Gastroprotective Effects of Pear (Pyrus Communis L.) Extract on Ethanol Induced Gastric Ulcer in Rats

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INTRODUCTION

Peptic ulcer is a serious health condition worldwide. 1 Peptic ulcer can be observed in esophagus, stomach or small intestine which is characterized with irritating symptoms such as heartburn, nausea, vomiting or bloating. 2 Acute gastric ulcer is often initiated with excessive alcohol consumption or high doses of nonsteroidal anti-inflammatory drug (NSAID) usage. 3,4 Ethanol not only directly damages the gastric mucosa, but also sensitizes the mucosa against injury. 5 Ethanol can induce gastric ulcer through different ways. Ethanol increases the generation of reactive oxygen species (ROS) by enhancing the cytochrome P450 enzyme activity and changing the levels of certain materials. 6 In addition, it has been stated that some inflammatory cytokines can play crucial roles in acute phase inflammation as well as in maintaining and regulating the severity of gastric ulcers. Over-expression and translocation of nuclear factor kappa-B (NF-kB) subunits promote the upregulation of pro-inflammatory mediators. For this reason, inhibition of NF-kB activity can alleviate the severity of inflammatory diseases such as gastric ulcer. 5,7,8 Various agents were examined against gastric ulcer in previous studies. 9 Pyrus communis L. is within Rosaceae family and grows widely around the world. 10 PYR tree is common in Turkey and Europe. Fruits are yellowish green color and 2-4 cm long. 11,12 Phenolic molecules are one of the major active ingredients in PYR and the anti-ulcer properties of phenolics on PYR has also been stated. Initial studies have shown that the main phenolics in PYR are leukocyanidine, quercitrin, catechin, chlorogenic acid, epicatechin and quercetin. 13 Many researchers have shown that phytochemicals in fruits and vegetables are important for the against chronic diseases including obesity, diabetes mellitus, cardiovascular diseases and cancer. 1,4,7,14,15 Here, it was investigated the...
beneficial effects of PYR on gastric ulcer through ethanol-induced gastric ulcer model in rats.

MATERIALS and METHODS

Animals
Wistar albino rats (female, 250-280 g) were procured from Atatürk University Medical Experimental Application and Research Center (Erzurum, Turkey) and experimental studies were carried out in this center. All the procedures carried out in this study were carried out in line with the permission obtained from Atatürk University Animal Experiments Local Ethics Committee (Protocol no:19.04.2016/70). All rats were exposed to a 12 hours/12 hours light/dark cycle in rooms with constant temperature and humidity control. Rats were free access to water and food.

Groups and drug administration
There were 4 groups composed of 32 female Wistar (n=8) as group I (sham group), group II (ulcer group), group III and IV (PYR 4 ml/kg and PYR 8 ml/kg groups). In group I, the animals were administered normal saline by oral gavage. In other groups, 5 ml/kg 99% ethanol (absolute ethanol) (Sigma-aldrich, USA) was administered to animals by oral gavage to establish ulcer model as described in previous studies 16,17. All interventions in groups lasted ten days. On the eleventh day of the study, the animals were kept away from food for 8 hours, but they were allowed access to water. After 90 minutes, animals were sacrificed, gastric tissues were removed and examined to determine gastric lesions.

Plant material
The pears (Pyrus communis L.) used in the research belong to Santa Maria cultivar and obtained from Goksun district Bursa province of Turkey. Pears were harvested in July and stored in controlled atmospheric warehouses then served to the market in August. Fresh pear fruits were washed and cleaned then cut into small pieces and their seeds were removed. A homogenizer was used to extract pulpy pear juices 18,19.

HPLC analysis of PYR profile
Phenolic profiles of PYR were evaluated by HPLC coupled to a photodiode array (HPLC-PDA). HPLC-PDA results of PYR sample were given as mg/100 mL samples for all. Standard calibration curves were prepared by using gallic acid, 4-hydroxy benzoic acid, caffeic acid, vanillic acid, catechin, p-coumaric acid, chlorogenic acid, ferulic acid, syringic acid, delphinidin-3-glucosidase and cyanidin-3-glucosidase. These samples and stock solutions were filtered through a 0.45-µm membrane filter and 1 mL of the filtered sample was placed into vials and analyzed in a Waters W600 HPLC system with PDA (Waters 996) detector, for each sample. Luna C18 column (Phenomenex, Utrecht, The Netherlands), heated to 40 °C, was used as the stationary phase. Chromatograms were recorded at 280, 312, 360, and 520 nm. Identification was based on the retention times and characteristic UV spectra and quantification was done by external standard curves 20.

