.

43. Conforti F, Tundis R, Marrelli M, Menichini F, Statti GA, De Cindio B, et al. Protective effect of Pimpinella anisoides ethanolic extract and its constituents on oxidative damage and its inhibition of nitric oxide in lipopolysaccharide-

stimulated RAW 264.7 macrophages. J Med Food. 2010; 13: 137-141.

44. Zayachkivska OS, Konturek SJ, Drozdowicz D, Konturek PC, Brzozowski T, Ghegotsky MR. Gastroprotective effects of flavonoids in plant extracts. See comment in PubMed Commons below J Physiol Pharmacol. 2005; 56: 219-231.

45. Camuesco D, Comalada M, Rodríguez-Cabezas ME, Nieto A, Lorente MD, Concha A, et al. The intestinal anti-inflammatory effect of quercitrin is associated with an inhibition in iNOS expression. See comment in PubMed Commons below Br J Pharmacol. 2004; 143: 908-918.

46. Morikawa K, Nonaka M, Narahara M, Torii I, Kawaguchi K, Yoshikawa T, et al. Inhibitory effect of quercetin on carrageenan-induced inflammation in rats. See comment in PubMed Commons below Life Sci. 2003; 74: 709-721.

Austin J Pharmacol Ther - Volume 2 Issue 5 - 2014
ISSN: 2373-6208 | www.austinpublishinggroup.com

Citation: Mahmoud MF. Gastroprotective Effects of Standardized Ginkgo Biloba Extract (Egb761), Aniseed, Quercetin and Trans-Anethole on Ethanol-Induced Ulcers in Rats. Austin J Pharmacol Ther. 2014; 2 (5).7