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The Potential Curative and Prophylactic Effects of Grape Seed Extract on Aspirin Induced Gastric Ulcer in Adult Male Albino Rats

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ABSTRACT

Introduction: Aspirin {Acetyl Salicylic Acid (ASA)} is a potent Nonsteroidal Anti-Inflammatory Drug (NSAID). Gastric ulcer associated with the use of aspirin is a major problem. Grape Seed Extract (GSE), has been shown to produce effective antioxidant effects.

Aim: To evaluate the effect of grape seed extract on gastric ulcer induced by aspirin.

Materials and Methods: Fifty adult rats were classified into: Group I (control group); Group II (GSE group), which received a single dose of GSE (250 mg/kg/day) by gavage for 10 days; Group III (Gastric ulcer group), which received (300 mg/kg) of aspirin by gavage, Group IV (Prophylactic group), which received GSE (250 mg/kg/day) by gavage for 10 days, one hour after administration of the last dose each rat received a single dose of aspirin (300 mg/kg) by gavage and Group V (Curative group), which received a single oral dose (300 mg/kg) of aspirin, five hours after administration of aspirin each rat received an oral dose (250 mg/kg) of GSE for 10 days. At the end of the study, the rats were anaesthetized and the stomach from all groups were extracted and examined. ANOVA test followed by a post-hoc test (LSD) were employed to compare the results of studied animal groups.

Results: The gastric tissue of group III revealed extensive mucosal erosions and sloughing of gastric glands. GSE administration in the prophylactic group resulted in intact mucosa with minimal inflammatory infiltration while sloughing of gastric glands was still observed in some areas in the curative group. Significant increase in the percentage of iNOS and MDA level was found in Group III compared to control group, while significant decrease of iNOS and MDA level was noticed in Group IV and Group V as compared to Group III.

Conclusion: Results of the current study revealed that administration of GSE before induction of gastric ulcer could greatly ameliorate the condition than after the induction of the ulcer.

Keywords: Adverse effect, Histopathology, NSAID

INTRODUCTION

Aspirin {Acetylsalicylic Acid (ASA)} is an old drug widely used for the prevention and treatment of cardiovascular and cerebrovascular diseases [1]. Aspirin is often used as a pain reliever, as an antipyretic to reduce fever and as an anti-inflammatory medication [2]. Today, aspirin is one of the most widely used drugs in the world, consuming an estimated 40,000 tons of it every year [3].

Salicylate ingestion can lead to epigastric distress, nausea, and vomiting. Salicylate may also cause ulceration of the stomach, exacerbation of symptoms of peptic ulcer as heartburn and dyspepsia. Gastrointestinal hemorrhage and erosive gastritis have all been reported in patients with high-dose therapy, but can also occur with low-dose [4].

Peptic ulcer, a common pathological gastrointestinal condition, usually occurs due to the imbalance of gastric and duodenal mucosal aggressive and defensive factors. Aggressive factors include hydrochloric acid, pepsin, helicobacter pylori, NSAIDs and ethanol. Local mucosal defensive factors include endogenous protective agents such as prostaglandins and Epidermal Growth Factors (EGF), bicarbonate, mucus secretion, blood flow and cellular regeneration [5].

Magierowski M et al., reported that Nitric Oxide (NO) contributes to numerous physiological and pathophysiological processes, including gastrointestinal integrity and gastroduodenal protection mechanisms [6]. The therapeutic effects of NSAIDs are mediated by inhibiting prostanoid biosynthesis [7]. Prostanoid derivatives are the result of conversion of arachidonic acid by Cyclo-Oxygenase (COX) isoenzymes after cell injury [8]. COX-1 inhibition in the gastrointestinal tract results in a reduction in prostaglandin secretion and its cytoprotective effects in gastric mucosa. This therefore increases the sensitivity to mucosal injury [9].

Grape is a significant source of nutritional antioxidants such as polyphenols, anthocyanins and biologically active ingredients in the diet [10]. GSE from the seed *Vitis vinifera* is a natural extract. It is a rich source of one of the most beneficial plant groups [11]. Grapevine (*Vitis vinifera*) is grown in all regions of different temperatures of the world today [12]. Its seeds contain several active ingredients including flavonoids, polyphenols, anthocyanidin, proanthocyanidin, procyanidins and the derivative resveratrol [13].

