DOI: 10.56511/JIPBS.2017.v4.i03.pp01-08

Journal of Innovations in Pharmaceutical and Biological Sciences (JIPBS)
ISSN: 2349-2759
Available online at www.jipbs.com

Research article

Evaluation of the anti-ulcer effect of *Aerva javanica* aerial parts against ethanol-induced gastric lesions in albino rats

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Key words: *Aerva javanica*, ethanol, gastric ulcer, antioxidants, lactate dehydrogenase.

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Abstract

The aim of this study was introducing the Aerva plant especially the species javanica as an anti-ulcer agent for ethanol-induced gastric mucosal lesions and evaluating its impact to strengthen the stomach self-defense mechanisms. The idea of the study was based on the comparison between the effect of the prophylactic treatment of the plant and the therapeutic one versus the effect of the anti-ulcer drug; ranitidine. Antioxidant parameters such as reduced glutathione (GSH), nitric oxide (NO) and malondialdehyde (MDA) in stomach tissue were determined. Anti-ulcer parameters were also estimated such as titrable acidity in stomach in addition to detecting the severity and the number of lesions in stomach mucosa. Serum prostaglandins (PGE_2), heat shock protein 70 (HSP70) and lactate dehydrogenase enzyme (LDH) were measured. Results obtained in the present study indicated that the therapeutic effect of the plant was more effective than the prophylactic one. The plant possessed healing and antioxidant properties. This observation manifested itself in normalizing the titrable acidity and decreasing the severity and the number of gastric lesions in stomach mucosa, especially in the therapeutic group. The plant positively affected the level of GSH, MDA in stomach tissue besides improving the serum levels of PGE_2, HSP70, and NO in stomach tissue. The plant also showed a strong controlling effect on the leakage of LDH into serum as a result of ulcers formation. Therefore, the plant *Aerva Javanica* aerial parts (as a prophylactic or therapeutic treatment) can be considered a potential new natural agent therapy described for ethanol-induced gastric ulcer cases.

Introduction

Stomach disorders such as ulcers are commonly persisted in humans. Ulcers are associated mainly with an imbalance among the stomach self-defense mechanisms (prostaglandins, heat shock proteins, endothelial nitric oxide, reduced glutathione) accompanied with an increase or a decrease in gastric secretions (HCl, pepsin). The inter-relationships among all these defenses are still an enigma [1, 2].

Excess alcohol intake (e.g ethanol) has been linked to ulcer development. It causes deformation in the mucosa layer and affects badly the vasculature in this region [3]. Decreasing in the blood flow in stomach ulcerated areas may develop tissue necrosis [4].

Ethanol intake is claimed for the production of extracellular and/ or intracellular oxidative stress detected in the stomach tissue. The continuous generation of free radicals may attack the DNA of the stomach cells leading to gastric cancer [5].

Histamine (H_2) antagonist drugs such as ranitidine are recommended for the treatment of ulcers caused by ethanol intake. The drug competitively blocks the histamine H_2 receptor and decrease acid secretions. Histamine (H_2) antagonists drugs may lead to a secondary rise in acidity within a short time of administration causing high ulcer relapse rate. Tolerance to the prolonged treatment by the drug may occur [6]. These side effects can be observed during ulcer treatment with ranitidine besides its effect in worsening the general health [7].

Overcoming all these defects of drugs may be performed by the invading of the natural products to the therapeutic fields. The idea of our study is summarized in evaluating the effect of the plant *Aerva javanica* in augmentation of gastric defense mechanisms in experimental ethanol induced ulcer.

The plant is a short shrub with long benches and belongs to the family Amaranthaceae and mainly found in North Africa and South West Asia. Many applications of the plant in the folk medicine were estimated as it is used as a chest pain relief and is recommended for diarrhea and also for toothache [8]. *Aerva javanica* is cultivated in Egypt. An Egyptian study in (1999) had proved the presence of various constituents including steroids, triterpenes, lipids, flavonoids, tannins, saponins, alkaloids, carbohydrates and glycosides in the plant [9].

A very recent study [10] proved the anti-ulcer effect of the plant *in vitro*. The study showed that the plant contains many compounds that have anti-ulcer properties that can inhibit the action of the urease enzyme *in vitro* by values

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reaching 64.6%. The urease enzyme is mainly secreted by the bacteria *Helicobacter pylori* claimed for gastric ulcer formation.

Our study is considered one of the pioneering studies that aim to test the anti-ulceration efficacy of the Aerva plant of the species javanica *in vivo*.

The present study was performed by the comparison between the efficacy of the prophylactic and therapeutic supplementation of ethanol extract of the aerial parts of *Aerva javanica* versus the efficacy of the anti-ulcer drug ; ranitidine in balancing the stomach acidity, the recession in the number and the severity of the ethanol-induced ulcer lesions. The study also focussed on the impact of the plant in restoring the stomach defense mechanisms such as the secretion of serum PGE_2, HSP70 and tissue levels of NO, GSH and MDA in addition to determining the serum level of LDH.

Materials and Methods

Materials

Drugs and Chemicals

All chemicals used in the present study were of high analytical grade, products of Sigma (USA), Merck (Germany) Fluka (Switzerland) and El Nasr company (Egypt).

