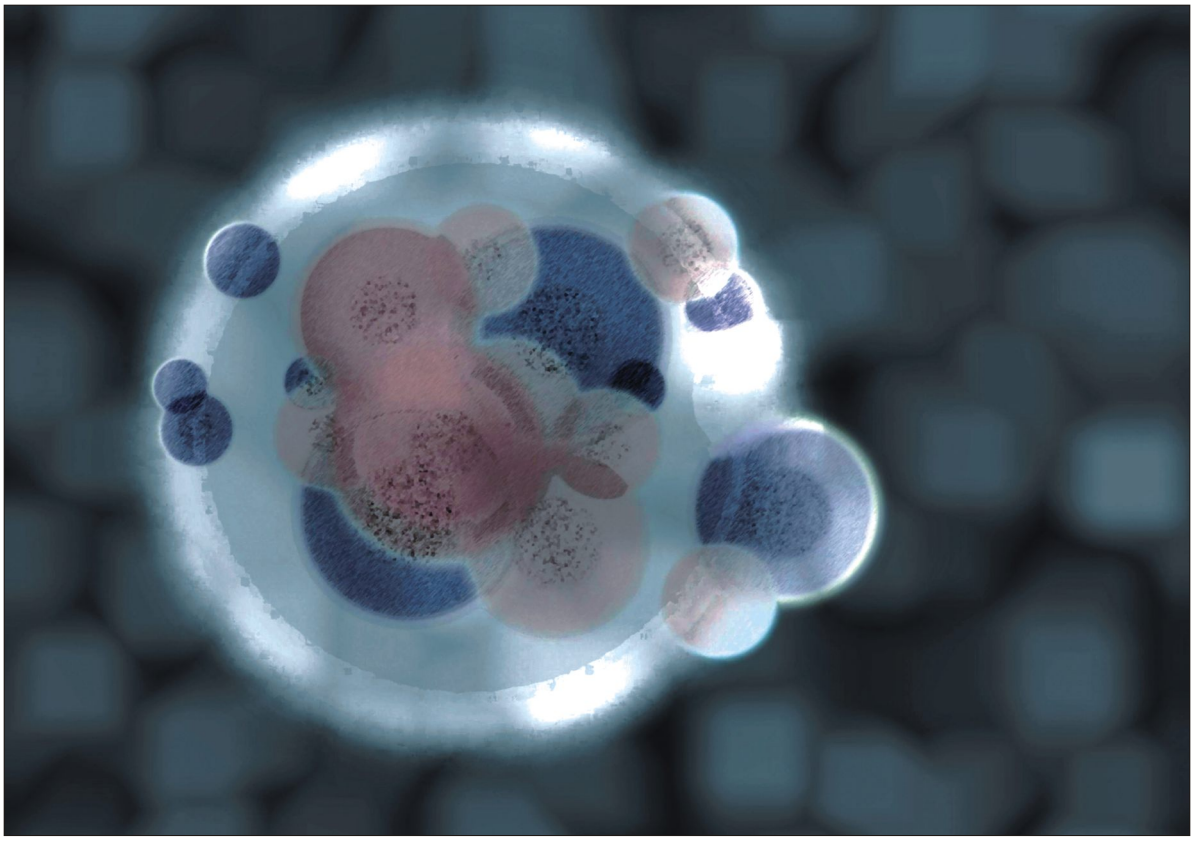

Journal of Biological Sciences

ISSN 1727-3048

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OPEN ACCESS Journal of Biological Sciences

ISSN 1727-3048
DOI: 10.3923/jbs.2019.161.172

Research Article
Protective and Curative Effects of *Bombax ceiba* Flower and *Ziziphus spina christi* Fruit Extracts on Gastric Ulcer

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Abstract

Background and Objective: *Bombax ceiba* L. and *Ziziphus spina christi* L. are medicinal plants with remarkable antioxidant and anti-inflammatory features. The current study aimed to investigate the protective and the curative effects of *Bombax ceiba* flower (BCF) and *Ziziphus spina christi* fruit (ZSCF) extracts against ethanol induced gastric injury in rats. **Materials and Methods:** Gastric ulcer was produced by single oral dose of 5 mL kg^{-1} absolute ethanol on 24 h empty stomachs. One group of animals was left untreated as normal control (NC) group. Other animal groups received orally Ranitidine 100 mg kg^{-1}/day, *Bombax* 300 mg kg^{-1}/day or *Ziziphus* 200 mg kg^{-1}/day for 7 days prior to set A or after ulceration (set B). All rats were sacrificed after 2 h in set A or after 1 week in set B. **Results:** Each extract succeeded to attenuate the severity of ethanol gastric mucosal damage, decreased the elevated ulcer index and cell organelle marker enzymes. Both extracts suppressed gastric inflammation by curbing of tumor necrosis factor-α (TNF-α) levels along with interleukin 1β (IL-1β) and halted gastric oxidative stress via inhibition of lipid peroxides with concomitant enhancement of glutathione (GSH) level. These favorable actions were associated with increase in the gastric cytoprotective factors such as prostaglandin E_2 (PGE_2), heat shock protein 70 (HSP_{70}), nitric oxide (NO_x) and decrease in vascular endothelial growth factor (VEGF) levels. **Conclusion:** The foregoing findings accentuated the protective and curative actions of both extracts in ethanol gastric injury.

Key words: Peptic ulcer, *Bombax ceiba* flower, *Ziziphus spina christi* fruit, antioxidant activity, cytoprotective biomarkers

Received: October 25, 2018 Accepted: November 28, 2018 Published: January 15, 2019

Citation: Maged Mohamed Abd-Elhakim Barakat, Noha Ahmed El-Boghdady, Ebtehal Kamal Eldin Farrag, Ataa Abdelhalim Said and Sylvia Edward Shaker, 2019. Protective and curative effects of *Bombax ceiba* flower and *Ziziphus spina christi* fruit extracts on gastric ulcer. J. Biol. Sci., 19: 161-172.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Peptic ulcers are one of the most prevalent disorders affecting a lot of people worldwide. Its increasing incidence and prevalence results from the disrupt in equilibrium between some endogenous offensive factors like hydrochloric acid, pepsin, reactive oxygen species (ROS) and the defensive factors, such as the mucus bicarbonate barrier, prostaglandin E_2 (PGE_2), mucosal blood flow, cell renewal and migration, enzymatic and non enzymatic antioxidants[1].

Many factors are associated with the pathogenesis of gastric ulcer including bacterial infection with Helicobacter pylori, non steroidal anti-inflammatory drugs and over ingestion of alcohol[2]. Ethanol administration increases the permeability of gastric mucosa leading to increased leakage of hydrogen ions from the lumen and back diffusion of acid[3]. Ethanol gastric mucosal damage develops as a result of increased various mediators such as cyclo-oxygenase, cytokines, free radicals and related signaling molecules leading to inflammation or apoptosis[4].

Antacids, anticholinergics, H_2 receptor blockers and proton pump blockers represent several drug categories available for the treatment of peptic ulcers[5]. However, most of these drugs produce several adverse reactions like arrhythmia, impotence, gynaecomastia and hematopoietic changes. Therefore, there are great needs to search for new alternatives with gastroprotective and ulcer-healing properties from medicinal plants[6]. Many natural products have been examined for their anti-ulcerogenic effects. But up to our knowledge, there are no publications concerning the antiulcerogenic activities of *Bombax ceiba* flower (BCF) and *Ziziphus spina christi* fruit (ZSCF) until now.

