Proanthocyanidin ameliorates ethanol-induced gastric mucosal erosion by attenuating inflammatory response, oxidative stress, and apoptosis in rats

Yakout A. El Senosi¹, Mohamed K. Mahfouz¹, Samy A. Hussein¹, Reem A. Abd el-raout²

¹ Department of Biochemistry, Faculty of Veterinary Medicine, Benha University, Egypt
² 2 Veterinarian

ARTICLE INFO

Keywords
Apoptosis
Gastric erosion
Inflammatory mediators
Oxidative stress
Proanthocyanidin

ABSTRACT

Gastric ulcer is a common chronic disease in human digestive system. Massive alcohol drinking can lead to gastric ulcer. The gastroprotective effect and molecular mechanisms of Proanthocyanidin in a rat model of ethanol-induced gastric mucosal erosion were investigated. Thirty-five male rats were divided into five equal groups. Group 1 (Control normal): rats received no drugs. Group 2 (Early ulcer): rats received absolute ethanol (0.5 ml/100g) orally on empty stomach and sacrificed one hour later. Group 3 (Early ulcer + Proanthocyanidin protected): rats received proanthocyanidin orally at a dose of (300 mg/kg b. wt/day) for 3 weeks before ethanol administration then sacrificed after one hour. Group 4 (Late ulcer): rats received ethanol like group 2 and sacrificed after 21 days. Group 5 (Late ulcer + proanthocyanidin treated): rats first administered ethanol (0.5 ml/100g) and after one-hour proanthocyanidin was administered for 21 days. A significant increase in stomach L-MDA concentration with marked decrease in CAT activity and GSH concentration were observed in gastric erosion-induced rats. However, a significant depletion of gastric L-MDA level and marked increase in CAT activity and GSH concentration were observed after Proanthocyanidin treatment when compared to ulcerated rats. A significant up-regulation of gene expression level of BAX, NF-κB and IL-1β with down-regulation of Bcl-2 gene were observed in stomach of gastric erosion-induced rats. However, a significant down-regulation of BAX, NF-κB and IL-1β with up-regulation of Bcl-2 gene were observed after proanthocyanidin treatment. Conclusively, proanthocyanidin protects rat gastric mucosa against ethanol-induced gastric erosion via anti-inflammatory, anti-apoptotic and anti-oxidative mechanisms.

1. INTRODUCTION

Gastric ulcers are characterized by necrosis, induction of oxidative stress and secretion of inflammatory factors (de Souza Almeida et al., 2011). The pathogenesis of gastric ulcers is based on a multifactorial and complex interaction between protective and aggressive factors, including mucosal integrity, secretion of gastric acid, Helicobacter pylori, free oxygen radicals and excess alcohol consumption (Bhattacharya et al., 2007). Disturbing the balance between aggressive and protective factors that control cell apoptosis and proliferation results in gastric ulceration, which then activates the repairing system in the gastric mucosa (Li et al., 2016). Gastric lesions are resultant of mucosal damage produced by several factors and are associated with cellular influx, free radical generation, cytokines, and growth factors. Acute inflammatory marker, myeloperoxidase (MPO), and pro-inflammatory cytokines (tumor necrosis factor-alpha [TNF-α], interleukin-6 [IL-6], and IL-1 beta [IL-1β]) play a major role in gastric ulceration (Kumar et al., 2013).

Ethanol consumption has been shown to be a major cause of gastric ulcer. Oxidative stress and depletion of antioxidants have been considered a crucial step in alcohol-induced mucosal damage. Ethanol treatment induces intracellular oxidative stress and produces mitochondrial permeability transition and mitochondrial depolarization, which precede cell death in gastric mucosal cells. Thus, considering that ethanol is involved in the formation of oxidative stress generated extracellular and/or intra-cellular (SJ and AI, 2015). Administration of ethanol causes gastric necrotic damage and subsequent inflammatory cell infiltration and reduces the secretion of bicarbonate, gastric mucus, and nitric oxide. In addition, ethanol reduces the gastric blood flow and induces the oxidative stress by increasing the production of malondialdehyde and reducing glutathione production (El-Maraghy et al., 2015). Intragastric administration of ethanol causes severe oxidative stress in stomach tissue by significant inhibition of the activity of antioxidant enzymes such as CAT, GPx, and SOD (Guzmán-Gómez et al., 2018). Additionally, there was a significant increase in the level of MDA (Antonisamy et al., 2015), decrease in the gastric level of nitric oxide (Abdulla et al., 2010). Pro-inflammatory cytokines such as
TNF-α, IL-1β, and IL-6 play important roles in the regulation of acute gastric ulcer induced by ethanol (Almasaudi et al., 2017). Ethanol led to produce reactive oxygen species which prevent the expression of Hsp70 and upsurge Bax expression (Hajrezaie et al., 2015).

Oxidative stress has been implicated in the development of Ethanol-induced gastric injury where reactive oxygen species generated by activated leukocytes triggers mucosal damage through lipid peroxidation and depletion of antioxidants defenses such as reduced glutathione, catalase, and total antioxidant capacity (Liu et al., 2012). In addition, depletion of mucosal cytoprotective moieties, including PGE2 and glycoproteins, has been linked to ethanol consumption. (Golbabapour et al., 2013). Although the mechanisms underlying Ethanol-induced gastric ulcer have not been fully elucidated yet, mounting evidence have indicated that pro-inflammatory cytokines, oxidative stress, and apoptosis play important roles in its pathogenesis (Arab et al., 2015).

An alternative treatment with fewer side effects that also reduces the inflammatory response and thereby reduces pain is believed to be Grape seed extract, a waste product of wine making. (Spruce Street and St Louis; Germany. Proanthocyanidin was manufactured by HANZHONG TRG Biotech co. LTD with Molecular formula: C_{12}H_{30}O_{8} and Molecular Weight: 592.553 g/mol Proanthocyanidin was dissolved in DMSO, completed with normal saline and administrated orally using a stomach tube at a dose of 300 mg/kg body weight per day (Koga et al., 1999).