It has been shown that GSE rich in flavonoids, mainly proanthocyanidin, have effective antioxidant effects [14]. Experimental studies showed that oral administration of GSE reduced the production of reactive oxygen species and plasma protein carbonyl groups while enhancing the endogenous antioxidant system activity [14-16].

Therefore, the aim of this study was to compare between curative and prophylactic effect of grape seed extract on gastric ulcer induced by acetyl salicylic acid in adult albino rats.

MATERIALS AND METHODS

Experimental Animals and Groups

The study was conducted at the Human Anatomy and Embryology Department, Faculty of Medicine, Menoufia University. A total of 50 adult healthy male Sprague-Dawly rats, 10-12 weeks of age, weighing 180-200 g, were used throughout the study. They were obtained from the Egyptian Organisation for Biological Products and Vaccines (Cairo, Egypt). They were housed individually for a 2-week

acclimatisation period prior to the experiment. Rats were fed *ad libitum* by standard laboratory pellet and tap water. A 12-hr light, 12-hour dark cycle was maintained. Room temperature was at 23±2°C with a relative humidity of 45-55%. The entire experimental protocol was approved by institutional ethical committee (1407/14/6/2014) and utmost care was taken during the experimental procedure, as well as at the time of sacrifice following the principles of the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines, EU Directive 2010/63/EU for animal experiments. After the 2 week-period of acclimatisation, the animals were randomly divided into five main groups (n=10 per group):

Group I (Control group): They were further subdivided into two subgroups: **Subgroup Ia (negative control)**, consisted of 5 rats, where animals did not receive anything. **Subgroup Ib (positive control)**, consisted of 5 rats, each rat received a single dose of 3 mL of 1% carboxymethyl cellulose by gavage.

Group II (GSE group): Each rat received GSE in a dose of 250 mg/kg/day orally by gavage for 10 days. One hour after the last dose rats were sacrificed [17].

Group III (Gastric ulcer group): They were fasted 24 hours before administration of aspirin. Each rat received a single dose of 300 mg/kg of aspirin dissolved in 3 mL of 1% carboxymethyl cellulose orally by gavage. Five hours later, rats were sacrificed [17].

Group IV (Prophylactic group): Each rat received GSE in a dose of 250 mg/kg/day orally by gavage for 10 days. One hour after administration of the last dose each rat received a single dose of aspirin 300 mg/kg dissolved in 3 mL of 1% of carboxymethyl cellulose orally by gavage. Five hours later rats were sacrificed [17].

Group V (Curative group): Each rat received a single oral dose of 300 mg/kg of aspirin dissolved in 3 mL of 1% carboxymethyl cellulose after 24 hours of fasting. Five hours after administration of the last dose, each rat received an oral dose of 250 mg/kg of GSE for 10 days. One hour after administration of the last dose of GSE rats were sacrificed.

At the end of the experiment, just before scarification, blood samples were taken from the retro-orbital sinus in collecting heparinized capillary tubes for biochemical studies. Rats were anaesthetized by diethyl ether inhalation then sacrificed, abdomen was opened to extract the stomach. The stomach was opened along the greater curvature then washed by saline. The forestomach was selected to undergo histological and immunohistochemical studies.

Chemicals

Acetylsalicylic acid powder was provided from Sigma-Aldrich chemical Co., St. Louis. Mo. (USA). It is available in the form of powder, each bottle of 250 mg.

GSE was provided from Sigma-Aldrich chemical Co., St. Louis. Mo. (USA) in the form of oil.

Immuno-histochemical markers used include Inducible Nitric Oxide Synthase (iNOS) to detect oxidative stress and EGF to detect the cofactors of ulcer healing. Both are rabbit polyclonal antibodies (immunoglobin GIgG). They were obtained from LabVision/Neomarkers Corporation as liquid ready to use.

Light Microscopic Study

The collected stomach tissues were fixed in aqueous Bouin's fixative, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and then impregnated in paraffin wax. Sections of 4-5 µm thickness were taken and stained with Haematoxylin & Eosin (H&E), Periodic Acid-Schiff (PAS) and Alcian Blue (AB) and Mallory trichrome stain, examined and photographed under light microscope [18].