Plant collection

Aerva javanica plant was collected from the desserts of Kingdom of Saudi Arabia.

Aerial parts of *Aerva javanica* were examined as anti-ulcer agent. The plant was authenticated by an expert plant taxonomist at the herbarium of the university under the specimen number (16281).

Plant extraction

The aerial parts of *Aerva javanica* were extracted exhaustively in a rotary vapor apparatus with 95% ethanol. After complete extraction, the aqueous ethanol was evaporated to dryness under vacuum at 40°C yielding semisolid residues and the concentrates were stored under refrigeration until further use. For the experiments, the extract was freshly prepared as a suspension in distilled water.

Animals

Healthy male rats (150-200 g) were chosen for the experiment and were purchased from the Animal house of the National Research Centre, Giza, Egypt. Animals were housed in cages with mesh bottoms in an air-conditioned room with a 12-h dark-light cycle and were allowed free access to water and food. Rats were caged in groups and left for one week as an acclimatization period.

Ethics

All experiments were carried out according to the recommendation of the ethical conditions approved by the Ethics Committee of National Research Center of Experimental Animals which is matched with international ethics for the handling of experimental animals (approval no.13163).

Doses and route of administration

For ulcer induction, Rats fasted for 24h before starting the experiments. Rats received a single dose of absolute ethanol (5 ml/kg) by oral gavage [11]. Ranitidine as a reference antiulcer drug was orally administrated at a dose of 100 mg/kg for 1 week before or after ethanol administration [12]. Plant extract was orally given at a dose of 300 mg/kg for one week before or after ethanol administration.

Methods

Acute toxicity study of the plant

Acute toxicity study of *Aerva javanica aerial parts* was done according to OECD [13] rules which reported that in the typical protocol for acute toxicity study if just one dose level at 5g/kg is not lethal no longer requirement for determination of an LD50 value. No mortality was recorded 24 hours later. After 15 days blood was obtained from all groups of rats after being lightly anaesthetized with ether by puncturing retro-orbital plexus [14], the blood was allowed to flow into a clean dry centrifuge tube and left to stand 30 minutes before centrifugation to avoid hemolysis. Then blood samples were centrifuged for 15 minutes at 2500 rpm the clear supernatant serum was separated and collected by Pasteur pipette into a dry clean tube to use for determination serum levels IU/L of: Alanine aminotransferase (ALT) (SGPT), Aspartate aminotransferase, (AST) (SGOT), Urea and Creatinine [15-17].

Efficacy study

Experimental groups

The rats were randomly divided into six groups, 8 rats in each group. The first group G1: served as negative control one. The second group G2: the rats administered absolute ethanol and sacrificed after 2 hours (Positive control or ulcer group). The third group G3: rats were administrated the reference drug ranitidine one week before ethanol induction then sacrificed after 2 hours (Prophylactic ranitidine). The fourth group G4: rats were administrated ranitidine for one week after ethanol induction (therapeutic ranitidine). The fifth group G5: rats were administrated the *Aerva javanica* extract one week before ethanol induction then sacrificed after 2 hours (Prophylactic *Aerva j.*). The sixth group G6: rats were administrated *Aerva javanica* extract for one week after ethanol induction (therapeutic or treated *Aerva j.*).

Anesthesia

Rats were anesthetized in a dissecator containing an appropriate amount of ether. The rat's abdomen was dissected and the esophagus nearest to the cardio and the distended stomach on the pyloric sphincter was immediately tied in a knot using a string to avoid leakage of the gastric contents [18]. The stomach was rapidly removed and immersed in water.

Measurement of gastric secretion or titrable acidity

The stomach juice containing food particles was discarded. The amount of gastric juice was measured using a measuring cylinder. The total acid concentration was determined in the supernatant by titration to PH=7 with 0.01 N NaOH using phenolphthalein as an indicator [19].

Quantification of Ulceration

The funds and corpus of each stomach was opened longitudinally along the greater curvature and examined macroscopically using a magnifying lens. The number and severity of lesions in the glandular mucosa were scored from 0 to 5 [20].

Score	Macroscopic Feature
0	No lesion
0.5	Diffuse hyperemia
1	1 to 2 small ulcers
1.5	3 to 6 small ulcers
2	7 to 10 small ulcers
2.5	More than 10 small ulcers
3	1 marked ulcer plus 0 to 4 small ulcers
3.5	1 marked ulcer plus 5 or more small ulcers
4	2 marked ulcers plus 0 to 4 small ulcers
4.5	2 marked ulcers plus 5 or more small ulcers
5	3 or more marked ulcers

Preparation of Tissue Homogenate

Longitudinal sections weighing 0.5 g from each stomach were homogenized in phosphate buffered saline (pH 7.4) using a glass-Teflon homogenizing tube (Janke & Kunkel, IKA-WERK, Germany). The homogenate was centrifuged at 3000 rpm for 10 min and the supernatant was stored for further estimation of the different parameters.