2.2.2. Absolute ethyl alcohol: It was manufactured by SIGMA-ALDRICH Pharmaceutical Chemicals co. 3050 Spruce Street and St Louis; Germany.

Induction of gastric erosion:
Rats were fasted for 18 hours and allowed free access of water prior to the oral administration of ethanol for induction of gastric erosion at a dose level of 0.5ml/100 g of rat (Li et al., 2016).

2.3. Animal grouping:
Rats were randomly divided into five main equal groups, each group contained 7 rats, placed in individual cages, and classified as follow:
- **Group 1 (Control Normal group):** Rats received no drugs, served as control non-treated for all experimental groups.
- **Group 2 (Early ulcer non-treated group):** Rats received absolute ethanol (0.5ml/100g rat) orally on empty stomach and sacrificed one hour later after ethanol administration.
- **Group 3 (Proanthocyanidin protected group):** Rats received proanthocyanidin (300 mg/kg body weight/day) orally for 21 successive days prior to ethanol administration. One hour after administration of ethanol the animals were sacrificed.
- **Group 4 (Late ulcer non-treated group):** Rats received absolute ethanol (0.5 ml/100g rat) on empty stomach and were left free and sacrificed 21 days later after ethanol administration.
- **Group 5 (Late ulcer + Proanthocyanidin treated group):** Rats first administered absolute ethanol (0.5 ml/100g rat) on empty stomach at the first day of experiment then after one an hour, proanthocyanidin was administered (300 mg/kg body weight/day) for 21 successive days then sacrificed.

2.4. Sampling:
Gastric tissue specimen was collected from all animal groups (control and experimental groups) once after the end of 3 weeks.

2.4.1. Gastric tissue for biochemical analysis:
After 21 days of treatment with proanthocyanidin, the rats were sacrificed by cervical decapitation. The stomach was quickly removed, and opened along the greater curvature using a scraper, cleaned by rinsing with cold saline and stored at –20 °C for subsequent biochemical analysis. Briefly, gastric tissues were cut, weighed, and minced into small pieces, homogenized with a glass homogenizer in 9 volume of ice-cold 0.05 mM potassium phosphate buffer (pH 7.4) to make 10% homogenates. The homogenates were centrifuged at 6000 rpm for 15 minutes at 4 °C then the resultant supernatant was used for the determination of L-Malondialdehyde (L-MDA) concentration and catalase (CAT) enzyme activity. Also, 0.2 g of stomach tissues were minced into small pieces homogenized with a glass homogenizer in 0.4 ml of 25% metaphosphoric acid (MPA) (ref. No.: 253-433-4, Sigma-Aldrich, Germany), then 1.4 mL of distilled water was added, mixed and incubated for 1
hour and centrifuged for 10 min at 3000 rpm then the clean supernatant was removed and used for determination of GSH concentration.

2.4.2. Gastric tissue for molecular analysis:
At the end of experiment, rats gastric tissues were immediately excised and frozen in liquid nitrogen and then in -80 °C until used for Pro apoptotic protein (BAX), B cell lymphoma-2 (Bcl-2), Nuclear factor kappa B (NF-κB) and Interleukin-1β (IL-1β) gene expression analysis by qPCR.

2.4.3. Gastric tissue for histopathological examination:
Gastric tissue specimens were taken from different parts of the stomach for histopathological examination. The specimens were preserved in 10% buffered neutral formalin. The fixed tissue was rinsed in tap water, dehydrated through graded series of alcohols, cleared in xylene, and embedded in paraffin wax. 5 μm thick sections were cut and stained with hematoxylin and eosin (H&E) (Bancroft and Stevens, 1996) and then the tissues were examined and evaluated by light microscopy.

2.5. Analysis:
2.5.1. Biochemical analysis:
Gastric tissue L-MDA, CAT and GSH were determined according to the methods described by Okahawa et al. (1979), Aebi (1984) and Beutler et al. (1963), respectively.

2.5.2. Molecular analysis
Total RNA was isolated from stomach tissue of rats using RNA extraction kit (Thermo Scientific, Fermentas, #K0731) according to the manufacturer’s protocol. Following determination of RNA concentration and purity by Quawell nanodrop Q5000 (USA), 5 mg of total RNA from each sample was reverse transcribed using Revert Aid H minus Reverse Transcriptase. The produced cDNA was used as a template to determine the relative expression of Pro apoptotic protein (BAX), B cell lymphoma-2 (Bcl-2), Nuclear factor kappa B (NF-κB) and Interleukin-1β (IL-1β) genes using Step One Plus real time PCR system (Applied Biosystem, USA) and gene specific primers. The reference gene, βactin, was used to calculate fold change (FC) in target genes expression. The thermal cycling conditions, melting curves temperatures, and calculation of relative expression was done. For the treated groups, assessment of 2^{-ΔΔCt} determined the fold change in gene expression relative to the control.

2.6. Statistical Analysis:
All the data were expressed as means± SE. The statistical significance was evaluated by One-way analysis of variance (ANOVA) using SPSS, 18.0 software, 2011 and the individual comparisons were obtained by Duncan’s multiple range test (DMRT). Values were considered statistically significant when p<0.05.