Bombax ceiba known as silk cotton tree belongs to family Bombacaceae. It is an important medicinal plant widely cultivated in Pakistan, India and Australia[7]. It was introduced into Egypt several decades ago as an ornamental plant and a shade tree. All parts of the plant have been reported to be of medicinal significance. Its flowers were reported as having diuretic, laxative, tonic and restorative properties due to its chemical constituents. previous research[8] isolated and identified 7 phenolic compounds from BCF including; isovanillic acid, mangiferin, protocatechuic acid, rutin, quercetin 3-O-β-D glucopyranoside, apigenin 7 -O-β-D glucopyranoside, quercetin 3-O-β-D galactopyranoside. Additional phytoconstituents were previously found by several authors; Joshi *et al.*[9] showed that BCF contains Vicenin-2, Saponarin and Cosmetin. Vicenin-2 and Saponarin have antioxidant and anti-inflammatory activities[10,11]. Cosmetin possesses, in addition to the antioxidant effect, an inhibiting activity on gastritis and reflux oesophagitis[12]. Meena and Chaudhary[13] reported that BCF contains Cyanidin-3-glucoside and Hentriacontane. Cyanidin-3-glucoside blocks ethanol induced intracellular accumulation of ROS[14]. Hentriacontane ameliorate the expression of inflammatory mediators as tumor necrosis factor-α (TNF-α)- and interleukin-1 β (IL-1 β)[15].

Ziziphus spina christi commonly known as Christ's Thorn Jujube belongs to the Rhamnaceae family. Fruits are used to treat pulmonary ailments, fevers, promote the healing of fresh wounds, relief digestive disorders, obesity, urinary troubles and as a potent anti-microbial agent. New flavonoid; quercetin 3-xylosyl (1→2) rhamnoside-accompanying with rutin, hyperin, quercetin, apigenin-7-O-glucoide, isovitexin and quercetin-3- lucoside-7-O-rhamnoside were isolated from ZSCF[16]. For these chemical compositions of both BCF and ZSCF, it was hypothesized that both extracts may posses' gastric antiulcer activity.

Therefore, the aim of the present study was to investigate the protective and healing effects of BCF (family: Bombacaceae) and ZSCF (family: Rhamnaceae) extracts against ethanol induced gastric ulcer in rats.

MATERIALS AND METHODS

This work was done in Lab. of Therapeutical Chemistry Department, National Research Center, El Buhouth St., Dokki, Cairo, Egypt as a PhD study. The total time duration was 2 years starting from February, 2015 till February, 2017.

Drugs and chemicals: Ranitidine hydrochloride was kindly provided by medical union pharmaceuticals (MUP, Ismailia, Egypt). The ELISA kits used for measuring prostaglandin E_2 (PGE_2), heat shock protein 70 (HSP_{70}), vascular endothelial growth factor (VEGF) and IL-1β were purchased from Cloud Clone Corp (USA). TNF-α ELISA kit was purchased from Thermo Scientific (USA).

Plant collection and extraction: The BCF and ZSCF were collected from Orman botanical garden, Giza, Egypt. After complete extraction of fresh flowers and fruits (1 kg) with 70% ethanol, the aqueous ethanol was evaporated under vacuum. For the experiments the semi solid residues of extracts were freshly prepared as a suspension in distilled water and administered orally to rats.

Phytochemical screening: The ethanolic extracts for both BCF and ZSCF were tested for the presence of carbohydrates and/or glycosides, tannins, alkaloids, flavonoids, sterols, saponins and coumarins[17].

Total phenolic assay: The total phenolic content (TP) was determined using Folin-Ciocalteu colorimetric method previously described by Karawya and Aboutabl[18]. The TP content was expressed as milligrams of gallic acid equivalents (g of the dry plant material.

Total flavonoid assay: The total flavonoid content (TF) was measured using an aluminum chloride colorimetric assay[19]. The TFC was expressed as milligrams of rutin equivalents/g of the dry plant material.

HPLC analysis of flavonoids and phenolic compounds: Flavonoids and phenolic compounds of BCF and ZSCF were detected and determined according to the method described by Goupy *et al.*[20] and Mattila *et al.*[21] using HPLC instrument. The HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using C18 column (4.6×250 mm i.d., 5 µm).

Acute toxicity test: The acute toxicity study was used to determine the safe dose for both extracts. About 50 fasted rats (25 males and 25 females) were assigned equally into 5 groups and orally received; vehicle (distilled water), 2 and 5 g kg^{-1} of each extract. Food was withheld for a further 3-4 h after dosing. The animals were observed for 48 h after the administration of the extracts for the onset of clinical or toxicological symptoms. Mortality, if any, was reported over a period of 2 weeks.

Experimental design: About 72 adult male albino Wistar rats weighing 150-200 g were fasted for 24 h before the experiment. They were allowed free access to water during the fasting period until 2 h before the start of the experiment[22]. They were divided into 9 groups each consisting of 8 animals (Fig. 1):

Group 1: Normal control (NC) group: Animals were left untreated

- Set A (prophylactic groups): from group 2-5

Group 2: Prophylactic ethanol control (PE) group: Animals administered single oral dose of absolute ethanol (5 mL kg^{-1})[23] and left for 2 h without treatment then sacrificed

Group 3: Prophylactic ranitidine (PR) group: Animals administered Ranitidine 100 mg kg^{-1}/day[24] for 1 week before ulceration

Group 4: Prophylactic *Bombax* (PB) group: Animals administered *Bombax* 300 mg kg^{-1}/day[25] for 1 week before ulceration

Group 5: Prophylactic *Ziziphus* (PZ) group: Animals administered *Ziziphus* 200 mg kg^{-1}/day[26] for 1 week before ulceration

- Set B (Treated groups): From group 6-9

Group 6: Treated ethanol control (TE) group: Animals administered single oral dose of absolute ethanol (5 mL kg^{-1})[23] and left for 1 week without treatment then sacrificed

Group 7: Treated ranitidine (TR) group: Animals administered Ranitidine 100 mg kg^{-1}/day[24] for 1 week after ulceration

Group 8: Treated *Bombax* (TB) group: Animals administered *Bombax* 300 mg kg^{-1}/day[25] for 1 week after ulceration

Group 9: Treated *Ziziphus* (TZ) group: Animals administered *Ziziphus* 200 mg kg^{-1}/day[26] for 1 week after ulceration

All rats were sacrificed after 2 h, from ethanol administration, in set A or after 1 week in set B. All experiments were carried out after approval of the protocol by the ethics committee of Faculty of Pharmacy, Cairo University and of NRC (Ethical approval No. is BC 1010 and 13163, respectively) and conformed to the ethical guidelines of the 1975 Helsinki Declaration.

Measurement of gastric secretion: Rats were anesthetized, the abdomen was dissected and the stomach contents were collected, measured, centrifuged and subjected to analysis for titratable acidity against 0.01 N NaOH[27,28].

Quantification of ulceration: The glandular portion comprising of the fundic and corpus region of each stomach was opened longitudinally along the greater curvature and examined macroscopically using a 10×magnifying lens to assess the formation of ulcerations. The number and ulcer score (US) in the glandular mucosa were scored from 0-10 according to the method of Tan *et al.*[29].

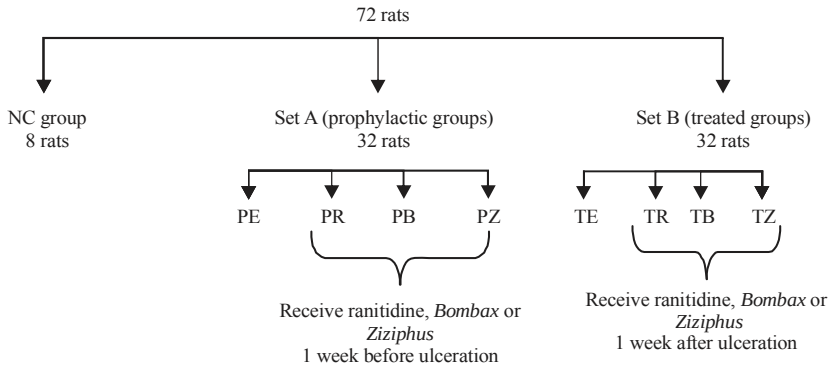

Fig. 1: Experimental design

Preparation of tissue homogenate: Longitudinal sections weighing 0.5 g from each stomach were homogenized in 5 mL phosphate buffered saline (pH 7.4). The homogenate was centrifuged at 4000 rpm for 10 min at 4°C and the supernatant was stored at -20°C for further studies.