Biochemical analysis

Oxidative stress markers, glutathione (GSH), Malondialdhyde (MDA), nitric oxide (NO) and total protein content

GSH levels of gastric tissue were determined according to the method of Beutler *et al.* [21]. MDA was determined as an indicator of lipid peroxidation according to Buege and Aust [22]. The protein concentration of the samples was determined following the method described by Gornal *et al.* [23].

NO concentration was estimated in the stomach homogenate according to the method described by Miranda *et al.* [24].

Determination of the serum Prostaglandin (PGE_2) and Heat Shock Protein (HSP70) and Lactate Dehydrogenase (LDH)

PGE2 level in serum was estimated according to the method described by Yamaguchi *et al.* [25]. HSP70 was determined in serum according to ELISA principals. LDH activity was kinetically measured through the oxidation of lactate to pyruvate according to Kachmar and Moss [26].

Statistical analysis

The results were presented as a mean ± standard error (S.E.). Results were analyzed statistically by one-way analysis of variance (ANOVA) using SPSS (Statistical Package for the Social Sciences, version 9) software. Values obtained were considered statistically significant at $p < 0.05$.

Results and Discussion

Results

Acute toxicity of the plant

There was no toxicity signs detected in rats given the plant orally at doses range up to 5000 mg/kg/b.wt. Accordingly the plant is considered safe and secure when supplemented to animals.

Gastric Examination Results

With respect to the normal control group (14.5 mEq/L) the titrable acidity showed marked rising levels by values reached 97.5 mEq/L (537%) in the positive control group (2-hrs after ethanol administration group) as shown in figure (1).

Similar decreasing acid levels were remarked in both prophylactic ranitidine and *Aerva javanica* extract treated groups (-61.5%) compared to positive control group.

Competitive anti-secretory results against acid secretion were obtained from both the therapeutic ranitidine and *Aerva javanica* groups. Both groups were able to normalize the acidity in the stomach. The acid levels were improved by values equal -83.3 % and -84.6% respectively compared to positive control group.

The 2-hrs after ethanol administration group (positive control or the ulcer group) was characterized by the existence of numerous and severe gastric lesions. The prophylactic groups treated with ranitidine and *Aerva javanica* extract showed different healing results towards the severity and the number of lesions (-54% and -61%) respectively in case of severity; (-60% and -41%) respectively in case of the lesions number compared to positive control group.

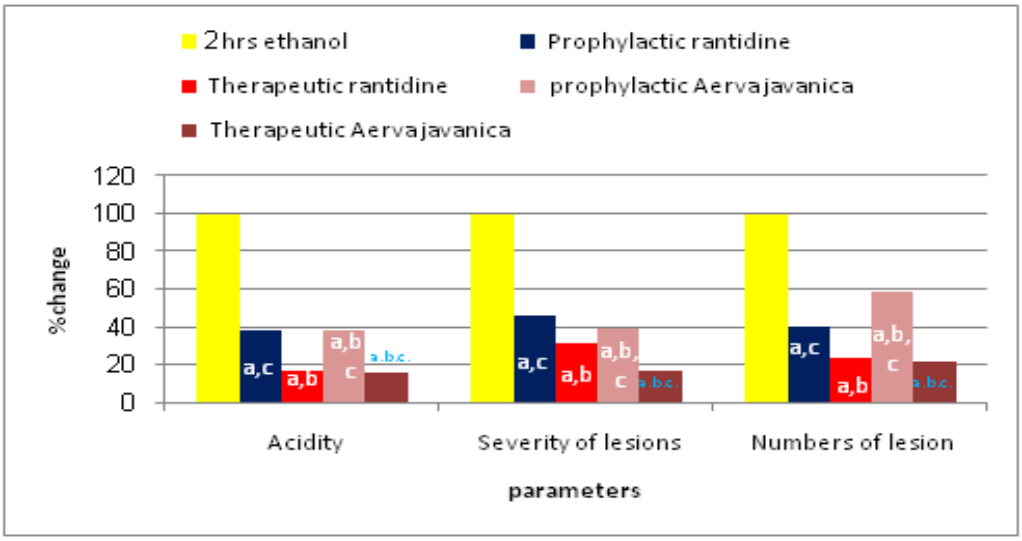


Figure 1. Percent change in acidity, severity, and number of lesions in treated groups compared to 2-hrs ethanol (positive control or ulcer) group.

[a] $P < 0.05$ compared to 2-hrs ethanol group (positive control or ulcer group), [b] $P < 0.05$ compared to prophylactic ranitidine and [c] $P < 0.05$ compared to therapeutic ranitidine.

The anti-ulcer property of the plant was noticed in the therapeutic group. A remarked decrease in the severity of lesions (–83%) and the number of lesions (-78.4%) in this group were obtained compared to positive control. The therapeutic ranitidine group results reached only a decrease in the severity of lesions by -69% and -77% in the number of lesions compared to positive control.

So we can notice here the therapeutic effect of the plant and its ability to normalize the acid secretion and siege the ulcer more than all of the other groups.

Biochemical Results

The anti-oxidant property of the plant was observed in measuring NO, GSH and MDA levels in stomach homogenate as shown in table (1).

Negative control group rats exhibited the normal levels of NO and GSH by values reached 1195.25µM/g tissue and 28.91 mg/g tissue respectively. Decreasing levels of NO and GSH were observed by values reached -92 % and -47% in the 2-hrs after ethanol administrated group compared to negative control group.