Immunohistochemical study:

a. Immunostaining of iNOS

iNOS was demonstrated using an immunoperoxidase immunohistochemical staining procedure on 5 µm sections cut from the paraffin blocks. Sections were deparaffinized in xylene, rehydrated through graded ethanols, washed with 0.01 mol/l Phosphate-Buffered Saline (PBS) and distilled water. Sections were boiled in 10 mM citrate buffer, pH 6.0 (Thermo Scientific/Lab Vision, Fremont, CA) and washed with 3% hydrogen peroxide (Merck, Darmstadt, Germany) at room temperature to inhibit endogenous peroxidase activity. To prevent nonspecific binding of antibodies, sections were incubated in blocking serum (Thermo Scientific/Lab Vision) for 15 min. Sections were incubated in primary antibody, iNOS (ab 15323; Abcam, Cambridge, UK) diluted 1:100 with antibody diluent solution (Dako, Glostrup, Denmark), at room temperature in a humid chamber with due regard for the incubation periods used in initial trials and the recommendation of the manufacturer. Sections were rinsed in PBS and incubated with biotinylated secondary antibody (Ultra vision Detection System HRP kit; Thermo Scientific/Lab Vision) and streptavidin peroxidase (Ultra vision Detection System-HRP kit; Thermo Scientific/Lab Vision) for 30 min. 3-Amino-9-ethylcarbazole (AEC, Thermo Scientific/Lab Vision) was used as the chromogen to visualise the immunohistochemical labelling, and Mayer's hematoxylin was used as a nuclear counter stain [19].

b. Immunostaining of epidermal growth factor

Paraffin sections (five-µm) were incubated in blocking reagent for 30 minute, treated with primary antibody at a 1/1000 dilution for 30 minute at 37°C, washed three times in PBS, and incubated with the biotinylated secondary antibody for 30 minute at Room Temperature (RT). They were then washed in PBS three times and then incubated for 30 minutes at RT in avidin biotin peroxidase complex. Following rinsing in PBS for 3x5 minutes, the sections were rinsed in distilled water, then incubated in DAB (Zymed, 00-2020) for 5 mins to reveal peroxidase. Counterstaining was carried out with haematoxylin. Sections were then cleaned in xylene and mounted with Entellan (Merck, 7961). Negative controls were processed using antibody diluent reagent solution rather than the primary antibody [20].

c. Biochemical studies for determination of oxidative stress

Blood samples were obtained from the retro-orbital sinus of each rat, then transferred into plain tube, left for 30 minutes for clotting and centrifuged for 10 minutes. The obtained serum was used for estimation of Malondialdehyde (MDA), Catalase (CAT) and Superoxide Dismutase (SOD).

Morphometric Study

Five different Mallory trichrome stained sections of the fundus of the stomach from animals of each group were used to measure the percentage of the surface area of collagen fibers in the submucosa and bases of the fundic glands. Another five different iNOS immunostained sections from five animals of each group were used to measure the percentage of the surface area of positive iNOS immuno-reactions. Also, five different EGF immuno-stained sections from five animals of each group were used to measure the percentage of the surface area of positive EGF immuno-staining. This study was conducted at Anatomy and Embryology Department, Faculty of Medicine, Menoufia University. The morphometry was done by automatic technique using image analyser software. Single-blinded investigator conducted the quantification to avoid inter-observer bias.

Technique

The data were obtained using LeciaQwin 500 image analyser computer system (England). The image analyser consisted of a coloured video camera, coloured monitor, hard disc of IBM personal computer connected to microscopic and controlled by LeciaQwin 500 software. The image analyser was automatically calibrated using the units of measurement (pixels) produced by the program of image analyser.