Biochemical determinations in stomach homogenate: *Glutathione* (GSH) in the tissue homogenate was measured spectrophotometrically using the method of Moron *et al.*[30]. *Lipid peroxide*, *malondialdehyde* (MDA), content in gastric mucosal tissues was determined by thiobarbituric acid reaction as described by Ruiz-Larrea *et al.*[31]. *Nitric oxide* (NO_x): NO_x metabolites were assayed colorimetrically, after griess reaction, as described by Miranda *et al.*[32]. Protein concentration was determined following the method described by Bradford[33] using bovine serum albumin as standard.

Succinate dehydrogenase (SDH), Glucose-6-phosphatase (G-6-Pase) and acid phosphatase (AP) activities were estimated in stomach homogenate by the method of Rice and Shelton[34], Swanson[35] and Wattiaux and De Duve[36], respectively.

PGE_2 was measured by solid phase competitive ELISA kit while VEGF, HSP_{70}, TNF-α and IL-1β levels were measured by solid phase sandwich ELISA kits according to manufacturer instructions.

Histopathological evaluation: Stomach was immediately immersed in 10% formalin-saline, embedded in paraffin and 5 µm sections were prepared, stained with hematoxylin and eosin (H and E) and examined microscopically[37].

Statistical analysis: Data were expressed as means±standard error (SEM) of 8 readings. The results were analyzed statistically by one-way analysis of variance (ANOVA) using SPSS (Statistical Package for the Social Sciences, version 16.0.1, Chicago, IL) software. Comparison of numerical variables between the studied groups was done using Scheffe's test as a post hoc pair wise comparisons. Differences were considered significant at $p<0.05$.

RESULTS

Phytochemical constituents: Phytochemical screening of BCF and ZSCF ethanolic extract revealed the presence of carbohydrates, tannins, flavonoids, sterols and coumarins. Saponins and alkaloids were present in ZSCF and absent in BCF (Table 1).

Total phenolics and flavonoids content: Table 2 showed that the total phenolic content of BCF and ZSCF were 0.68 and 0.51 mg of gallic acid equivalents g of the dry plant material, respectively. The total flavonoids were 0.55 and 0.21 mg rutin equivalents/g of the dry plant material, respectively.

HPLC analysis of phenolic and flavonoids compounds: The experiment revealed the identification of 12 phenolic compounds in BCF, the most abundant one was naringenin (11.77 mg g^{-1}) while ZSCF revealed the presence of 8 phenolic compounds. The most abundant one in ZSCF was gallic acid (0.09 mg g^{-1}). These results were illustrated in Table 3.

Acute toxicity study: During the period of 2 weeks, neither toxic symptoms nor mortality was observed. Likewise, no abnormal behavioral changes or body weight (b.wt.) alteration were observed at both mentioned doses. Both

Table 1: Phytochemical screening of BCF and ZSCF

Constituents	BCF	ZSCF
Carbohydrates and/or glycosides	+	+
Tannins	+	+
Alkaloids	-	+
Flavonoids	+	+
Sterols	+	+
Saponins	-	+
Coumarins	+	+

+: Presence of the constituent, -: Absence of the constituent, BCF: *Bombax ceiba* flower, ZSCF: *Ziziphus spina christi* Fruit

Table 2: Total phenolic and flavonoid content of BCF and ZSCF

Plant	Phenolic content (mg g^{-1})	Flavonoid content (mg g^{-1})
BCF	0.68	0.55
ZSCF	0.51	0.21

BCF: *Bombax ceiba* flower, ZSCF: *Ziziphus spina christi* Fruit

Table 3: HPLC analysis of phenolic and flavonoid compounds of ethanolic extract of BCF and ZSCF

Peak No.	R_t (min)	Identified phenolic compound	Conc. (mg g^{-1}) in BCF	Conc. (mg g^{-1}) in ZSCF
1	3.126	Gallic acid	0.29	0.09
2	4.935	Caffeic acid	1.35	0.02
3	5.281	Syringic acid	0.17	0.00
4	5.479	Rutin	0.82	0.08
5	7.500	P-Coumaric acid	0.15	0.02
6	8.161	Vanillin	0.04	0.00
7	8.859	Ferulic acid	0.22	0.00
8	9.484	Naringenin	11.77	0.00
9	10.637	Quercetin	0.29	0.05
10	11.128	Cinnamic acid	0.01	0.01
11	10.220	Propyl gallate	0.04	0.01
12	10.372	4,7-dihydroxy isoflavone	0.03	0.01

R_t: Retention time

Table 4: Symptoms and death of animals treated with BCF and ZSCF

Groups	Dose	Death	Symptoms
Control	Distilled water	0	none
BCF	2 g kg^{-1}	0	none
ZSCF	2 g kg^{-1}	0	none
BCF	5 g kg^{-1}	0	none
ZSCF	5 g kg^{-1}	0	none

10 animals for control and each dose

extracts proved to be safe up to 5 g kg^{-1} b.wt. These results were illustrated in Table 4.

Biochemical analysis

Effect of BCF and ZSCF extracts on gastric ulcer indices: Data in Table 5 showed that the greatest decrease in measured gastric ulcer indices was due to BCF extract administration which recorded better results than ZSCF and ranitidine. These results were accentuated by BCF therapeutic effect where it showed the best decrease in the measured gastric ulcer indices.

Effect of BCF and ZSCF extracts on cell organelle marker enzymes in stomach: Stomach ulcer protected by BCF (PB) and ZSCF (PZ) ameliorated the significant increase in all cell organelle marker enzymes as compared with PE group. Here again BCF declared the best significant decrease in all measured enzymes. Therapeutic effect of BCF (TB) and ZSCF (TZ) after ulcer induction confirmed the above results where BCF achieved the best improvement in SDH, G-6-Pase and AP (Table 6).

Effect of BCF and ZSCF extracts on oxidative stress markers in stomach: Gastric ulcer protected for 1 week with either BCF or ZSCF extract improved the significant increase in MDA due to ulcer formation. Conjointly, the 2 extracts ameliorated the significant decrease in both GSH and NO_x levels due to ulceration (Table 7). Treatment of gastric ulcer with BCF (TB) or ZSCF (TZ) for one week affirmed the protected results where BCF succeeded in reaching the best improvement. It corrected the significant increase in MDA and the significant decrease in both GSH and NO_x levels.

Table 5: Effect of BCF and ZSCF extracts on gastric ulcer indices in stomach

	Parameters			
Groups	Gastric juice volume (μL)	Total acidity (mEq L^{-1})	Ulcer number	Ulcer score (US)
NC	139.50±10.8	16.6±0.73	-	-
Prophylactic groups				
PE	2105.00±88.33^{a}	39.2±2.05^{ad}	13.7±1.1	11.1±0.99
PR	1307.00±89.1^{ab}	22.9±1.79^{ab}	7.4±0.77^{ab}	7.2±0.67^{ab}
PB	1080.00±72.65^{abd}	17.7±0.93^{bd}	4.9±0.37^{abd}	4.3±0.47^{abd}
PZ	1402.00±9.11$^{a\,b}$	23.2±1.21^{ab}	7.1±0.57^{ab}	5.5±0.49^{abd}
Treated groups				
TE	206.00±14.32^{e}	25.6±1.8^{a}	3.1±0.34^{a}	1.6±0.17^{a}
TR	138.88±11.09^{c}	17.4±1.41^{c}	1.4±0.12^{ac}	1.0±0.13^{a}
TB	133.00±8.7^{c}	16.1±0.69^{c}	0.6±0.11^{ace}	0.5±0.16^{ace}
TZ	186.50±12.1^{ae}	19.2±1.47^{ac}	1.1±0.13^{ac}	0.8±0.13^{ac}

Data are Means±SE of eight rats in each group, $^{a}p<0.05$ compared to NC group, $^{b}p<0.05$ compared to PE group, $^{c}p<0.05$ compared to TE group, $^{d}p<0.05$ compared to PR group, $^{e}p<0.05$ compared to TR group