Prophylactic rantidine and *Aerva javanica* extract treated groups showed approximately similar improvement in NO and GSH levels by values reached 652.4% and 43% respectively in case of prophylactic rantidine treated group and values reached 584% and 32.4% respectively in case of prophylactic *Aerva javanica* treated group compared to positive control group.

The anti-oxidant impact of the plant *Aerva javanica* was noticed in the therapeutic group in which the levels of NO and GSH reached an increase equal 653% and 66.3% respectively. Therapeutic rantidine group recorded the highest improvement in NO level by an increase equal to 685% compared to positive control group.

Also it was found that due to the decrease in the anti-oxidant levels in stomach homogenate expressed in decreasing values of GSH and NO, there was a corresponding increase in the MDA level in the ethanol administrated group by values equal 85.06% compared to negative control group.

An improvement in MDA level was recorded in rantidine and *Aerva javanica* prophylactic groups (-32% and -28% respectively) compared to positive control group. Therapeutic rantidine group recorded the highest improvement in MDA values that reached -45% while the therapeutic *Aerva javanica* group recorded only a decrease equal to -34.3% compared to positive control group.

We can observe from these data the competition between the therapeutic effects of *Aerva javanica* with the therapeutic effect of rantidine in restoring antioxidants level. On the other hand, the prophylactic effect of both rantidine and *Aerva javanica* also recorded nearly the same results.

Recording PGE_2 and HSP70 serum levels may clarify the anti-inflammatory effect of the plant as shown in table (2).

The lowest serum levels of PGE_2 and HSP70 were recorded in the negative control group by values equal to 21.6 pg/ml and 1.01 ng/ml respectively.

Increasing levels were recorded by values reached 231.9% and 532.6% in the 2-hrs after ethanol administrated group in PGE_2 and HSP70 levels respectively compared to control group. The prophylactic rantidine group showed a great improvement in decreasing the values of PGE_2 and HSP70 by values reached -55.3% and -59.7% respectively compared to positive control group. The prophylactic *Aerva javanica* group showed moderate improvement by values reached -24.1% and -46% for the PGE_2 and HSP70 levels respectively.

Table 1. Effect of *Aerva javanica* extract on antioxidant parameters in stomach homogenate of ethanol induced gastric ulcer in rats.

Parameters / Groups	NO (µM/g tissue)	% Change	GSH (µg/gtissue)	% change	MDA (nmol/gtissue)	% Change
Negative control	1195.25±29.9^{bcd}	_	28.91±0.66^{bc}	_	17.14±0.32^{bc}	_
Positive control(ulcer group)	97.89±18.9^{acd}	-92	15.23±0.55^{acd}	-47	31.72±0.81^{ac}	85.06
Prophylactic ranitidine (100mg/kg)	736.58±43.8^{ab}	652.4	21.84±1.17^{ab}	43	21.68±0.49^{ab}	-32
Therapeutic ranitidine (100mg/kg)	768.04±47.3^{ab}	685	23.68±0.97	55.4	17.59±0.47^{bc}	-45
Prophylactic *Aerva javanica* (300mg/kg)	669.13±44.1^{abd}	584	20.17±1.16^{ab}	32.4	22.92±0.72^{abd}	-28
Therapeutic *Aerva javanica* (300mg/kg)	737.3±23.1^{ab}	653	25.33±0.97^{bc}	66.3	20.83±0.93^{abd}	-34.3

Data are means ± SE of 8 rats in each group; ^{a} $P < 0.05$ compared to clean control group , ^{b} $P < 0.05$ compared to positive (ulcer) group, ^{c} $P < 0.05$ compared to prophylactic ranitidine and ^{d} $P < 0.05$ compared to therapeutic ranitidine. % change is preformed compared to positive control group.

Table 2. Effect of *Aerva javanica* extract on serum PGE_2 and HS P70 in ethanol induced gastric ulcer in rats.

Parameters / Groups	PGE_2 (pg/ml)	%Change	HSP70 (ng/ml)	Change%
Negative control	21.6±1.18^{bcd}	_	1.01±0.06^{bcd}	_
Positive control (ulcer group)	71.71±1.25^{acd}	231.9	6.39±0.17^{acd}	532.6
Prophylactic rantidine (100mg/kg)	32.05±0.78^{abd}	-55.3	2.57±0.20^{ab}	-59.7
Therapeutic rantidine (100mg/kg)	43.37±1.02^{abc}	-39.5	3.42±0.22^{ab}	-46.4
Prophylactic *Aerva javanica* (300mg/kg)	54.41±1.68^{abc}	-24.1	3.46±0.24^{ab}	-46
Therapeutic *Aerva javanica* (300mg/kg)	48.71±1.86^{abc}	-32	3.53±1.49^{ab}	-45

Data are means ± SE of 8 rats in each group ^{a} $P < 0.05$ compared to clean control group , ^{b} $P < 0.05$ compared to positive (ulcer) group, ^{c} $P < 0.05$ compared to prophylactic ranitidine and ^{d} $P < 0.05$ compared to therapeutic ranitidine.% change is preformed compared to positive control group.