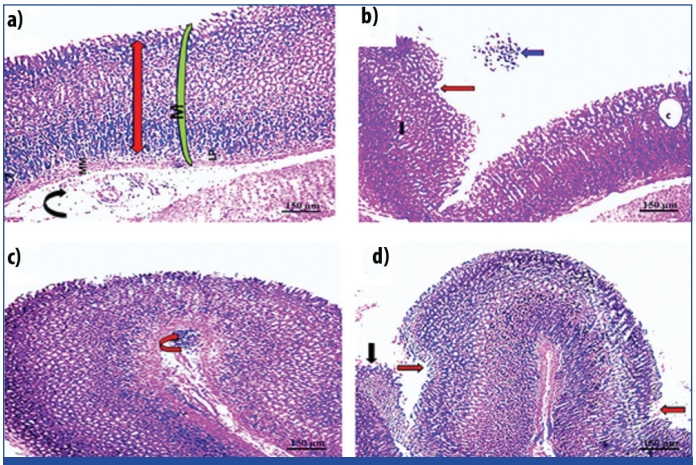
STATISTICAL ANALYSIS

The analysis was done with SPSS 17.0. The morphometric data of each animal group were statistically analysed and the ANOVA test followed by a post-hoc test (LSD) were employed to compare the studied animal groups. Data were expressed as the mean (±) SD. Significance of the data was determined by p-values where a $p<0.05$ was considered significant.

RESULTS

Light Microscopic Results

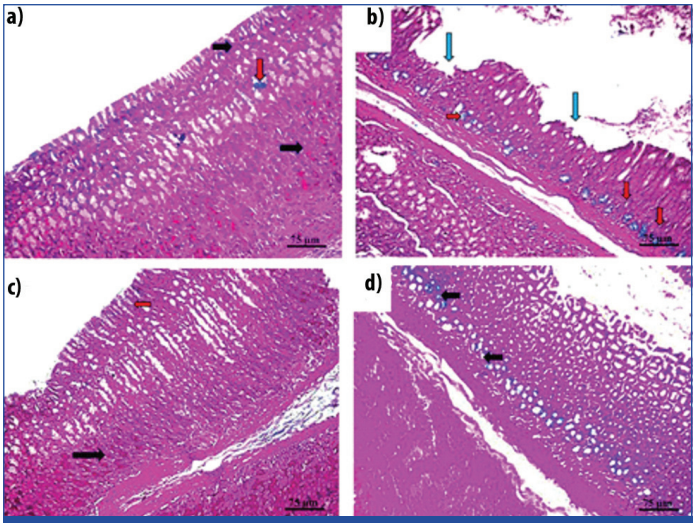
Hematoxylin and eosin stained sections of the fundus of stomach of the control and grape seed extract groups revealed normal architecture with intact mucosa and normal submucosa [Table/Fig-1a]. On the other hand, rats with gastric ulcer showed extensive gastric mucosal erosions in the glandular part of the stomach, sloughing of gastric glands and exfoliated cells appearing in the lumen. In areas without erosion, vacuolated cytoplasm of the gastric mucosal epithelium and cystic dilatation of the glands were detected [Table/Fig-1b]. Rats of the prophylactic group showed intact mucosa, regular gastric glands and normal submucosa with mild inflammatory infiltration [Table/Fig-1c]. The microscopic examination of stomach of the curative group showed that gastric epithelium was protected to some extent, sloughing of gastric glands and exfoliated cells were still observed in some areas [Table/Fig-1d].



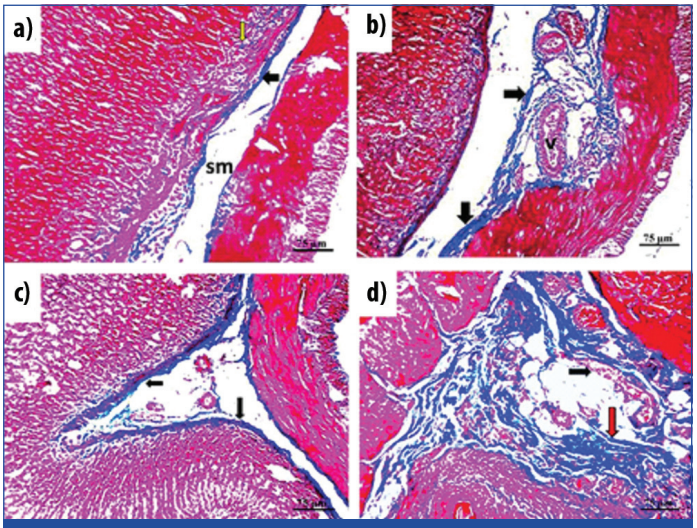
[Table/Fig-1]: Sections of the fundus of the stomach, (a) Control group showing normal mucosal architecture (M), intact epithelial surface (red arrow), lamina propria (LP) and muscularis mucosa (MM) with normal submucosal (curved black arrow) (H&E, x100); (b) Gastric ulcer rats elicited erosion of the surface mucous cells (red arrow), massive sloughing of the superficial parts of gastric glands (blue arrow) with exposed muscularis mucosa, dilatation of fundic glands (c) and vacuolation of cells (black arrow) (H&E, x100); (c) Prophylactic group showed preservation of gastric mucosal architecture and an outline similar to that of control rats except presence of mild inflammatory infiltration (curved arrow) (H&E, x100); (d) Curative group showed restoration of gastric mucosal cells to some extent, erosion of the surface mucous cells (red arrows) and sloughing of the gastric glands (black arrow) was detected in some areas (H&E, x100).