Table 1 Forward and reverse primers sequence for real time PCR

| Gene        | Forward primer (5'→3') | Reverse primer (5'→3') |
|-------------|------------------------|------------------------|
| Bax         | AGCCCTGAGCTGACTTGTG    | AGCCATGATGTTGTCGATC    |
| Bcl-2       | AGTACCTGAACCGGACATCG   | GGGTCCATGTTGAAGTCAC    |
| Bax         | CACCCTCAAGCAAGGAGCCAC  | GGCGTCCATGTTGAAGTCAC   |
| Bcl-2       | AGTACCTGAACCGGACATCG   | GGGTCCATGTTGAAGTCAC    |
| β-actin     | ACCCAACTGTGCCACCTCTA   | CTGCAACCCCTGTGAGT     |

3. RESULTS
Effect of proanthocyanidin administration on gastric tissue L-MDA, CAT and GSH of ethanol-induced gastric ulcer in male rats is presented in table (2). Gastric L-MDA concentration significantly increased, while CAT activity and GSH concentration significantly decreased in ethanol-induced gastric ulcerative rats in both early ulcer (after one hour) and late ulcer (after 21 days) when compared with the normal control group. Proanthocyanidin administration to ethanol-induced gastric ulcer rats exhibited a significant decrease in gastric-L-MDA concentration, and significant increase in CAT activity and GSH concentration as compared to untreated early ulcer and late ulcer groups. Effect of Proanthocyanidin administration on the relative mRNA gene expression levels of BAX, Bcl-2, NF-κB and IL-1β in stomach of ethanol-induced gastric erosion in rats is shown in table (3). The obtained qPCR results revealed a significant up-regulation of BAX, IL-1β and NF-κB gene expression levels with significant down-regulation of Bcl-2 gene expression level in stomach of ethanol-induced gastric ulcerative rats (G2 early ulcer and G4 late ulcer ) as compared to control normal group (G1). This expression was significantly downregulated with upregulation of Bcl-2 gene expression level following administration of proanthocyanidin either before (G3) or after (G5) induction of ulcer, with lower expression in protected group (G3).

Table 2 Effect of proanthocyanidin administration on gastric tissue L-MDA, GSH concentrations and CAT activity of ethanol-induced gastric mucosal erosion in male rats

| Animal groups          | L-MDA (nmol/g tissue) | CAT (μg/ tissue) | GSH (mg/g. tissue) |
|------------------------|-----------------------|------------------|-------------------|
| Group I: Normal control| 1.53±0.14             | 0.93±0.04        | 4.48±0.32         |
| Group II: Early ulcer group | 10.20±0.52           | 0.39²±0.02       | 1.19±0.12         |
| Group III: Early ulcer + proanthocyanidin protected | 2.83±0.25           | 0.76³±0.03       | 3.25±0.28         |
| Group IV: Late ulcer group | 12.29±0.73           | 0.37±0.03        | 0.81±0.05         |
| Group V: Late ulcer + proanthocyanidin treated | 4.82±0.4         | 0.64³±0.02       | 1.75±0.14         |

Data are presented as (Mean ± S.E). Mean values with different superscript letters in the same column are significantly different at (P<0.05).

Table 3 Effect of proanthocyanidin administration on the relative expression of BAX, Bcl-2, IL-1β and NF-κB gene levels in stomach of ethanol-induced gastric mucosal erosion in male rats

3
Histopathological examination

The examined stomach mucosa of control normal rats revealed no histopathological changes (Fig. 1a). Multifocally, there was necrosis of the surface epithelium characterized by shrunken, hyper-eosinophilic cytoplasm with pyknotic nuclei were observed in stomach of early ulcer non-treated rats (Fig. 1b). The region of stomach mucosa showed normal histological appearance like control group. Occasionally, variable sized erosions were observed in the mucosa of stomach of PGSE protected rats (Fig. 1c).

Multifocally, there were marked areas of erosions primarily affecting the surface and deep mucosa. These erosive areas characterized by necrosis and loss of the surface epithelium with presence of necrotic debris in the gastric lumen of late ulcer non-treated rats (Fig. 1d). Rarely, small erosions were observed in stomach of PGSE treated rats characterized by necrosis and loss of the surface epithelium. No erosions or ulcers affecting the middle and deep mucosa were observed (Fig. 1e).

4. DISCUSSION

In recent times, it has been revealed that ethanol-induced lipid peroxidation and oxidative stress has a major role in the pathogenesis of acute gastric injuries. The production of reactive oxidative species (ROS) in gastric mucosal tissue continues at a normal level, due to the equilibrium between pro-oxidant and antioxidant systems. Conversely, the equilibrium is altered in many circumstances, including drinking alcohol (Antonisamy et al., 2016). The obtained results indicated that gastric L-MDA concentration significantly increased and gastric CAT activity, GSH concentration significantly decreased in ethanol-induced gastric ulcerative rats in both early ulcer and late ulcer. Ethanol exposure causes significant rises of MDA level, and reductions in SOD, CAT, and GSH-px activities (Paulrayer et al., 2017). Ethanol is commonly used to induce ulcers in experimental rats; ethanol leads to intense gastric mucosal damage, directly and indirectly through such mediators as reactive oxygen species (ROS) and cytokines (Abdel-Salam et al., 2001). The cells of the gastrointestinal tract have an antioxidant defense system that is capable of preventing the cytotoxicity of ROS through mechanisms that involve the
action of enzymes and compounds with the potential to scavenge free radicals and prevent their destructive action. The major anti-oxidative enzyme is SOD, which catalyzes the dismutation of O$_2$ into less noxious H$_2$O$_2$, which is further degraded by catalase or GSH-Px (Brzozowski et al., 2001). The decreases in the amounts of the GSH-Px and glutathione reductase (GR) enzymes and the antioxidant compound glutathione (GSH), associated with the elevation of MPO production, support an important role for oxidative stress in the pathogenesis of ethanol-induced gastric ulcers (Rozza et al., 2014). An unrestrained intake of alcohol may result in an imbalance between offensive and defensive factors of stomach and lead to gastric ulcer. Therefore, ethanol-stimulated gastric lesions model is commonly used to investigate the pathogenesis of gastric ulceration and evaluate the gastroprotective effect of drugs (Wu et al., 2018), the authors also showed a marked increase of MDA level and decrease of SOD, GSH, and CAT levels in rats after ethanol exposure. Also, Zheng et al. (2016) proved that ethanol-induced (MDA) overproduction. The gastric ulcer caused by ethanol is related to high production of reactive oxygen species (ROS), which can trigger oxidative stress by inhibiting the effects of antioxidants including glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) (Rezaie et al., 2007). GSH, SOD, and CAT are vital endogenous antioxidants which protect bio membrane from oxidative damage by scavenging ROS. GSH is considered as an antioxidative barrier which protects the gastric mucosa against oxidative stress caused by free radicals and peroxides (Brzozowski et al., 2004) It also coordinates with some other antioxidant enzymes then alleviates oxidative damage. CAT can scavenge ROS by triggering the rapid conversion of peroxyl radical (H$_2$O$_2$) into water and oxygen (Wong et al., 2013). MDA is a metabolite for oxidative stress which is generated by unsaturated fatty acids through ROS-activated lipid peroxidation. Thus, MDA is deemed as the biomarker of lipid peroxidation and used to quantify and activated lipid peroxidation. Thus, MDA is deemed as the biomarker of lipid peroxidation and used to quantify and evaluate oxidative and pro-inflammatory enzymes and free radicals, resulting in oxidative stress (Chatterjee et al., 2007). (Park et al., 2008) reported that ethanol administration depleted the gastric GSH, GPx and TAC antioxidant defenses which scavenge free radicals and prevent their noxious effects.