Table 3. Effect of *Aerva javanica* extract on serum lactate dehydrogenase in ethanol induced gastric ulcer in rats

Parameters / Groups	LDH (U/L)	% change
Negative control	547.59±13.79^{bcd}	_
Positive control (ulcer group)	2740.47±16.11^{acd}	400
Prophylactic rantidine (100mg/kg)	924.77±26.07^{abd}	-66.2
Therapeutic rantidine (100mg/kg)	1969.89±33.35^{abc}	-28.1
Prophylactic *Aerva javanica* (300mg/kg)	786.49±13.23^{abd}	-71.3
Therapeutic *Aerva javanica* (300mg/kg)	557.49±22.91^{bcd}	-79.6

Data are means ± SE of 8 rats in each group; data are expressed as U/L for lactate dehydrogenase (LDH). ^{a} $P < 0.05$ compared to clean control group , ^{b} $P < 0.05$ compared to positive (ulcer) group, ^{c} $P < 0.05$ compared to prophylactic ranitidine and ^{d} $P < 0.05$ compared to therapeutic ranitidine. % change is performed compared to positive control group.

The therapeutic effect of both rantidine and *Aerva javanica* extract showed nearly the same effect in improving the HSP70 by values reached -46.4% and 45% respectively.

The therapeutic effect of rantidine showed more improvement in case of PGE_2 (-39.5%) than that was found in the therapeutic *Aerva javanica* group (-32%) compared to positive control group.

The data recorded may clarify the rapid response to the prophylactic and therapeutic treatments of rantidine and

Aerva javanica in restoring the serum level of HSP70 more than that of the PGE_2.

Serum lactate dehydrogenase seemed to be a valuable parameter in detecting the curing efficacy of the plant *Aerva javanica*. Normal enzyme level was recorded in the control group as shown in table (3).

A great jump was detected in the 2-hrs after ethanol administrated group (positive control or ulcer group), the enzyme level jumped by values reached 400% compared to negative control. Decreasing results in LDH jumping levels were reported in the prophylactic ranitidine group by values reached -66.2% compared to positive control group. Prophylactic *Aerva javanica* treated group showed different levels of improvement by decreasing values equals to -71.3% compared to positive control.

Excellent normalized levels of LDH were recorded in the therapeutic *Aerva javanica* group by values reached -79. 6% compared to positive control. These detected values were not reached by the therapeutic ranitidine group.

Discussion

High gastric acidity is known to be a factor in the etiology of gastric ulcer [27]. Ranitidine the drug of choice in the present study performs its action by decreasing HCl concentration [28]. Decreasing HCl under normal levels was proved to be harmful to human health and contribute to long-term health challenges conditions and diseases [29]. A study was documented by Ramaswamy *et al.* [7] showed that 71% of patients treated with anti-ulcer drugs such as ranitidine had fungal candida growth in the stomach. This is beside the probability of causing high ulcer relapse rate and tolerance to the prolonged treatment [6].

In the present study, two hours after administration of a single dose of ethanol to normal rats showed highly detectable levels of secreted HCl.

Our study demonstrated that the therapeutic effect of *Aerva javanica* ethanol extract showed a competitive anti-secretary effect and cytoprotective activity with the therapeutic effect of ranitidine. A noticed similar decreasing titrable acidity levels were obtained in both groups. A number of ulcers and the degree of its severity were also affected. The gastric ulcer lesions in the case of the therapeutic *Aerva javanica* group nearly reached values close to normal during a week of supplementation while in the therapeutic ranitidine group the curative values were somehow retarded. Mohua *et al.* [30] documented the accelerative healing process of ranitidine in curing gastric ulcers, however, our plant seemed to have more accelerative healing properties.

The prophylactic groups of ranitidine and *Aerva javanica* extract in our study also showed an observed progress in affecting titrable acidity and ulcer number and severity but the values recorded were not reaching the curing levels.

The curative effect of the plant *Aerva lanata* aqueous extract against ethanol-induced gastric ulcer was observed by Indukuri *et al.* [31]. They found that the pre-treatment of the plant with different doses had cytoprotective properties with different ranges.

The anti- ulcer effect of the plant in our study may be discussed due to the presence of flavonoids and triterpenes and other antioxidant constituents in the plant extract [32, 33, 34].

In the present study, the capability of the *Aerva javanica* extract in restoring the stomach self-defence mechanisms was greatly manifested. As compared to the control normal rats, ethanol administration led to oxidative stress and lipid peroxidation (LPO) represented in an increase in the levels of malondialdehyde (MDA) in the stomach tissue causing decreasing levels of antioxidants such as reduced glutathione (GSH). GSH is a main cytoplasmic antioxidant and is considered as the first defense for the cells against LPO [35, 36]. Our study showed that in the prophylactic and the therapeutic *Areva javanica* extract groups, there was an increase in the GSH levels accompanied by a corresponding decrease in MDA levels. These results were in accordance with Kumar *et al.* [37]. They attributed the anti-ulcer effect of *Arava lanata* to the plant antioxidant properties by capturing reactive oxygen species.