In combined PAS-Alcian blue stained sections of the control group showed that the surface of the mucosa was covered by a thin film of PAS positive mucous coat which was extended to fill the gastric pits and lumina of the glands, the mucous neck cells were also found to contain AB (alcian blue) positive mucous [Table/Fig-2a]. In gastric ulcer group, the surface epithelium lacked the PAS positive mucous coat [Table/Fig-2b]. However, in the prophylactic group, the superficial cells as well as the cells in the bases of the fundic glands showed PAS positive reaction similar to the control group [Table/Fig-2c]. In the curative group, absent PAS positive mucous on the surface was noticed [Table/Fig-2d].

In Mallory trichrome stained sections of the control group, gastric ulcer group and prophylactic group revealed few collagenous fibers in the submucosa, lamina propria and bases of the fundic glands. While increase in the amount of collagen fibers was seen in the curative group that may be explained by start of process of ulcer healing [Table/Fig-3].



[Table/Fig-2]: Sections of the fundus of the stomach, (a) Control group showing PAS positive reaction in the surface columnar cells and cells on the bases of the glands (black arrows), the mucous neck cells contain AB (alcian blue) positive mucous (red arrow) (PAS & alcian blue, x200); (b) Gastric ulcer rats showing absent PAS positive mucous coat on the eroded surface (blue arrows) but still present in the basal part of the gland (red arrows) (PAS & alcian blue, x200); (c) Prophylactic group showing PAS positive reaction in the superficial cells (red arrow) as well as the cells in the bases of the fundic glands (black arrow) (PAS & alcian blue, x200); (d) Curative group showing absent PAS positive reaction on the surface and AB positive mucous (black arrows) (PAS & alcian blue, x200).



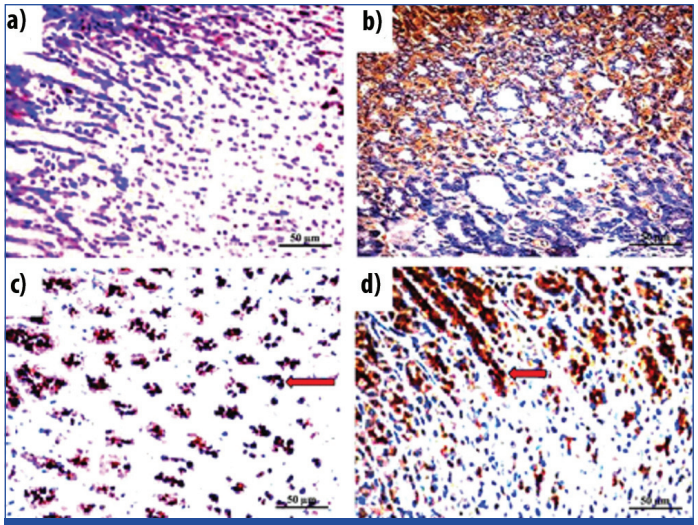
[Table/Fig-3]: Sections of the fundus of the stomach, (a) Control group showing few collagenous fibers (black arrow) in the submucousa (sm) and bases of the fundic glands (yellow arrow) (Mallory trichrome, x200); (b) Gastric ulcer rats showing few amount of collagenous fibers (black arrows) around dilated congested blood vessels (V) (Mallory trichrome, x200); (c) Prophylactic group showing few amount of collagenous fibers in the submcosa (black arrows) (Mallory trichrome, x200); (d) Curative group showing massive amount of collagenous fibers (red arrow) around dilated congested blood vessel (black arrow) (Mallory trichrome, x200).