In the current study PGSE administration to ethanol-induced gastric ulcerated rats exhibited a significant decrease in gastric L-MDA concentration and significantly increased GSH concentration and CAT activity as compared to ulcerated untreated groups. GSPE acted on the expression of Heme Oxygenase-1 (HO-1) protein as a non-stressful stimulant, which led to the gastroprotective effect as due to the presence of HO-1, which was induced, carbon monoxide dilates the blood vessels and suppresses the aggregation of platelets. Thus, it plays a role in increasing the blood flow in the gastric mucosa (Kim et al., 2013), the authors also showed that pretreatment with GSPE in indomethacin (IND)-induced gastric mucosal injury in rats elevated GSH concentration compared to disease control group. The present finding was corroborated with the reports of (Abbas and Sakr, 2013) who reported that, GSE have a protective effect against IND-induced gastric ulcers through prevention of lipid peroxidation, increase of GSH, significantly decreased the gastric ulcer index, MDA, and TNF, increase antioxidants (superoxide dismutase, catalase, and glutathione peroxidase) activities of radical scavenging enzymes, prostaglandin (PGEl) generation, and anti-inflammatory activity. Also, El-Shitany and Eid, (2017) stated that Proanthocyanidin pretreatment reduced indicators of oxidative stress in the liver, including nitric oxide (NO) and malondialdehyde (MDA). It also increased the antioxidants, reduced glutathione (GSH) concentration, glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT) activities in the liver. GSP protects cells from damage by controlling the oxidative damage by reducing the tissue injury and maintains antioxidant status and reduces the release of pro-inflammatory mediators (Aysun et al., 2006). Moreover, (Liu et al., 2016) showed that GSPE increased the expression of Nuclear factor erythroid 2-related factor 2 (NRF2) and its target antioxidant genes superoxide dismutase and catalase and decreased the production of malondialdehyde and hydrogen peroxide in the liver of mice exposed to perfluorooctanoic acid.

A significant up-regulation of Bax, IL-1β and NF-κB gene expression levels with significant down-regulation of Bcl-2 gene were observed in stomach of ethanol-induced gastric ulcerative rats. Enhanced apoptotic death of gastric epithelial cells has been partly implicated in ethanol-induced gastric mucosal injury (Ye et al., 2013). Inflammatory signals along with oxidative stress have been reported to instigate the expression of several genes responsible for cellular death by apoptosis (Mei et al., 2012). Apoptosis is initiated by pro-apoptotic signals such as Bax (Abdel wahab, 2013) which promote the release of Cyt C from the mitochondria to the cytosol, with following activation of caspase-9 and yet caspase-3, the major tormentor caspase (Luo et al., 2013). Bax promotes apoptosis (Emily et al., 2001), while Bcl-2 inhibits this process. Apoptosis may be caused by an imbalance in the expression of Bcl-2 family antiapoptotic proteins and apoptotic Bax proteins in stress ulcers (Konturek et al., 1999). As confirmed with (Wang et al., 2018) who reported that, gene expression of caspase-3 and Bax were significantly upregulated in the ethanol group compared with the control group, while the expression of Bcl-2 was significantly down regulated. Moreover, chronic excessive alcohol intake also increases the level of inflammatory cytokines, such as TNF-α, IL-1β, and IL-6 (Wang et al., 2016). The release of these cytokines is mainly associated with the activation of toll-like receptor 4 (TLR4) and its downstream nuclear factor kappa B (NF-κB), which remains the key inflammatory pathway playing a vital role in alcohol-induced acute liver disease model (Lee et al., 2015). The present finding was corroborated with the reports of (Almasaudi et al., 2016) who reported that treatment of rats with ethanol caused a significant increase in plasma TNF-α, IL-1β, and IL-6 levels as compared to the control group. Also, (Liu et al., 2017) showed that, in contrast to the normal control group, the concentrations of the proinflammatory factors IL-1β, TNF-α and IL-6, were significantly higher in the ethanol-challenged mice.