Ethanol-induced oxidative stress may lead to decreasing levels of nitric oxide (NO).This can be discussed by shifting of NO towards scavenging free radicals [38]. This observation was in agreement with our results as a great drop in NO levels was observed in non-treated group administrated ethanol.

When the gastric mucosa is exposed to ethanol, endothelial cells lining the micro vessels of the stomach produce NO and a massive and rapid increase in blood flow occurs. By this mechanism of action, the gastric mucosa can be self-protected and the ethanol is diluted or removed. [4, 39].

Berg *et al.* [40] demonstrated that NO participates in decreasing gastric acid secretion in gastric glands isolated from human. These results were in agreement with our results as we found that there was an observed decrease in titrable acidity was accompanied by an increase in NO levels especially in the therapeutic ranitidine and the therapeutic *Aerva javanica* groups.

Prostaglandins (PGs) are considered a very important self-defence mechanism in the stomach tissue. PGs were reported to be the headmaster that controls almost all the mucosal self-defence mechanisms. It was also reported that PGs inhibited the platelets adhesion to the vascular epithelium and attenuated the activity of mast cells and leukocytes in the ulcerated area [41].

In our study, an observed increase in PGE_2 serum levels was reported in the non-treated group administrated ethanol. This can be discussed by the formation of increasing levels of inflammatory mediators due to the attack of the immune cells to the ulcerated stomach lesions [42]. The anti-inflammatory effect of the plant *Aerva javanica* was greatly shown in the prophylactic and therapeutic groups. Decreasing levels of PGE_2 were noticed. This may indicate a decrease in the attack of the immune cells and attenuation of

inflammation in ulcerated lesions. Wallace [43] reported that most of the inflammatory mediators are under controlled by PGs.

On the other hand, the prophylactic and the therapeutic ranitidine groups showed more progressive results in attenuating the inflammation and decreasing PGE_2 serum levels. These results were in agreement with Chandranath *et al.* [44] who discussed the mode of action of the drug. They denoted that the anti-ulcer effect of ranitidine is mainly performed through influencing NO and PGs pathways.

Heat shock proteins HSPs represent another factor as a gastroprotective mechanism. In our study, an increase in serum HSP70 was observed in the ethanol non-treated group compared to control group. These results were in agreement with Choi *et al.* [45] who reported in their research that HSP70 was induced due to various stresses such as acidosis, hypoxia, cytokines and energy depletion.

Similar improvement levels of HSP70 were observed in the prophylactic and the therapeutic *Aerva javanica* extract groups and in the therapeutic ranitidine group. We can attribute this improvement in HSP70 serum levels observed in the plant treated groups to the effect of the plant in decreasing the titrable acidity and the ethanol oxidative stress effect by increasing the GSH levels in stomach cells. Also decreasing serum levels of PGE_2 and attenuating the inflammation in the ulcerated area performed by the anti-inflammatory influence of the plant can be a second reason in improving HSP70 levels.

Detecting the serum levels of lactate dehydrogenase enzyme (LDH) in ethanol-induced ulcer rats seems to be valuable and a matter of importance. In our study, the enzyme levels dramatically increased in the ethanol administrated non-treated group compared to control group. This may indicate the highly destructive action of ethanol administration on epithelial stomach cells. The release of the enzyme in the serum may be an indicator of the profound injury in the mucosa and the epithelial stomach layers.

Our study revealed the healing property of the plant *Aerva javanica* which appeared glory in the therapeutic group followed by the prophylactic one. The plant was capable of restoring the normal level of LDH enzyme in serum. Arbab *et al.* [8] demonstrated that treatment with *Aerva javanica* plant possessed a hepatoprotective effect and restored serum levels of liver enzymes (ALT, AST, ALP) in rats administrated carbon tetrachloride. The authors also denoted the ability of the plant in enhancing cell proliferation of hepatocytes after the exposure to toxicity. Accordingly, we can deduce the ability of the plant in normalizing LDH serum levels. We also can conclude that the *Aerva javanica* supplementation may induce the stomach cells proliferation and regeneration which in turn resulted in decreasing and normalizing LDH enzyme.

Further studies are needed to discuss the mode of action of the plant in normalizing the acidity in the stomach and restoring its self-defence mechanisms.

Conclusion

Valuable observations were concluded from this study. Post-treatment (therapeutic group) of *Aerva javanica* plant to ethanol induced gastric ulcer expressed anti-secretary effect against high levels of gastric HCl. It also agumented the anti-oxidant parameters such as NO and GSH in stomach tissue. Decreasing MDA levels were observed due to the anti-oxidant influence of the plant. An anti-inflammatory effect was observed and was shown in balancing the serum levels of PGE_2 and HSP70. Finally, The healing property of *Aerva Javanica* post-treatment appeared glory in restoring serum LDH normal levels which may be an indicator for the ability of the plant in the regeneration process of the stomach epithelial cells.