Immuno-histochemical Results

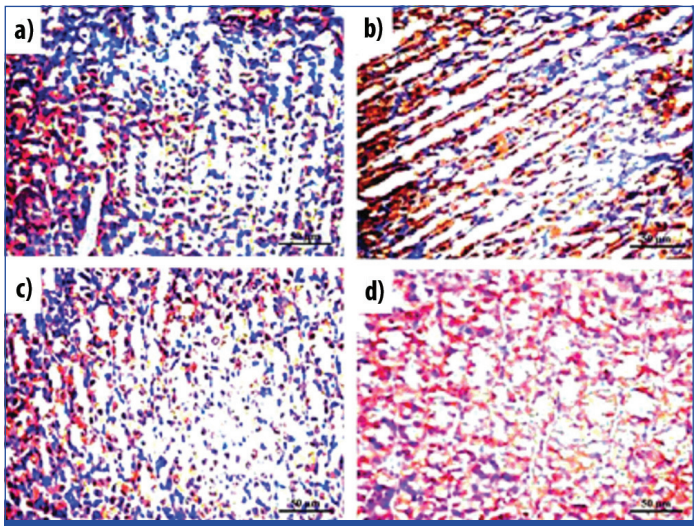
Examinations of rat gastric mucosa revealed that aspirin administration significantly increased the expression of iNOS in the cytoplasm of gastric glandular cells. However, in the prophylactic group, there was significant decrease of iNOS expression when compared to gastric ulcer group. iNOS expression in the curative group also showed significant decrease compared to gastric ulcer group but not to that extent showed in the prophylactic group [Table/Fig-4]. Examinations of rat gastric mucosa of EGF stained sections showed minimal expression of EGF in the gastric mucosa of control group. Gastric ulcer and prophylactic groups revealed minimal increase in EGF immunoreactivity compared to control group while increase in EGF immunoreactivity was detected in the curative group [Table/Fig-5].

Morphometric Results

Non-significant difference in percentage of collagenous fibers was evident in gastric ulcer and prophylactic groups while significant increase in percentage of collagen fibers was found in the curative group compared with control group [Table/Fig-6].



[Table/Fig-4]: Sections of the fundus of the stomach, (a) Control group showing minimal expression of iNOS in the gastric mucosal cells (iNOS, x400); (b) Gastric ulcer rats showing extensive expression in iNOS immunoreactivity in the cytoplasm of gastric epithelial cells (iNOS, x400); (c) Prophylactic group demonstrating a significant reduction in iNOS immunostaining (red arrow) compared to group III (iNOS, x400); (d) Curative group showing reduction in iNOS immunoreactivity (red arrow) (iNOS, x400). Brown color indicates iNOS positivity.



[Table/Fig-5]: Sections of the fundus of the stomach, (a) Control group showing minimal expression of EGF in the gastric mucosal cells (EGF, x400); (b), (c) Gastric ulcer and prophylactic groups respectively, showing minimal increase in EGF immunoreactivity in the cytoplasm of gastric epithelial cells compared to control group (EGF, x400); (d) Curative group showing marked increase in EGF immunoreactivity (EGF, x400). Brown color indicates EGF positivity.

Significant increase in the percentage of iNOS immuno-reactivity surface area in gastric ulcer group compared with control group while significant decrease in the percentage of iNOS immunoreactivity surface area was found in prophylactic and curative groups as compared with group III. Non-significant increase in the percentage of EGF immunoreactivity surface area in gastric ulcer and prophylactic groups when compared with the control group while significant increase in the percentage of EGF immunoreactivity surface area was found in curative group as compared with gastric ulcer group [Table/Fig-6].