Nuclear factor-κB (NF-κB) is a transcription factor that regulates the transcription of DNA to control the expression of protein-encoding genes for numerous biological processes. Due to the response with the existence of cellular stimuli, the inflammatory signaling is activated resulting in...
the transcribing proinflammatory genes thereby generating proinflammatory mediators and cytokines including nitric oxide (NO), cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), interleukin 6 (IL-6), interleukin 1β (IL-1β), tumor necrosis factor alpha (TNFα), and prostaglandin (PGE2) (Fard et al., 2015). LPS activates the signaling pathway such as NF-κB via the stimulation of Toll-like receptor 4 (TLR4) which consequently releases proinflammatory cytokines including IL-1β, TNFα, and IL-6 and mediators such as NO and PGE2 (Arulselvan et al., 2016). In a normal/resting state without the presence of any threat, the NF-κB subunit p65 is in the cytoplasm in an inactive form allied with an inhibitory protein, IκBα. On the other hand, when threat such as LPS is detected, a cascade reaction takes place that results in the phosphorylation and degradation of IκB. The complex degradation consequence is the release of the cytoplasmic NF-κB p65 to be translocated into the nucleus and binds to the enhancer elements of target genes thereby encouraging the transcription of the target proinflammatory genes (Muniandy et al., 2018). Ethanol reduced cytoplasmic p65 NF-κB while increased nuclear p65 NF-κB, indicating that ethanol stimulated nuclear translocation of p65 NF-κB. Ethanol also enhanced IκB-α phosphorylation and decreased the levels of IκB-α. These results indicated that ethanol exposure activated NF-κB signaling (Wang et al., 2015), when NF-κB activity is regulated by reactive oxygen species (ROS) (Pelucchi et al., 2011). Also, Wang et al. (2015) stated that ethanol may stimulate the NF-κB pathway by inducing ROS production. The present finding was corroborated with the reports of (Szabo et al., 2007) who reported that prolonged alcohol exposure resulted in an increase in NF-κB and TNFα production in response to TLR4 stimulation with LPS and (Yoo et al., 2018), who stated that exposure to ethanol significantly increased gastric NF-κB, Cox2, and inos mRNA expression levels.

In the current study the obtained qPCR results revealed a significant downregulation of Bax, IL-1β and NF-κB gene expression levels with significant upregulation of Bcl-2 gene expression level in stomach of ethanol-induced gastric ulcerative rats following administration of Proanthocyanidin either before or after induction of ulcer. The antioxidant effects of GSPE are seen in the blood vessels and tissue in the submucosal layer, which eventually suppresses the ischemia reperfusion injury caused by the inflammatory actions of the neutrophils and vascular local inflammation. This is assumed to suppress the progression of apoptosis and cellular damage. (Kim et al., 2013). The present finding was corroborated with the reports of (El-Shitany and Eid, 2017), who reported that Proanthocyanidin administration downregulated the expression of the apoptotic marker Bax, while upregulated the anti-apoptotic marker Bcl-2. Also, (Nazima et al., 2015) stated that GST treatment downregulated gene expression caspase-3, and Bax and upregulated Bcl-2 protein expression. Moreover, Liu et al., (2016) showed that PGSE treatment up-regulated the expression of anti-apoptotic protein Bcl-2 and down-regulated the expression of pro-apoptotic proteins Bax and p53, with a reduction of activity of caspase-3 in the liver of perfluorooctanoic acid (PFOA)-treated mice. Grape seed proanthocyanidin extract was shown to inhibit the NF-κB signaling pathway, reducing the expression levels of TNF-α, p-IKKα/β, p-IκBα, and the translocation of NF-κB to the nucleus of colonic epithelial cells (Li et al., 2011). GSPE-treated mice had fewer TLR4-expressing cells in their synovium in comparison to those that were not treated with GSPE. This suggests that GSPE inhibits TLR4 expression in vivo. TLR4 ligation leads to the activation of a downstream transcription factor, NF-κB, which is linked by MyD88, a key adaptor protein in TLR4-NF-κB signaling (Kim et al., 2018). The authors also showed that GSPE treatment diminishes the phosphorylation of IκBα and inhibits nuclear translocation of p65 and p50 NF-κB subunits. The results indicate that GSPE inhibits NF-κB activation through the suppression of IκBα phosphorylation in addition to MyD88. (Rajput et al., 2019) also reported that GSPE blocked the phosphorylation of NF-κB and the degradation of IκBα protein, which was the primary protein to activate NF-κB. Moreover, (Hu et al., 2019) showed lower levels of IKKα, IKKβ, NF-κBp65, and NF-κBp50 mRNA and protein in the groups pretreated with GSPE with higher levels of IκB-α than that in the arsenic groups.

5. CONCLUSION

These findings suggest that oral treatment with Proanthocyanidin shows a significant gastroprotective effects in ethanol induced gastric erosion models confirmed by antioxidant and anti-inflammatory activities. Also, the gastroprotective effect of Proanthocyanidin might be mediated by adjustment of inflammatory mediators, apoptosis and increasing antioxidants as well as attenuating oxidant/antioxidant imbalance. Moreover, Proanthocyanidin administration may have the potential as an alternative treatment for gastric ulcer because of its cytoprotective and anti-inflammatory effects.