References

1. Holzer P: Role of visceral afferent neurons in mucosal inflammation and defense.Curr.Opin.Pharmacol. 2007; 7(6): 563-569.
2. Okokon JE and Nwafor PA: Antiulcer and anticonvulsant activity of Croton Zambesicus. J.Pharm.Sci. 2009; 22: 384-390.
3. Al Mofleh IA, Alhaider AA, Mossa J, Al-Sohaibari M, Al-yahya M, Rafatullah S and Shaik SA: Gastroprotective effect of an aqueous suspension of black cumin Nigella Sativa on necrotizing agents-induced gastric injury in experimental animals. Saud. J. Gastroenterol. 2010; 14(3): 128-134.
4. Holzer P: Neural regulation of gastrointestinal blood flow In: physiology of the gastrointestinal tract. Johnson LR, Academic press, New York 2006; 817-839.
5. Fox JG and Wang TC: Inflamation, atrophy and gastric cancer. J. Clin. Invest. 2007; 117(1): 60-69.
6. Khayyal MT, Seif El-Naser MA, El-Gazaly MA, Okpanyi SN, Kelber O and Weiser D: Mechanism involved in the gastro –protective effect of STW5 (Iberogast s) and its components against ulcers and rebound acidity. Phytomed. 2006; SV:56-66.
7. Ramaswamy K, Correa M and Koshy A: Non healing gastric ulcer associated with candida infection. Indian J. Med. Microbio. 2007; 25: 57-58.
8. Arbab H A, Parvez MK, Al-Dosan MS, Al-Rehaily AJ, Ibrahim KE, Alam P, Alsaid MS and Rafatullah S: Therapeutic efficacy of ethanolic extract of Aerva javanica aerial parts in the ameliorationof CCl4 induced hepatotoxicity and oxidative damage in rats. Food Nutr. Res. 2016; 60.
9. Movaliya V and Zaveri M: A review on pashanbheda plant Aerva javonica. Int. J. Pharm. Sci. Rev. Res. 2014; 25(2): 268-275.
10. Khan AW, Jan S, ParaeenS, Khan RA, Saeed A Tnveer AJ and Shad AA: Phytochemical analysis and enzyme inhibition assay of Aerva javonica for ulcer.Chems.Centr.J. 2012; 6; 76.
11. Moustfa YM, Khoder DM, El-Awady DD and Zaitone AA: Sildenafil citrate protects against gastric mucosal damage induced by indomethacin in rats. Eur. Review Med. Pharm. Sci. 2013; 17:179-188.
12. Mard SA, Bahari Z, Eshaghi N and Farbood Y: Antiulcerogenic effect of Securigera securidaca L. seed extract on various experimental gastric ulcer models in rats. Pak. J. Biol. Sci. 2008; 11: 261-263.
13. OECD 2000. Guidance Document on Acute Oral Toxicity. Environmental Health and Safety Monograph Series on Testing and Assessment No 24.
14. Sorg DA and Buckner B: A simple method of obtaining venous blood from small laboratory animals. Proc. Soc. Exp. Biol. Med. 1964; 115:1131-2.
15. Reitman S and Frankel S: A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol.1957; 28(1):56-63.
16. Fawcett JK and Scott JE: A rapid and precise method for the determination of urea. J. Clin. Pathol. 1960; 13:156-9.
17. Schirmeister J: Creatinine forms a colored complex with picrate in an alkaline medium. Dtsch. med Wschr, 1964; 89.
18. Shu MH, Appleton D, Zandi K and AbuBakar S: Anti-inflammatory, gastroprotective and anti-ulcerogenic effects of red algae Gracilariachangii (Gracilariales Rhodophyta) extract. BMC Complement Altern. Med. 2013; 13: 61.