Biochemical Results

Determination of oxidative stress markers:

Rats with gastric ulcer showed significant changes in oxidative stress markers with a significant increase in blood MDA level compared to control rats. Treatment of gastric ulcer rats with GSE in both prophylactic and curative groups significantly reduced the MDA level compared to gastric ulcer group. Significant decrease in levels of SOD and CAT enzymes were observed after aspirin administration when compared with control rats. Both prophylactic and curative groups showed significant increase in levels of SOD and CAT as compared to gastric ulcer group [Table/Fig-7].

DISCUSSION

Gastric ulcer grasps all interest as it is a widespread disease and its complications are great, this is in agreement with Chaturvedi A et al., who stated that gastric ulcer is a major health hazard in terms of morbidity and mortality [21]. Also, Tortora G et al., mentioned that untreated gastric ulcer is capable of inducing upper gastrointestinal bleeding [22].

In the present work, histological study of the fundus of the stomach of gastric ulcer group, revealed ulceration, desquamation and exfoliation of the surface epithelial cells, this is in agreement with Zhang JY et al., who demonstrated that aspirin induced severe congestion and multiple haemorrhagic erosion in the stomach tissue particularly in mucous secreting cells, characterised by gastric pit damage and vacuolation of the glandular portion [23]. The lamina propria showed mononuclear cellular infilteration, and this was supported by Abdelgawad et al., who mentioned that oral administration of a single dose (200 mg/kg) of aspirin to rats caused edema in lamina propria with mononuclear cellular infiltration [24].

In the present study, gastric ulcer group showed depletion of PAS positive cells with absent reaction on the surface but there was increase in alcian blue stained cells in the neck of the fundic glands, this is in agreement with Al Sagaaf S et al., who noticed the presence of interrupted faint PAS-AB positive mucous film with predominance of AB positive mucous secreting cells in the neck region and some scattered in the bases of the gland after a single toxic dose of NSAIDs [25].

In the current study, the amount of collagen fibers were markedly increased in the curative group. Kumar S et al., explained the increased collagen deposition at the site of inflammation as that many growth factors were elaborated by inflammatory mononuclear cells which were attracted to the site of injury [26].

Morphometric parameter	GI (Control group)	GII (GSE group)	Group III (Gastric ulcer group)	Group IV (Prophylactic group)	Group V (Curative group)
Collagen fibers area percentage	10.98±0.46	10.92±0.60	12.47±0.57	8.08±6.27	36.72±0.31*a
iNOS area percentage	15.56±0.43	15.32±0.37	75.51±1.13*a	17.92±0.56*b	60.10±0.62*b
EGF area percentage	16.98±0.27	20.79±0.28	20.70±0.45	21.06±0.58	86.78±0.64*b

[Table/Fig-6]: Mean±SD of the morphometric parameters among the different groups.
*p≤0.05
[a]compared to the control group; [b]compared to the gastric ulcer group

Biochemical parameter	GI (Control group)	GII (GSE group)	Group III (Gastric ulcer group)	GroupIV (Prophylactic group)	GroupV (Curative group)
MDA nmol/mg	0.97±0.20	1.11±0.26	12.4±0.48*a	1.93±0.17*b	8.16±0.56*b,c
CAT µmol/mg	37.9±0.94	38.5±0.66	28.7±1.62*a	35.9±0.56*b	32±0.37*b,c
SOD um/mg	46.96±4.70	47.26±4.70	39.54±0.97*a	43.36±3.30*b	40.60±3.73*b

[Table/Fig-7]: Mean±SD of the biochemical parameters among the different groups.
*p≤0.05
[a]compared to the control group; [b]compared to the gastric ulcer group; [c]compared to the prophylactic group

Concerning the immunohistochemical study, the present study demonstrated great increase in the surface area of iNOS in gastric ulcer group. This finding was supported by Konturek PC et al., who stated that one of the mechanisms by which aspirin damages the gastric mucosa is the increased production of NO due to the over expression of iNOS [27]. Also Whittle BJ et al., Hsu DZ et al., stated that excessive release of NO from gastric epithelial cells induced by aspirin exerted detrimental effects [28, 29].

In addition, there was minimal increase in the EGF immunoreactivity after a single toxic dose of aspirin but there was marked increase in EGF immunoreaction in the curative group, this was in accordance with Choi GH et al., who reported that there was an increase in the expression of EGF receptors in the early stages of ulcer healing [30].