6. REFERENCES

1. Abbas, A.M. and Sakr, H.F. 2013. Effect of selenium and grape seed extract on indomethacin-induced gastric ulcers in rats. Journal of Physiology and Biochemistry, 69(3), 527–537.
2. Abdel wahab, S.I. 2013. Protective mechanism of gallic acid and its novel derivative against ethanol-induced gastric ulcero genesis: Involvement of immunomodulation markers, Hsp70 and Bcl-2-associated X protein. Int Immunopharmacol, 16 (2):296–305.
3. Abdel-Salam OME, Criminer J, Debrecceni A, Szolcsa ny J, Mo’zak G. 2001. Gastric mucosal integrity: gastric mucosal blood flow and microcirculation. Anoverview. J Physiol Paris 95(1–6):105–127.
4. Abdulla, M., Ahmed, K.A.-A., Al-Bayaty, F.H., Masood Y. 2010. Gastroprotective effect of Phyllanthus niruri leaf extract against ethanol-induced gastric mucosal injury in rats. Afr. J. Pharm. Pharmacol., 4 (5), 226-230.
5. Aebi, H. 1984. Methods Enzymol 105, 121–126.
6. Almasaudi SB, Abbas AT, Al-Hindi RR, El-Shitany NA, Abdel-Dayem UA, Ali SS, Saleh RM, et al. 2017. Manuka honey exerts antioxidant and anti-Inflammatory activities that promote healing of acetic acid-induced gastric ulcer in rats. Evid Based Complement Alternat Med 2017: 5413917.
7. Almasaudi, S.B., El-Shitany, N.A., Abbas, A.T., Abdel-dayem, U.A., Ali, S.S., Al Jaouni, S.K. and Harakeh, S. 2016. Antioxidant, Anti-inflammatory, and Antiulcer Potential of Manuka Honey against Gastric Ulcer in Rats. Oxidative Medicine and Cellular Longevity, –10.
8. Antonisamy P., Duraiapandian V., Aravinthan A., Al-Dhabi N.A., Ignacimuthu S., Choi K.C., Kim J.H. 2015. Protective Effects of Friedelin Isolated from Azima Tetractantha Lam. against Ethanol-Induced Gastric Ulcer in Rats and Possible Underlying Mechanisms. Eur. J. Pharmacol.750: 167–175.

9. Antonisamy, P.; Subash-Babu, P.; Albert-Baskar, A.; Alshatwi, A.A.; Aravinthan, A.; Ignacimuthu, S. 2016. Experimental study on gastrointestinal efficacy and mechanisms of luteolin-7-O-glucoside isolated from Ophiopogon mungos Linn, in different experimental models. J. Funct. Food, 25, 302–313.

10. Arab HH, Salama SA, Omar HA, Arafah ES, Maghrabi IA. 2015. Diosmin protects against ethanol-induced gastric injury in rats: Novel antioxidant actions. PLoS One;10(3): e0122417.

11. Arulselvan, P., Tan, W., Gothai, S.et al. 2016. “Anti-inflammatory potential of ethyl acetate fraction of Moringa oleifera in downregulating the NF-xB signaling pathway in lipopolysaccharide-stimulated macrophages,” Molecules, 31; 21(11):1452

12. Aysun, C., Leylagul, K., Ismaili, K., Sibel, H., Recep, S., Ahmet, O. et al. 2008. The effect of grape seed extract on radiation-induced oxidative stress in the rat liver. Turkey J. Gastroenterol. 19, 92–98

13. Bagchi D, Bagchi M, Stohs S, Ray SD, Sen CK, Preuss HG 2002. Cellular protection with proanthocyanidins derived from grape seeds. Ann NY Acad Sci 957: 260–270.

14. Bancroft, J.D. and Stevens, S.A., 1996. Theory and Practice of Histological Techniques. Churchill-Livingstone, New York. 435-470.

15. Beutler E., Duron O., Kelly MB. J. Lab Clin. Med. 1963, 61, 882

16. Bhattacharya S, Banerjee D, Bauri A., Bhattacharya S, Banerjee D, Bandyopadhyay SK. 2007. Healing property of the Piper betel phenol, allylpyrocatechol against inflammatory potential of ethyl acetate fraction of Moringa oleifera in downregulating the NF-κB signaling pathway. Experimental Biology and Medicine, 244(3), 213–226.

17. Ibrahim, I. A. A., Abdulla, M. A., Hajrezaie, M.et al., 2016. “The gastroprotective effects of hydroalcoholic extract of Momordica charantia against ethanol-induced gastric mucosal injuries in Sprague Dawley rats,” Drug Design, Development and Therapy 10, 93–105.

18. Kim, S.-H., Bang, J., Son, C.-N., Baek, W.-K. and Kim, J.-M. 2018. Grape seed proanthocyanidin extract ameliorates murine autoimmune arthritis through regulation of TLR4/MyD88/NF-κB signaling pathway. The Korean Journal of Internal Medicine, 33(3), 612–621.

19. Kim, T.H., Jeon, E.J., Cheung, D.Y., Kim, C.W., Kim, S.S., Park, S.-H., Han, S.W., Kim, M.J., Lee, Y.S., Cho, M.-L., Chang, J.H., Min, J.K. and Kim, J.I. 2013. Gastroprotective Effects of Grape Seed Proanthocyanidin Extracts against Nonsteroidal Anti-Inflammatory Drug-Induced Gastric Injury in Rats. Gut and Liver, 7(3), 282–289.

20. Koga T, Moro K, Nakamori K, et al. 1999. Increase of antioxidative potential of rat plasma by oral administration of proanthocyanidin-rich extract from grape seeds. J Agric Food Chem.; 47:1892–1897.

21. Konkurek PC, Brzozowski T, Konturek S, Pajdo R, Konturek J, et al. 1999. Apoptosis in gastric mucosa with stress-induced gastric ulcers. J Physiol Pharmacol. 1999 Jun; 50(2):211-25.

22. Kumar M, Gautam MK, Singh A, Goel RK. 2013. Healing effects of Musa sapientum var. Paradisiaca in diabetic rats with co-occurring gastric ulcer: Cytokines and growth factor by PCR amplification. BMC Complement Altern Med; 13:305.

23. Lee, D. H., Kim, D. H., Hwang, C. J. et al. 2015. Interleukin-32γ attenuates ethanol-induced liver injury by the inhibition of cytochrome P450 2E1 expression and inflammatory responses, Clinical Science 128(10), 695–706.

24. Fard, M. T., Arulselvan, P., Karthikeyan, G., Adam, S. K and Fukaruzi, S. 2015. Bioactive extract from Moringa oleifera inhibits the pro-inflammatory mediators in lipopolysaccharide stimulated macrophages, Pharmacognosy Magazine, 11 (44), 556.