Table 6: Effect of BCF and ZSCF extracts on cell organelle marker enzymes in stomach

	Parameters		
Groups	SDH (μmol mg^{-1} protein)	G-6-Pase (μmol mg^{-1} protein)	AP (μmol mg^{-1} protein)
NC	3.12±0.23	1.43±0.128	5.60±0.32
Prophylactic groups			
PE	8.86±0.39[a]	15.12±0.186[a]	20.25±1.12[a]
PR	4.14±0.14[b]	7.24±0.138[ab]	11.84±0.22[ab]
PB	3.66±0.21[b]	4.57±0.136[abd]	8.45±0.31[abd]
PZ	4.94±0.23[ab]	6.709±0.12[ab]	11.35±0.72[ab]
Treated groups			
TE	4.39±0.32[a]	9.26±0.3[a]	14.23±0.89[a]
TR	3.44±0.28	5.62±0.11[ac]	8.54±0.31[ac]
TB	2.93±0.2[c]	3.57±0.28[ace]	7.22±0.23[ac]
TZ	3.81±0.25	4.76±0.36[ac]	8.64±0.43[ac]

Data are Means±SE of 8 rats in each group, [a]p<0.05 compared to NC group, [b]p<0.05 compared to PE group, [c]p<0.05 compared to TE group, [d]p<0.05 compared to PR group, [e]p<0.05 compared to TR group, SDH: Succinate dehydrogenase, G-6-Pase: Glucose-6-phosphatase, AP: Acid phosphatase

Table 7: Effect of BCF and ZSCF extracts on oxidative stress markers in stomach

	Parameters		
Groups	MDA (nmol mg^{-1} protein)	GSH (μg mg^{-1} protein)	NO_x (μmol mg^{-1} protein)
NC	750.0±37.85	18.48±0.83	25.98±1.93
Prophylactic groups			
PE	4356.0±254.4[a]	3.93±0.22[a]	6.88±0.26[ad]
PR	1519.0±98.29[ab]	14.94±0.96[ab]	10.50±0.12[ab]
PB	1434.0±93.19[ab]	16.86±0.79[b]	16.32±0.69[abd]
PZ	1618.0±89.59[ab]	13.68±0.82[ab]	14.23±0.73[abd]
Treated groups			
TE	3031.0±275.83[a]	7.71±0.22[a]	11.65±0.11[a]
TR	1142.0±80.88[ac]	17.78±1.04[c]	15.00±0.708[ac]
TB	1212.0±89.93[ac]	18.71±0.74[c]	19.13±0.21[ace]
TZ	1429.0±92.53[ac]	15.79±0.62[ac]	12.53±0.32[ae]

Data are Means±SE of 8 rats in each group, [a]p<0.05 compared to NC group, [b]p<0.05 compared to PE group, [c]p<0.05 compared to TE group, [d]p<0.05 compared to PR group, [e]p<0.05 compared to TR group, MDA: Malondialdehyde, GSH: Glutathione, NO_x: Nitric oxide

Effect of BCF and ZSCF extracts on some inflammatory cytokines in stomach: Data in Fig. 2 showed that BCF and ZSCF significantly decreased the elevated TNF-α, IL-1β and VEGF. This outcome was ascertained by the therapeutic groups.

Effect of BCF and ZSCF extracts on some gastroprotective factors in stomach: Administration of BCF recorded the best increase in the reduced levels of PGE_2 and HSP_{70}, due to ulceration. Similar results were found therapeutically where BCF achieved the best amelioration in PGE_2 and HSP_{70} (Fig. 3). By comparing prophylactic and treated groups in all measured parameters. The treated groups, either for BCF or ZSCF showed better results than the prophylactic groups. These results were ascertained by the histopathological data.

Histopathological results: Histopathological results presented in Fig. 4 showed that the stomach of normal rats revealed no alterations. Normal histological structure of the gastric mucosa was recorded (a). By contrast, stomach sections from rats of PE group showed disruption of surface epithelium with ulcer formation, marked edema and leucocytes infiltration of the submucosal layer (b).

Prophylactic treatment of PR, PB and PZ improved the alterations in gastric morphology evidenced by the appearance of almost normal surface epithelium, no leucocytes infiltration of the submucosal layer and normal muscle layer for the 3 treatments. Mild edema was showed in PR and PB groups (c and d, respectively), while marked edema was observed in PZ group (e).

Rats treated with ethanol, ranitidine, BCF and ZSCF for 1 week showed almost normal surface epithelium, no leucocytes infiltration of the submucosal layer and normal muscle layer. The TR, TB and TZ showed no edema (g, h and i, respectively) while TE showed mild edema (f).

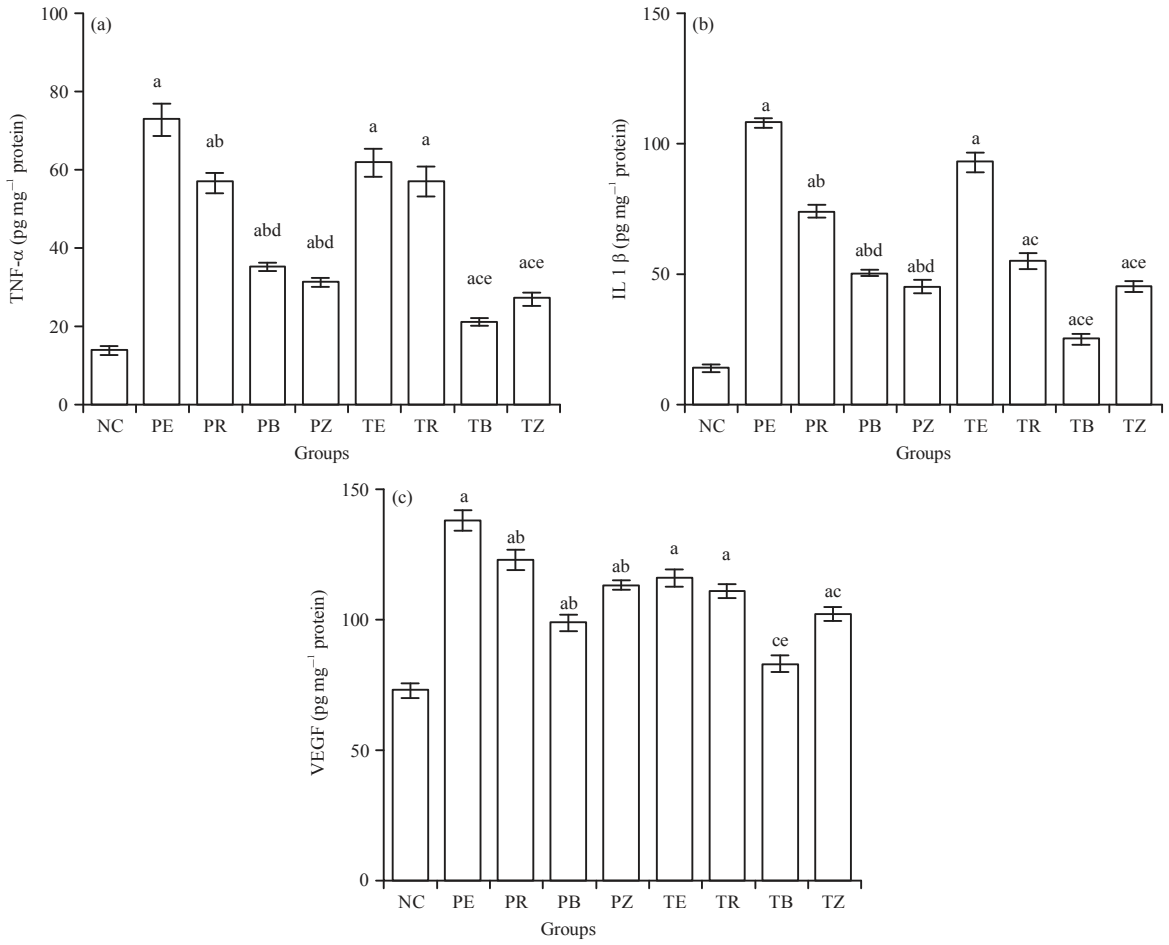

Fig. 2(a-c): Effect of BCF and ZSCF extracts on some inflammatory cytokines in stomach, (a) TNF-α: Tumor necrosis factor alpha, (b) IL-1β: Interleukin 1β and (c) VEGF: Vascular endothelial growth factor