Biochemical analysis of blood samples obtained from animals of gastric ulcer group showed increase level of MDA, this was in agreement with Kim JH et al., who reported that gastric antral ulceration develops mainly through the production of oxygen free radicals and lipid peroxides [31]. This is also supported by Cuevas V et al., who proved that aspirin increased MDA gastric concentration significantly as compared to control animals [17].

In agreement with this study, Chattopadhyay L et al., reported that NSAIDs induced reactive oxidative metabolites in animal models, which may contribute to mucosal injury [32]. These free radicals also damage the cellular antioxidant enzymes such as CAT and SOD, acting as the first line of cellular defense against oxidative injury and this might lead to aggravated tissue damage during gastric ulceration as mentioned by El-Missiry MA et al., [33]. This was apparent in our results from the stimulated lipid oxidation leading to increased accumulation of MDA as well as reduction in the gastric activity of CAT and SOD.

Immunohistochemical study of rats of group IV revealed decrease of the surface area of inducible iNOS immunoreactivity. This was in agreement with Brzozowski T et al., who reported that GSE ameliorated ethanol and stress induced gastric ulcer through suppression of inducible nitric oxide synthase [34]. Also, group V showed increase in the surface area of EGF immunoreaction. This was supported by Xu X et al., who mentioned that procyanidins accelerated gastric ulcer healing via increasing the content of EGF which would facilitate epithelial proliferation during ulcer healing stage [35].

Biochemical analysis of blood samples obtained from animals of prophylactic and curative groups showed increase in SOD and CAT. This was in agreement with Rajput SA et al., who found that dietary proanthocyanidins ameliorated oxidative stress by inhibiting the accumulation of MDA and enhancing the activity of antioxidant enzymes such as SOD and CAT [36]. This was also supported by Cetin A et al., who stated that GSE was a potent antioxidant via stimulation of endogenous antioxidant enzymes as CAT and SOD [37]. Also, there was decrease in the amount of MDA in the prophylactic and curative groups. This finding in agreement with Cuevas V et al., who reported that oral administration of GSE prevented aspirin and ethanol induced gastric mucosal ulceration and reduced the increase of gastric MDA elicited by these aggressive agents [17]. In addition, Abbas AM et al., stated that pretreatment with GSE in indomethacin induced gastric mucosal lesion improved the condition via decreasing MDA as an indicator of lipid peroxidation [38].

The results obtained from the fundus of the stomach of rats of the prophylactic group was better than that of the curative group. This improvement might be due to receiving GSE early before aspirin exposure that lead to creation of antioxidant enzymes, decrease lipid peroxidation and immunity response than receiving GSE after aspirin exposure. This is supported by the study of GSE in other organs as Enginar H et al., who indicated that GSE enhanced the antioxidant status and decreased the incidence of free radical induced lipid peroxidation in blood samples of rats exposed to x-radiation [39]. In addition, Ayad SK stated that exposure to borate caused deleterious damage in kidney but treatment with GSE showed a protective and ameliorative effects against nephrotoxicity and oxidative damage [40]. Also, Devi A et al., found that intake of procyanidin in moderately low quantity is effective in up-regulating the antioxidant defense mechanism [16]. Moreover, Sehirli O et al., reported that GSE could reduce organ injury through its ability to balance oxidant-antioxidant status and to regulate the release of inflammatory mediators [41].

LIMITATION AND RECOMMENDATION OF THE STUDY

As this study was of short duration, other studies of longer duration and multiple dosages of grape seed extract need to be planned to explore the therapeutic role of the extract in gastric ulcer. Also further clinical studies should be conducted in human beings.

CONCLUSION

The present study supports and validates the promising gastro-protective efficacy of grape seed extract against aspirin-induced gastric ulceration and affords gastro-protective mechanisms via maintenance of mucosal NO. Thus, the results open the way to important applications in humans, where it could be recommended that patients receiving aspirin should take GSE in a regular manner to reduce the incidence of gastric mucosal lesion and its complications. However, more studies are recommended to evaluate the therapeutic effect of GSE on gastric ulcer.

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