25. Golbabapour S, Gwaram NS, Hassanarvish P, Hajrezaie M, Kamalideghan B, Abdulla MA, et al. 2013. Gastroprotective Effects of Ophiorrhiza mungos Linn. in different experimental models. J. Funct. Food, 25, 302–313.

26. Guzmán-Gómez, O., García-Rodríguez, R., Quevedo-Corona, I., Pérez-Pastén-Botía, R., Rivero-Ramírez, N., Ríos-Castro, E., Pérez-Gutiérrez, S., Pérez-Ramos, J., and Chamorro-Cevallos, G. 2018. Amelioration of Ethanol-Induced Gastric Ulcers in Rats Pretreated with Phycobiliproteins of Arthrospira (Spirulina) Maxima. Nutrients, 10(6), 763.

27. Hajrezaie M, Salehian N, Karimian H, Zadehfard Shams M, Batran RA, et al. 2015. Biochanin A Gastroprotective Effects in Ethanol-Induced Gastric Mucosal Ulceration in Rats. PLoS ONE 10(3): e0121529.

28. Hu, Y., Wei, M., Niu, Q., Ma, R., Li, Y., Wang, X., Feng, G., Li, S. and Pang, I. 2019. Grape seed proanthocyanidin extract alleviates arsenic-induced lung damage through NF-κB signaling. Experimental Biology and Medicine, 244(3), 213–226.

29. Kim, S.-H., Bang, J., Son, C.-N., Baek, W.-K. and Kim, J.-M. 2018. Grape seed proanthocyanidin extract ameliorates murine autoimmune arthritis through regulation of TLR4/MyD88/NF-κB signaling pathway. The Korean Journal of Internal Medicine, 33(3), 612–621.

30. Kim, J.M., Park, S.-H., Han, S.W., Kim, M.J., Lee, Y.S., Cho, M.-L., Chang, J.H., Min, J.K. and Kim, J.I. 2013. Gastroprotective Effects of Grape Seed Proanthocyanidin Extracts against Nonsteroidal Anti-Inflammatory Drug-Induced Gastric Injury in Rats. Gut and Liver, 7(3), 282–289.

31. Konturek PC, Brzozowski T, Konturek S, Pajdo R, Konturek J, et al. 1999. Apoptosis in gastric mucosa with stress-induced gastric ulcers. J Physiol Pharmacol. 1999 Jun; 50(2):211-25.

32. Kumar M, Gautam MK, Singh A, Goel RK. 2013. Healing effects of Musa sapientum var. Paradisiaca in diabetic rats with co-occurring gastric ulcer: Cytokines and growth factor by PCR amplification. BMC Complement Altern Med; 13:305.

33. Lee, D. H., Kim, D. H., Hwang, C. J. et al. 2015. Interleukin-32γ attenuates ethanol-induced liver injury by the inhibition of cytochrome P450 2E1 expression and inflammatory responses, Clinical Science 128(10), 695–706.

34. Li, W., Wang, X., Zhang, H., He, Z., Zhi, W., Liu, F., Wang, Y. and Niu, X. 2016. Anti-ulcerogenic effect of cavidine against ethanol-induced acute gastric ulcer in mice and possible underlying mechanism. International Immunopharmacology, 38, 450-459.