Data are Means±SE of 8 rats in each group, [a]p<0.05 compared to NC group, [b]p<0.05 compared to PE group, [c]p<0.05 compared to TE group, [d]p<0.05 compared to PR group, [e]p<0.05 compared to TR group

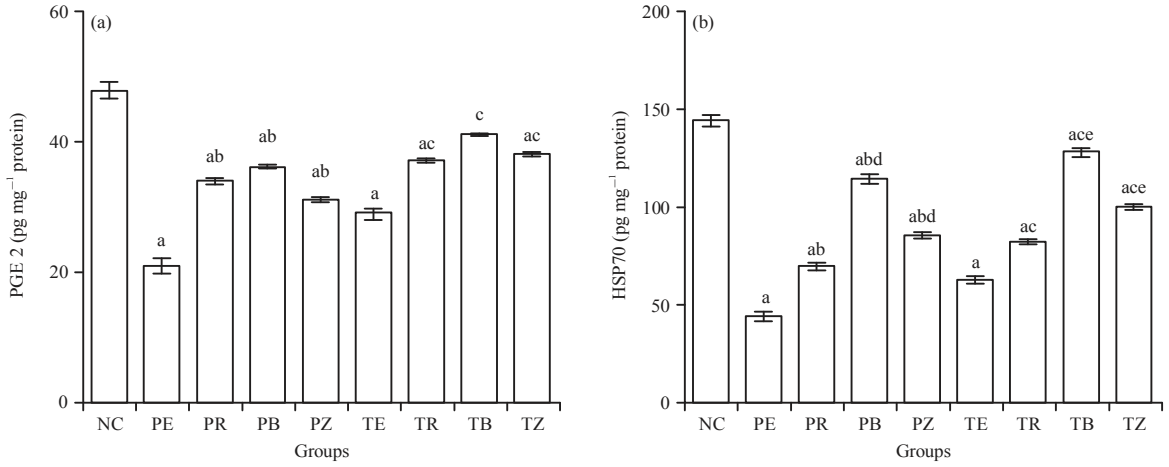

Fig. 3(a-b): Effect of BCF and ZSCF extracts on gastroprotective factors in stomach, (a) PGE_2: Prostaglandin E_2 and (b) HSP_{70}: sHeat shock protein 70

Data are Means±SE of 8 rats in each group, [a]p<0.05 compared to NC group, [b]p<0.05 compared to PE group, [c]p<0.05 compared to TE group, [d]p<0.05 compared to PR group, [e]p<0.05 compared to TR group

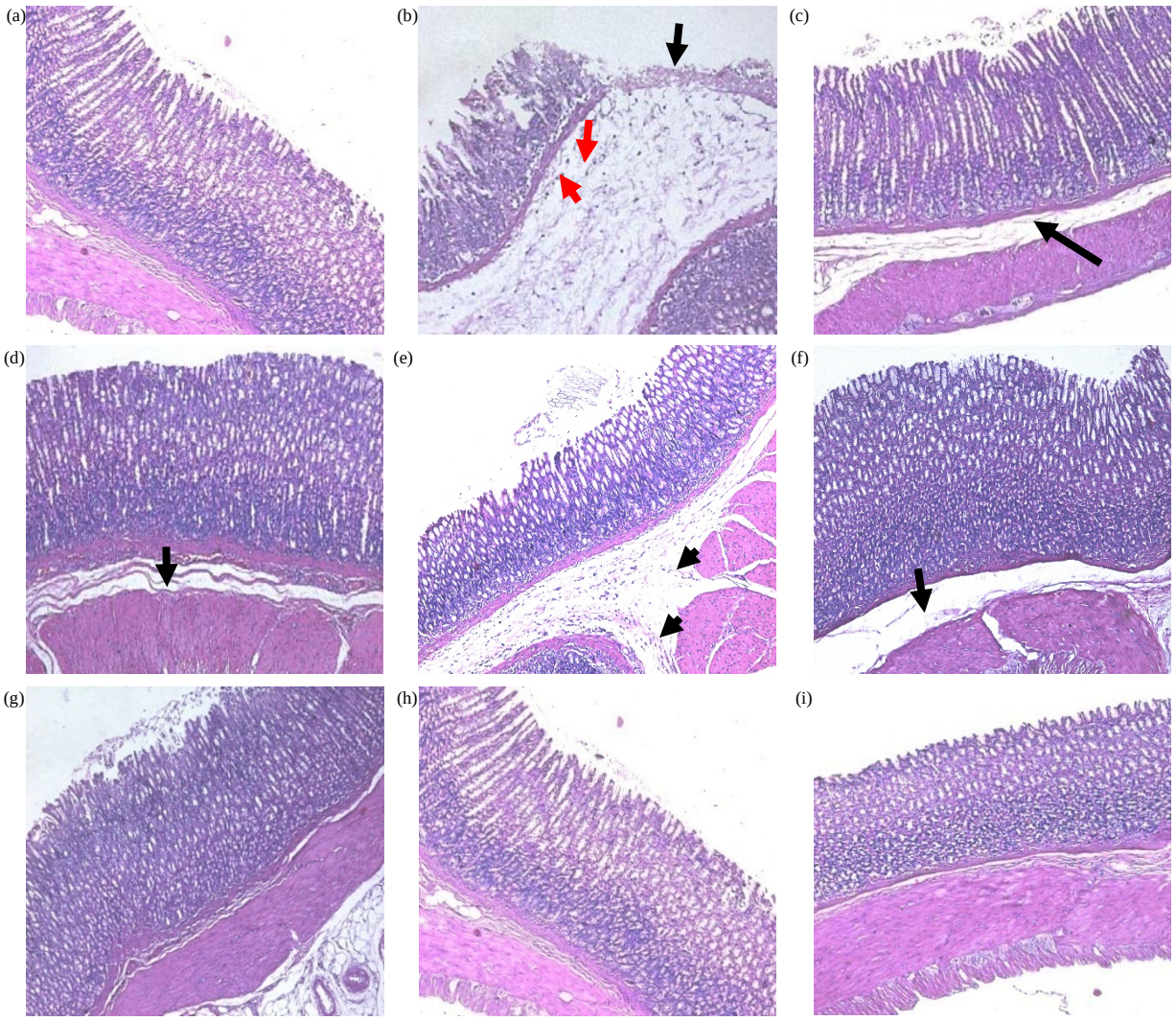

Fig. 4(a-i): Photomicrographs of gastric sections (H and E stain, x100), (a) NC: Normal histological structure of the gastric mucosa, (b) PE: Disruption of surface epithelium with ulcer formation (black arrow), marked edema and leucocytes infiltration of the submucosal layer (red arrow), (c) PR: Almost normal surface epithelium appearance with mild edema (black arrow), (d) PB: Showed almost normal surface epithelium with mild edema (black arrow), (e) PZ: Showed almost normal surface epithelium with moderate to marked edema (black arrow), (f) TE: There was almost normal surface epithelium with mild edema (black arrow) and (g-i) For groups TR, TB and TZ, respectively, there was almost normal histological structure

DISCUSSION

Ethanol administration augments oxidative stress and inflammation while weakens the mucosal protective factors[38]. The oxidative stress and inflammation were evidenced, in the present study, by depletion of gastric GSH and elevation of MDA, IL-1β and TNF-α-content[39,40]. The enzyme leakage was proved by increasing the activity of SDH, G-6-Pase and AP in ulcerated rats.

IL-1β induces acute phase response and release of TNF-α[41,42]. The TNF-α enhances ROS generation, expression of nuclear factor kappa (NF-κB) and the production of other cytokines. It suppresses the gastric micro-circulation around ulcerated mucosa thus delaying its healing[43,44].