19. Guedes MM, Carvalho AC, and Lima AF: Gastroprotective mechanisms of centipedic acid, a natural diterpene from Egletes viscose LESS. Biol. Pharm. Bull. 2008; 31(7): 1351-1355.
20. Clementi G, Caruso A, Cutuli VM, De Bernardis E, Prato A, Mangano NG and Amico-roxas M: Effect of centrally or peripherally injected adrenomedullin on reserpine-induced gastric lesions. Eur. J. Pharmacol. 1998; 360(1): 51-54.
21. Beutler E, Duron O and Kelly BM: Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 1963; 61:882-888.
22. Buege JA and Aust SD: Microsomal lipid peroxidation. Methods Enzymol. 1978; 52: 302-310.
23. Gornal, AC, Bardawill, CJ and David, MM: Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. 1949; 177: 751-766.
24. Miranda KM, Espey MG and Wink DA: A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 2001; 5(1):62-71.
25. Yamaguchi T, Hidaka N, Suemaru K and Hiroaki Araki H: The co-administration of paroxetine and low-dose aspirin synergistically enhances gastric ulcerogenic risk in rats. Biol. Pharm. Bull. 2008; 31: 1371-1375.
26. Kachmar JF and Moss DW: In fundamentals of clinical chemistry, 2nded. NW Tietz, Editor. WB Saunders, Philadelphia, 1976; 652.
27. Hajrezaie M, Golbabapour S, Hassandarvich P, Gwaram NS, HadiHA, Mohd Ali H, Majid N and Abdulla MA: Acute toxicity and gastroprotection studies of a new Schiff base derived copper (II) complex against ethanol –induced acute gastric lesions in rats. Plos one 2012; 7(12):.e51537.
28. Nartey E, Ofosuhene M, kudzi W and Agbale CM: Antioxidant and gastric cytoprotective prostaglandins properties of Cassia Sieberiana roots bark extract as an anti-ulcerogenic agent. BMC Comp. Altern. Med. 2012; 12: 65.
29. Schinke T, Schilling AE, Baranowsky A, Seitz S, Marshall RP, LinnT, Blaeker M, Huebner AK, Schulz A, Simon R, GebauerM, Priemel M, Kornak U, Perkovic S, Barvencik F, BeilFT, Del Fattore A, Frattini A, Streichert T, Pueschel K, Villa A, Debatin KM, Rueger JM, teti A, Zustin J, Sauter G, Amling M: Impaired gastric acidification negatively affects calcium homeostasis and bone mass. Nature Med. 2009; 15(6): 674-681.
30. Mohua S, Palash M, Mita S, Suhrita P and Mradu G: Evaluation of anti-ulcer activity of aqueous extract of black cumin (Nigella sativa L) on experimental albino rats. J. Drug Delivery Therap., 2013; 3(1): 25-28.
31. Indukuri R, Bahanuprakash, Priadarchini RL, Vattipalli M, Rajukumar P and Bikku: Evaluation of anti-ulcer activity of Aerva lanata stem extract in rats. Indo. American J. Pharm. Reserch 2013; 3(12):1702-1708.
32. Al-Howiriny T, Al-Sohaibani M, Al-Said M, Al-Yahya M, El-Tahir M and Rafatullah S: Effect of Commiphora Opobalsamum (L) Eng (Balessan) on experimental gastric ulcers and secretion in rats. J. Ethnopharmacol. 2005; 98: 287-294.
33. Hiruma-Lima CA, Calvo TR, Rodrigues CM, Andrade FD, Vilegas W and Brito AR: Anti-ulcerogenic activity of Alchornea Castaneaefolia effects on somatostatin, gastrin and prostaglandin. J. Ethnopharmacol. 2006; 104: 215-224
34. Kouakou KL, Goze NB, Bleyere NM, Konan BA, Amonkan KA, Abo Kouakou JC,Yapo AP and Ehile EE: Acute toxicity and anti-ulcerogenic activity of an aqueous extract from the stem bark of Terminalia superb Engl. and Diels (Combretaceae). Worl. J. Pharm.Sci. 2013; 1(4): 117-129.
35. Rozza AL, DeMelloMoraesF, Kushima H, Tanimato A, Marques MO, Bauab TM, Hiruma-Lima CA and Pellizon CH: Gastroprotective mechanisms of Citrus lemon (Rutaceae) essential oil and its majority compounds limonene and beta-pinene: Involvement of heat shock protein 70-vasoactive intestinal peptide, glutathione, sulfhydryl compounds, nitric oxide and prostaglandin E2. Chem. Biol. Interact. 2011; 189: 82-89.
36. Suleyman B, Halici Z, Odabasoglu F and Gocer F: The effect of lacidipine on indomethacin induced ulcer in rats. Int. J. Pharmacol. 2012; 8: 115-121.
37. Kumar G, KarthikL and Rao KV: Phytochemical composition and in vitro antioxidant activity of aqueous extract Aerva Lanata (L) Jussex Schult. stem (Amaranthaceae). Asian Pacific J.Trop.Med. 2013; 6(3): 180-187.
38. Kato S, Tanaka K, Kunikata T, Mizoguchi H and Takeuchi K: The roles of nitric oxide and prostaglandins in alterations of ulcerogenic and healing responses in adjuvant induced arthritic rat stomachs. Alimen. Pharmacol. Therap. 2000; 14(1):18-25.
39. Kochar NI, Chandewal AV, Ravindra I, Kochar B and Kochar P: Nitric oxide and the gastrointestinal tract. Inter. J. Pharm. 2011; 7(1): 31-39.
40. Berg AS, Redeen AC, Sjostrand E and Sjostrand SE: Nitric oxide an endogenous inhibitor of gastric acid secretion in isolated human gastric glands. BMC Gastroenterol 2004; 4: 16-20.
41. Brzozowski T, Konturek PC, Konturek SJ, Brzozowska I and Pawlik T: Role of prostaglandins in gastroprotection and gastric adaptation. J. Physiol. Parmacol. 2005; 56 (5): 33-55.
42. El-Far AH, Ginidi RR and Abd- ElMaksoud HAA: Some biochemical changes followed experimental gastric ulceration. Inter. J. Biol. Biomol. Agricul. Food Biotech. Enginer. 2012; 6(10): 959-964.
43. Wallace JL: Prostaglandins, NSAIDs and gastric mucosal protection: why does not the stomach digest itself?.Phsiol. Rev. 2008; 88:1547-1565.
44. Chandranath S, Bastaki SM, D Souza A, Adem A and Singh J: Attenuation of stress-induced gastric lesions by lansoprazole, PD-136450 and rantidine in rats. Mol.Cell Biochem. 2011; 349(1-2): 205-212.
45. Choi SR, Lee SA, Kim YJ, Ok CY, Lee HJ and Hahm KB: Role of heat shock proteins in gastric inflammation and ulcer healing. J. Physiol. Pharmacol. 2009; 60(7): 5-17.