35. Li, X., Yang, X., Cai, Y., Qin, H., Wang, L., Wang, Y., Huang, Y., Wang, X., Yan, S., Wang, L., Zhao, X., Li, W., Li, S., Chen, J. and Wu, Y. 2011. Proanthocyanidins
from Grape Seeds Modulate the NF-κB Signal Transduction Pathways in Rats with TNBS-Induced Ulcerative Colitis. Molecules, 16(8), 6721–6731.
38. Liu Y, Tian X, Gou L, Fu X, Li S, Lan N, et al. 2012. Protective effect of 1-citirulline against ethanol-induced gastric ulcer in rats. Environ Toxicol Pharmacol. 34(2): 280–287.
39. Liu, J., Wang, J., Shi, Y., Su, W., Chen, J., Zhang, Z., Wang, G. and Wang, F. 2017. Short Chain Fatty Acid Acetate Protects against Ethanol-Induced Gastric Mucosal Lesion in Mice. Biological & Pharmaceutical Bulletin, 40(9), 1439–1446.
40. Liu, W., Xu, C., Sun, X., Kuang, H., Kuang, X., Zou, W., Yang, B., Wu, L., Liu, F., Zou, T. and Zhang, D. 2016. Grape seed proanthocyanidin extract protects against perfluorooctanoic acid-induced hepatotoxicity by attenuating inflammatory response, oxidative stress and apoptosis in mice. Toxicology Research, 5(1), 224–234.
41. Luo XJ, Liu B, Dai Z, Li TR, Li NS, Zhang XJ, et al. 2013. Expression of apoptosis-associated microRNAs in ethanol-induced acute gastric mucosal injury via JNK pathway. Alcohol.47(6):481–493.
42. Mei X, Xu D, Xu S, Zheng Y, Xu S. 2012. Novel role of Zn (II)-curcumin in enhancing cell proliferation and adjusting proinflammatory cytokine-mediated oxidative damage of ethanol-induced acute gastric ulcers. Chem Biol Interact 197: 31-39.
43. Muniandy, K., Gohthai, S., Badran, K.M.H., Suresh Kumar, S., Esa, N.M. and Arulselvan, P. 2018. Suppression of Proinflammatory Cytokines and Mediators in LPS-Induced RAW 264.7 Macrophages by Stem Extract of Alternanthera sessilis via the Inhibition of the NF-κB Pathway. Journal of Immunology Research, 2018, pp.1–12.
44. Nazima, B., Manoharan, V. and Miltonprabu, S. 2015. Grape seed proanthocyanidins ameliorates cadmium-induced renal injury and oxidative stress in experimental rats through the up-regulation of nuclear related factor 2 and antioxidant responsive elements. Biochemistry and Cell Biology, 93(3), 210–226.
45. Ohkawa, H., Ohishi W, and Yagi K. Anal. Biochem. 1979. 95, 351.
46. Park SW, Oh TY, Kim YS, Sim H, Park SJ, Jung EL, et al. 2008. Artemisia asiatica extracts protect against ethanol-induced injury in gastric mucosa of rats. J Gastroenterol Hepatol,23(6):976–984.
47. Paulayer, A., Adithan, A., Lee, J., Moon, K., Kim, D., Im, S., Kang, C.-W., Kim and N, J.H. 2017. Aronia melanocarpa (Black Chokeberry) Reduces Ethanol-Induced Gastric Damage via Regulation of HSP-70, NF-κB, and MCP-1 Signaling. International Journal of Molecular Sciences, 18(6), 1195.
48. Pelucchi C, Tramaceare I, Boffetta P, Negri E, La Vecchia C. 2011. Alcohol consumption and cancer risk. Nutr Cancer.63(7):893–90, doi: 10.1080/01635581.2011.596642.
49. Rajput, S., Zhang, C., Feng, Y., Wei, X., Khalil, M., Rajput, I., Baloch, D., Shaukat, A., Rajput, N., Qamar, H., Hassan, M. and Qi, D. 2019. Proanthocyanidins Alleviates AflatoxinB1-Induced Oxidative Stress and Apoptosis through Mitochondrial Pathway in the Bursa of Fabricius of Broilers. Toxins.
50. Rezaie, A. R. D. Parker, and M. Abdollahi. 2007. “Oxidative stress and pathogenesis of inflammatory bowel disease: an epiphenomenon or the cause?” Digestive Diseases and Sciences 52 (9): 2015–2021.
51. Rozza AL, Meira de Faria F, Souza Brito AR, Pellizzon CH. 2014. The gastroprotective effect of menhool: involvement of anti-apoptotic, antioxidant and anti-inflammatory activities. PLOS ONE 9, e86686.
52. Saito M, Hosoyama H, Ariga T, Kataoka S, Yamaji N 1998. Antilucret activity of grape seed extract and procyanidins. J Agric Food Chem 46: 1460-1464.
53. Shi J, Yu J, Pohryl JE, Kakud Y. 2003. Polyphenolics in grape seed biochemistry and functionality. J Med Food 6: 291-299.
54. Mary SJ and Merina AJ. 2015. Gastroprotective Effect of Guttarda speciosa against Ethanol Induced Gastric Ulcer in Rats. Medicinal & Aromatic Plants, 05(01):1-3.
55. Szabo, G., Mandrekar, P., Oak, S. and Mayerle, J. 2007. Effect of Ethanol on Inflammatory Responses. Pancreatology, 7(2–3), 115–123.
56. Wang, F., Yang, J.-L., Yu, K., Xu, M., Xu, Y., Chen, L., Lu, Y., Fang, H., Wang, X., Hu, Z., Li, F., Kan, L., Luo, J. and Wang, S.-Y. 2015. Activation of the NF-κB pathway as a mechanism of alcohol enhanced progression and metastasis of human hepatocellular carcinoma. Molecular Cancer, 14(1), p.10.
57. Wang, J.-W., Chen, X.-Y., Hu, P.-Y. et al. 2016. “Effects of linderae radix extracts on a rat model of alcoholic liver injury.” Experimental and Therapeutic Medicine 11 (6): 2185–2192.
58. Wang, Z., Luo, H., & Xia, H. 2018. Theflavins attenuate ethanol-induced oxidative stress and cell apoptosis in gastric mucosa epithelial cells via downregulation of the mitogen-activated protein kinase pathway. Molecular Medicine Reports. doi: 10.3892/mmr.2018.9352.
59. Wong J.-Y., M. A. Abdulla, J. Raman et al. 2013. “Gastroprotective Effects of Lion’s Mane Mushroom Hericium erinaceus (Bull.Fr.) Pers. (Aphyllophoromycetidae) Extract against Ethanol-Induced Ulcer in Rats,” Evidence-Based Complementary and Alternative Medicine, vol. 2013, Article ID 492976, 9 pages.
60. Wren AF, Cleary M, Frantz C, Melton S, Norris L. 2002. 90-day oral toxicity study of a grape seed extract (IH636) in rats. J Agric Food Chem 50: 2180.
61. Wu, X., Huang, Q., Xu, N., Cai, J., Luo, D., Zhang, Q., Su, Z., Gao, C. and Liu, Y. 2018. Antioxidative and Anti-Inflammatory Effects of Water Extract of Acrostichum aureum Linn. against Ethanol-Induced Gastric Ulcer in Rats. Evidence-Based Complementary and Alternative Medicine, 2018, pp.1-10.
62. Ye HH, Wu KJ, Fei SJ, Zhang WX, Liu HX, Zhang JL, Zhang YM. 2013. Propofol participates in gastric mucosal protection through inhibiting the toll-like receptor-4/nuclear factor kappa-B signaling pathway. Clin Res Hepatol Gastroenterol.; 37(1): 3–15.
63. Ye X, Krohn RL., Liu W, Joshi SS, Kuszyski CA. 1999. The cytotoxic effects of a novel IH636 grape seed proanthocyanidin extract on cultures human cancer cells. Mol Cell Biochem 196: 99–108.
64. Yoo, J., Lee, J., Lee, Y., Xu, S. and Lee, H. 2018. Protective effect of bovine milk against HCl and ethanol–induced gastric ulcer in mice. J Dairy Sci 101(3):3758-3770.
65. Zheng, H., Chen, Y., Zhang, J., Wang, L., Jin, Z., Huang, H., Man, S. and Gao, W. 2016. Evaluation of protective effects of costunolide and dehydrocostuslactone on ethanol-induced gastric ulcer in mice based on multi-pathway regulation. Chemico-Biological Interactions, 250: 68–77.