The observed reduction in NO level may be explained by down regulation of gastric mucosal constitutive nitric oxide synthetase or the consumption of NO in the overproduction of peroxynitrites during ethanol metabolism[45,46]. Previously, it

was demonstrated that ethanol administration reduces PGE_2 synthesis in gastric mucosa[4,47]. The ROS converts prostaglandin into 8-iso-prostaglandin F_{2alpha} that causes vasoconstriction and platelet aggregation[48,49]. The GSH augments prostaglandin action and stabilizes mucus composition by controlling the thiol/disulfide ratio[50]. The observed decrease in HSP_{70} level is mainly attributed to its damage by the ROS generation[49,51]. The elevation of VEGF level may be explained by the increase of; IL-1, TNF-α or increase in hypoxia inducible factor-1α mRNA[52]. These factors reinforce the binding of NF-κB to the VEGF gene promoter resulting in the increase in VEGF expression[53]. The BCF and ZSCF extracts succeeded in protecting and treating gastric mucosa after ethanol injury more than ranitidine the reference drug[54].

Due to their antioxidant effect, both extracts protected the cell organelles integrity and combated oxidative stress by augmenting GSH[55] and quenched the superoxide anion which MDA consumes NO[56].

Due to their anti-inflammatory and protecting effect, the 2 plants decreased IL-1β, TNF-α and VEGF level while increased PGE_2 and HSP_{70} levels. Consequently, elevated prostaglandin attenuated the release of IL-1β and TNF-α[40]. HSP_{70} suppressed mitogenic activated protein kinase (MAPK) which transduces signals to initiate inflammatory cellular response[57]. Finally, the decrease in IL-1β, TNF-α and MAPK lead to decrease in VEGF level.

Both extracts contain rutin, quercetin and gallic acid previously reported to prevent gastric mucosal ulceration in several animal models[58,59]. Rutin inhibits Platelet activating factor (PAF) and the gastric proton pump[60]. Quercetin exhibits an antiradical property toward hydroxyl, peroxyl radicals and superoxides anions[61]. Gallic acid has the ability to form a protective pellicle by promoting precipitation of protein on the ulcer in order to prevent ulcer development. This pellicle helps in preventing toxic substance absorption and combat the attack of proteolytic enzymes[62].

The present study revealed that ZSCF was ranked after BCF in almost all measured parameters. This may be due to higher concentration of total phenolics and flavonoids in BCF, as well as it contains additional phytoconstituents. The BCF contains caffeic, ferulic, p-coumaric, syringeic and cinnamic acids which are previously reported of having potent anti ulcer, antioxidant and antilipid peroxidation activities[63-65].

Naringenin was the most abundant flavonoid identified in BCF. Cavia-Saiz *et al.*[66] found that naringenin is a strong scavenger of free radicals and prevent lipid peroxidation. Nishimura *et al.*[67] found that naringenin exerts its effects by controlling the effector mechanisms of ROS production through ROS scavenging activity[68,69].

Said *et al.*[8] isolated and identified mangiferin from BCF. Mangiferin (MF) possesses a gastroprotective effect via its antisecretory and antioxidant activities[70,71]. It maintains the cellular oxidation-antioxidant balance as it generates MF phenoxy radicals that binds to metal ions ($Fe^{2+/3+}$) and form a stable MF-iron complex. This complex does not allow the generation of hydroxyl ($OH^{\bullet}$) radicals and/or oxo-ferryl groups and scavenges lipid peroxy/alkoxy radicals.

CONCLUSION

It can be concluded that BCF and ZSCF have antiulcer effect, which is evidenced by the decreased levels of ulcer indicies, SDH, G-6-Pase, AP, MDA, TNF-α, IL-1β, VEGF and elevated levels of GSH, PGE_2 and HSP_{70} in the tissue homogenate and in this respect, it was found that BCF more efficient than ZSCF.

SIGNIFICANCE STATEMENT

This study highlight the use of BCF and ZSCF extracts in the alleviation of ethanol gastric injury. This can be beneficial for introducing new alternatives using natural sources. As both plants showed almost better effect on ulcerated rats than that exerted by ranitidine, they can be formulated as new antiulcer drugs after further studies.

ACKNOWLEDGMENTS

This work was supported by National Research Center, Cairo, Egypt as a PhD study with grant number 2/2/8. The authors would like to thank Professor Dr. Olfat Emam, Department of Pathology, Theodor Bilharz Rersearch Institute for evaluation of histopathological slides.

REFERENCES

1. Farzaei, M.H., M. Abdollahi and R. Rahimi, 2015. Role of dietary polyphenols in the management of peptic ulcer. World J. Gastroenterol., 21: 6499-6517.
2. Ibrahim, M.Y., N.M. Hashim, S.M. Dhiyaaldeen, M.M.J. Al-Obaidi and R.M. El-Ferjani *et al.*, 2016. Acute toxicity and gastroprotection studies of a new schiff base derived Manganese (II) complex against Hcl/Ethanol-induced gastric ulcerations in rats. Scient. Rep., Vol. 6.

3. Xie, J.H., Y.L. Chen, Q.H. Wu, J. Wu and J.Y. Su *et al*., 2013. Gastroprotective and anti-*Helicobacter pylori* potential of herbal formula HZJW: Safety and efficacy assessment. BMC Complement. Altern. Med., Vol. 13, No. 1. 10.1186/1472-6882-13-119.
4. Wang, Q.S., X.N. Zhu, H.L. Jiang, G.F. Wang and Y.L. Cui, 2015. Protective effects of alginate-chitosan microspheres loaded with alkaloids from *Coptis chinensis* Franch. and *Evodia rutaecarpa* (Juss.) Benth. (Zuojin Pill) against ethanol-induced acute gastric mucosal injury in rats. Drug Design Dev. Ther., 9: 6151-6165.
5. DeVault, K.R. and N.J. Talley, 2009. Insights into the future of gastric acid suppression. Nat. Rev. Gastroenterol. Hepatol., 6: 524-532.
6. Mota, K.S., G.E. Dias, M.E. Pinto, A. Luiz-Ferreira and A.R. Souza-Brito *et al*., 2009. Flavonoids with gastroprotective activity. Molecules, 14: 979-1012.
7. Saleem, R., M. Ahmed, S.A Hussain, A.M. Qazi and S.I. Ahmad *et al*., 1999. Hypotensive, hypoglycaemic and toxicological studies on the flavonol C-glycoside Shamimin from *Bombax ceiba*. Planta Med., 65: 331-334.
8. Said, A., E.A. Aboutabl, S.M. Nofal, H. Tokuda and M. Raslan, 2011. Phytoconstituents and bioctivity evaluation of *Bombax ceiba* L. flowers. J. Tradit. Med., 28: 55-62.
9. Joshi, K.R., H.P. Devkota and S. Yahara, 2013. Chemical analysis of flowers of *Bombax ceiba* from Nepal. Natl. Prod. Commun., 8: 583-584.
10. Velozo, L.S.M., M.J.P. Ferreira, M.I.S. Santos, D.L. Moreira, E.F. Guimaraes, V.P. Emerenciano and M.A.C. Kaplan, 2009. C-glycosyl flavones from *Peperomia blanda*. Fitoterapia, 80: 119-122.
11. Vitcheva, V., R. Simeonova, I. Krasteva, M. Yotova, S. Nikolov and M. Mitcheva, 2011. Hepatoprotective effects of saponarin, isolated from *Gypsophila trichotoma* Wend. on cocaine-induced oxidative stress in rats. Redox Rep., 16: 56-61.
12. Min, Y.S., S.H. Yim, K.L. Bai, H.J. Choi and J.H. Jeong *et al*., 2005. The effects of apigenin-7-O-β-d-glucuronopyranoside on reflux oesophagitis and gastritis in rats. Auton. Autacoid Pharmacol., 25: 85-91.
13. Meena, V. and A.K. Chaudhary, 2017. Shalmali (*Bombax ceiba*): Versatility in its therapeutics. Int. J. Green Pharm., 11: S401-S406.
14. Chen, W.M., M. Jin and W. Wu, 2002. Experimental study on inhibitory effect of rutin against platelet activation induced by platelet activating factor in rabbits. Chin. J. Integr. Tradit. Western Med., 22: 283-285.
15. Kim, S.J., W.S. Chung, S.S. Kim, S.G. Ko and J.Y. Um, 2011. Antiinflammatory effect of *Oldenlandia diffusa* and its constituent, hentriacontane, through suppression of caspase 1 activation in mouse peritoneal macrophages. Phytother. Res., 25: 1537-1546.
16. Asgarpanah, J. and E. Haghighat, 2012. Phytochemistry and pharmacologic properties of *Ziziphus spina christi* (L.) Willd. Afr. J. Pharm. Pharmacol., 6: 2332-2339.
17. Jyothiprabha, V. and P. Venkatachalam, 2016. Preliminary phytochemical screening of different solvent extracts of selected Indian spices. Int. J. Curr. Microbiol. Applied Sci., 5: 116-122.
18. Karawya, M.S. and E.A. Aboutabl, 1982. Phytoconstituents of *Tabernaemontana coronaria* Jacq. Willd and *Tabernaemontana dichotoma* Roxb. growing in Egypt part IV, the flavonoids. Bull. Fac. Pharm. Cairo Univ., 21: 41-49.
19. Antolovich, M., P.D. Prenzler, E. Patsalides, S. McDonald and K. Robards, 2002. Methods for testing antioxidant activity. Analyst, 127: 183-198.
20. Goupy, P., M. Hugues, P. Boivin and M.J. Amiot, 1999. Antioxidant composition and activity of barley (*Hordeum vulgare*) and malt extracts and of isolated phenolic compounds. J. Agric. Food Chem., 79: 1625-1634.
21. Mattila, P., J. Astola and J. Kumpulainen, 2000. Determination of flavonoids in plant material by HPLC with diode-array and electro-array detections. J. Agric. Food Chem., 48: 5834-5841.
22. Nordin, N., S.M. Salama, S. Golbabapour, M. Hajrezaie and P. Hassandarvish *et al*., 2014. Anti-ulcerogenic effect of methanolic extracts from *Enicosanthellum pulchrum* (King) Heusden against ethanol-induced acute gastric lesion in animal models. PloS One, Vol. 9, No. 11. 10.1371/journal.pone.0111925.
23. Moustafa, Y.M., D.M. Khoder, E.E. El-Awady and S.A. Zaitone, 2013. Sildenafil citrate protects against gastric mucosal damage induced by indomethacin in rats. Eur. Rev. Med. Pharmacol. Sci., 17: 179-188.
24. Mard, S.A., Z. Bahari, N. Eshaghi and Y. Farbood, 2008. Antiulcerogenic effect of *Securigera securidaca* L. Seed extract on various experimental gastric ulcer models in rats. Pak. J. Biol. Sci., 11: 2619-2623.
25. Ravi, V., S.S. Patel, N.K. Verma, D. Dutta and T.S.M. Saleem, 2010. Hepatoprotective activity of *Bombax ceiba* Linn against isoniazid and rifampicin-induced toxicity in experimental rats. Int. J. Applied Res. Nat. Prod., 3: 19-26.
26. Amin, A. and D. Mahmoud-Ghoneim, 2009. *Zizyphus spina-christi* protects against carbon tetrachloride-induced liver fibrosis in rats. Food Chem. Toxicol., 47: 2111-2119.
27. Shay, H., S.A. Komarow, S.S. Fels, D. Meranze, M. Gruenstein and H. Siplet, 1945. A simple method for the uniform production of gastric ulceration in the rat. Gastroenterology, 5: 43-61.
28. Shu, M.H., D. Appleton, K. Zandi and S. AbuBakar, 2013. Anti-Inflammatory, gastroprotective and anti-ulcerogenic effects of red algae *Gracilaria changii* (Gracilariales, Rhodophyta) extract. BMC Complement. Altern. Med., Vol. 13. 10.1186/1472-6882-13-61.

29. Tan, P.V., N.G. Nditafon, M.P. Yewah, T. Dimo and F.J. Ayafor, 1996. *Eremomastax speciosa*: Effects of leaf aqueous extract on ulcer formation and gastric secretion in rats. J. Ethnopharmacol., 54: 139-142.
30. Moron, M.S., J.W. Depierre and B. Mannervik, 1979. Levels of glutathione, glutathione reductase and glutathione *S*-transferase activities in rat lung and liver. Biochimica Biophysica Acta (BBA)-Gen. Subj., 582: 67-78.
31. Ruiz-Larrea, M.B., A.M. Leal, M. Liza, M. Lacort and H. de Groot, 1994. Antioxidant effects of estradiol and 2-hydroxyestradiol on iron-induced lipid peroxidation of rat liver microsomes. Steriods, 59: 383-388.
32. Miranda, K.M., M.G. Espey and D.A. Wink, 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide, 5: 62-71.
33. Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.
34. Rice, M.E. and E. Shelton, 1957. Comparison of the reduction of two tetrazolium salts with succinoxidase activity of tissue homogenates. J. Nat. Cancer Inst., 18: 117-125.
35. Swanson, M.A., 1955. Glucose-6-phosphatase from liver: Glucose-6-P+H_2O→Glucose+ PO_4. Methods Enzymol., 2: 541-543.
36. Wattiaux, R. and C. de Duve, 1956. Tissue fractionation studies. 7. Release of bound hydrolases by means of Triton X-100. Biochem. J., 63: 606-608.
37. Bancroft, J.D., A. Stevens and M.P. Dawswon, 1996. Theory and Practice of Histological Techniques. 4th Edn., Churchill Livingstone, Edinbergh, London and New York, pp: 273, 292.
38. Ali, M.J.B., F. Guesmi, A.H. Harrath, S. Alwasel and A. Hedfi *et al*., 2015. Investigation of antiulcer and antioxidant activity of *Juniperus phoenicea* L. (1753) essential oil in an experimental rat model. Biol. Pharm. Bull., 38: 1738-1746.
39. Motawi, T.K., M.A. Hamed, R.M. Hashem, M.H. Shabana and Y.R. Ahmed, 2012. Protective and therapeutic effects of *Argyreia speciosa* against ethanol-induced gastric ulcer in rats. Z Naturforsch C., 67: 47-57.
40. Sidahmed, H.M.A., N.M. Hashim, M.A. Abdulla, H.M. Ali and S. Mohan *et al*., 2015. Antisecretory, gastroprotective, antioxidant and anti-*Helicobcter pylori* activity of zerumbone from *Zingiber zerumbet* (L.) Smith. PloS One, Vol. 10, No. 3. 10.1371/journal.pone.0121060
41. Dinarello, C.A., 2011. A clinical perspective of IL 1β as the gatekeeper of inflammation. Eur. J. Immunol., 41: 1203-1217.
42. Warzecha, Z., P. Ceranowicz, M. Dembinski, J. Cieszkowski, G. Ginter, A. Ptak-Belowska and A. Dembinski, 2014. Involvement of cyclooxygenase-1 and cyclooxygenase-2 activity in the therapeutic effect of ghrelin in the course of ethanol-induced gastric ulcers in rats. J. Physiol. Pharmacol., 65: 95-106.
43. Mei, X., D. Xu, S. Xu, Y. Zheng and S. Xu, 2012. Novel role of Zn (II)-curcumin in enhancing cell proliferation and adjusting proinflammatory cytokine-mediated oxidative damage of ethanol-induced acute gastric ulcers. Chemico-Biol. Interact., 197: 31-39.
44. Hasgul, R., S. Uysal, H. Haltas, S. Akyol, Y. Yuksel, A. Gurel and F. Armutcu, 2014. Protective effects of Ankaferd blood stopper on aspirin-induced oxidative mucosal damage in a rat model of gastric injury. Toxicol. Ind. Health, 30: 888-895.
45. Caldas, G.F.R., A.R. da Silva Oliveira, A.V. Araujo, S.S.L. Lafayette and G.S. Albuquerque *et al*., 2015. Gastroprotective mechanisms of the monoterpene 1, 8-cineole (eucalyptol). PLoS One, Vol. 10, No. 8. 10.1371/journal.pone.0134558.
46. Zakaria, Z.A., T. Balan, A.K. Azemi, M.H. Omar and N. Mohtarrudin *et al*., 2016. Mechanism(s) of action underlying the gastroprotective effect of ethyl acetate fraction obtained from the crude methanolic leaves extract of *Muntingia calabura*. BMC Complement. Altern. Med., Vol. 16. 10.1186/s12906-016-1041-0.
47. Nartey, E.T., M. Ofosuhene, W. Kudzi and C.M. Agbale, 2012. Antioxidant and gastric cytoprotective prostaglandins properties of *Cassia sieberiana* roots bark extract as an anti-ulcerogenic agent. BMC Complement. Altern. Med., Vol. 12, No. 1. 10.1186/1472-6882-12-65
48. Zhao, W., F. Zhu, W. Shen, A. Fu and L. Zheng *et al*., 2009. Protective effects of DIDS against ethanol-induced gastric mucosal injury in rats. Acta Biochim. Biophys. Sin., 41: 301-308.
49. El-Maraghy, S.A., S.M. Rizk and N.N. Shahin, 2015. Gastroprotective effect of crocin in ethanol-induced gastric injury in rats. Chemico-Biol. Int., 229: 26-35.
50. El-Abhar, H.S., 2010. Coenzyme Q_{10}: A novel gastroprotective effect via modulation of vascular permeability, prostaglandin E_2, nitric oxide and redox status in indomethacin-induced gastric ulcer model. Eur. J. Pharmacol., 649: 314-319.
51. Choi, S.R., S.A. Lee, Y.J. Kim, C.Y. Ok, H.J. Lee and K.B. Hahm, 2009. Role of heat shock proteins in gastric inflammation and ulcer healing. J. Physiol. Pharmacol., 60: 5-17.
52. Taghizadeh, S., M. Sankian, A. Ajami, M. Tehrani and N. Hafezi *et al*., 2014. Expression levels of vascular endothelial growth factors a and C in patients with peptic ulcers and gastric cancer. J. Gastric Cancer, 14: 196-203.
53. Tarnawski, A.S., A. Ahluwalia and M.K. Jones, 2014. Angiogenesis in gastric mucosa: An important component of gastric erosion and ulcer healing and its impairment in aging. J. Gastroenterol. Hepatol., 29: 112-123.
54. Yakubu, M.T., I.O. Salau and N.O. Muhammad, 2003. Phosphatase activities in selected rat tissues following repeated administration of ranitidine. Nig. J. Biochem. Mol. Biol., 18: 21-24.

55. Chakraborty, S., S. Stalin, N. Das, S.T. Choudhury, S. Ghosh and S. Swarnakar, 2012. The use of nano-quercetin to arrest mitochondrial damage and MMP-9 upregulation during prevention of gastric inflammation induced by ethanol in rat. Biomaterials, 33: 2991-3001.
56. Arab, H.H., S.A. Salama, H.A. Omar, E.S.A. Arafa and I.A. Maghrabi, 2015. Diosmin protects against ethanol-induced gastric injury in rats: Novel anti-ulcer actions. Plos One, Vol. 10. 10.1371/journal.pone.0122417.
57. Hirata, I., Y. Naito, O. Handa, N. Hayashi and K. Mizushima *et al*., 2009. Heat-shock protein 70-overexpressing gastric epithelial cells are resistant to indomethacin-induced apoptosis. Digestion, 79: 243-250.
58. La Casa, C., I. Villegas, C.A. de la Lastra, V. Motilva and M.J.M. Calero, 2000. Evidence for protective and antioxidant properties of rutin, a natural flavone, against ethanol induced gastric lesions. J. Ethnopharmacol., 71: 45-53.
59. Rao, C.V., S.K. Ojha, R. Govindarajan, A.K.S. Rawat, S. Mehrotra and P. Pushpangadan, 2003. Quercetin, a bioflavonoid, protects against oxidative stress-related gastric mucosal damage in rats. Natl. Prod. Sci., 9: 68-72.
60. Dubey, S., A. Ganeshpurkar, A. Shrivastava, D. Bansal and N. Dubey, 2013. Rutin exerts antiulcer effect by inhibiting the gastric proton pump. Indian J. Pharmacol., 45: 415-417.
61. Abourehab, M.A., K.A. Khaled, H.A. Sarhan and O.A. Ahmed, 2015. Evaluation of combined famotidine with quercetin for the treatment of peptic ulcer: *In vivo* animal study. Drug Des. Dev. Ther., 9: 2159-2169.
62. Zakaria, Z.A., T. Balan, V. Suppaiah, S. Ahmad and F. Jamaludin, 2014. Mechanism(s) of action involved in the gastroprotective activity of *Muntingia calabura*. J. Ethnopharmacol., 151: 1184-1193.
63. De Almeida, C.L.F., S.A. Brito, T.I. de Santana, H.B.A. Costa and C.H.R. de Carvalho Junior *et al*., 2017. *Spondias purpurea* L. (Anacardiaceae): Antioxidant and antiulcer activities of the leaf hexane extract. Oxidat. Med. Cell. Longev., Vol. 2017. 10.1155/2017/6593073.
64. De Barros, M.P., M. Lemos, E.L. Maistro, M.F. Leite, J.P.B. Sousa, J.K. Bastos and S.F. de Andrade, 2008. Evaluation of antiulcer activity of the main phenolic acids found in Brazilian green propolis. J. Ethnopharmacol., 120: 372-377.
65. Liu, H.C., M.K. Ku, F.Y. Chung, C.C. Lin and S.R. Lin, 2012. Effectiveness of great burdock essence compounds in the adjuvant treatment of gastric ulcer patients infected with *Helicobacter pylori*. Genomic Med. Biomarkers Health Sci., 4: 81-84.
66. Cavia-Saiz, M., M.D. Busto, M.C. Pilar-Izquierdo, N. Ortega, M. Perez-Mateos and P. Muniz, 2010. Antioxidant properties, radical scavenging activity and biomolecule protection capacity of flavonoid naringenin and its glycoside naringin: A comparative study. J. Sci. Food Agric., 90: 1238-1244.
67. Nishimura, F.D.C.Y., A.C. De Almeida, B.A. Ratti, T. Ueda-Nakamura, C.V. Nakamura, V.F. Ximenes and S.D.O. Silva, 2013. Antioxidant effects of quercetin and naringenin are associated with impaired neutrophil microbicidal activity. Evidence-Based Complement. Altern. Med., Vol. 2013. 10.1155/2013/795916
68. Agati, G., E. Azzarello, S. Pollastri and M. Tattini, 2012. Flavonoids as antioxidants in plants: Location and functional significance. Plant Sci., 196: 67-76.
69. Ciz, M., P. Denev, M. Kratchanova, O. Vasicek, G. Ambrozova and A. Lojek, 2012. Flavonoids inhibit the respiratory burst of neutrophils in mammals. Oxidat. Med. Cell. Longev., Vol. 2012. 10.1155/2012/181295.
70. Carvalho, A.C., M.M. Guedes, A.L. de Souza, M.T. Trevisan, A.F. Lima, F.A. Santos and V.S. Rao, 2007. Gastroprotective effect of mangiferin, a xanthonoid from *Mangifera indica*, against gastric injury induced by ethanol and indomethacin in rodents. Planta. Med., 73: 1372-1376.
71. Mahmoud-Awny, M., A.S. Attia, M.F. Abd-Ellah and H.S. El-Abhar, 2015. Mangiferin mitigates gastric ulcer in ischemia/reperfused rats: Involvement of PPAR-γ, NF-κB and Nrf2/HO-1 signaling pathways. PloS One, Vol. 10, No. 7. 10.1371/journal.pone.0132497.