Spectrophotometric assays (evaluation of the content of PYR)
Free radical clearance activity was evaluated with 2,2-azino-di-(3-ethylbenzothiazoline-sulphonic acid (ABTS). ABTS activity measurement was modified according to previous studies 21. Antioxidant features of PYR content was evaluated with cupric reducing antioxidant capacity (CUPRAC) analysis 22. Total phenolic content was analyzed with Folin–Ciocalteu reagent (FCR) and the method developed by Folin and Singleton 23,24.

Immunohistochemical examination
Gastric tissues were cut along the large curvature, washed with saline and photographed. After the imaging process was completed, the tissues were placed in a 10% formalin (Sigma-aldrich, USA) solution and fixed. Then, they were embedded in paraffin and 5 µm sections were taken with microtome (Leica RM2235, Germany). Immunohistochemical (IHC) and hematoxylin and eosin (H&E) staining were carried out. IHC staining was done using Caspase-3 (Novus Biological, USA) and NF-κB (Abcam, England) antibodies. The samples were examined under light microscope (Olympus BH-40, Japan).

Statistical analysis
Statistical analysis was done using SPSS v.20.0 software (SPSS Inc., USA). The treatment groups were compared with ulcer group. One-way analysis of variance (ANOVA) and Tukey post hoc test were performed. Statistical significance was accepted as p<0.05. All data were expressed as mean±standard deviation (SD).
RESULTS

**HPLC Results**

Determination of the phenolic compounds of the PYR content was measured using HPLC and the results were given as average (ppm) ± SD in table 1.

| Phenolic Substances                  | Value (ppm) (Average ± SD) |
|--------------------------------------|-------------------------------|
| Gallic acid                          | 5,24 ±0,15                    |
| 4-Hydroxybenzoic                     | 10,94 ±0,89                   |
| Catechin                             | 34,10 ±1,64                   |
| Vanillic acid                        | 0,91 ±0,07                    |
| Syringic acid                        | 2,93 ±0,31                    |
| Arbutin                              | 0,33 ±0,06                    |
| Isorhamnetin 3-o-rutinoside          | 31,92 ±4,16                   |
| Abscisic acid                        | 25,65 ±5,40                   |
| p-coumaric acid                     | 1,55 ±0,01                    |
| Chlorogenic acid                     | 0,49 ±0,06                    |
| Caffeic acid                         | 0,16 ±0,03                    |
| Rutin                                | 0,44 ±0,06                    |

Table 1. PYR phenolic ingredient contents (average±SD)

**Antioxidant Properties of PYR**

The antioxidant capacity of PYR samples was found out according to two different procedures (CUPRAC and ABTS). The total phenolic content was detected according to the Folin Ciocalteu Reactive (FCR) method. All values were demonstrated in table 2.

| Analysis                           | Value (Average ± SD) |
|------------------------------------|----------------------|
| CUPRAC (mg TEAC/100ml)             | 4,46 ± 2,18          |
| ABTS (mg TEAC/100ml)               | 239,96 ± 15,25       |
| FCR (mg GAE/100 ml)                | 11,51 ± 0,56         |

Table 2. CUPRAC, ABTS and FCR values (average±SD)

**Histopathological and Immunohistochemical Assessment**

In figure 1, macroscopic and histopathological views of stomach samples of all groups were presented. Hemorrhagic and ulcerative lesions were not observed in the control group. In ulcer group, hemorrhagic ulceration lesions were observed. Serious erosion with hemorrhagic lesions extending deep into the mucosa was demonstrated in histological evaluation of the stomach. Additionally, histopathological findings such as widespread edema and leukocyte infiltration were observed. On the other side, mucosal damage decreased in PYR groups compared to ulcer group. Decline in mucosal damage was supported by decreased ulcer area, edema and leukocyte Infiltration.

In immunohistochemical evaluation of caspase-3 and NF-κB immunopositivity, the group with ulcer had higher immunopositivity compared to sham group. Moreover, PYR treatment groups demonstrated lower immunopositivity compared to ulcer group. The most significant difference was noted in the groups in which 8ml/kg PYR was applied.

**DISCUSSION**

Peptic ulcer appears due to disruption/loss of the mucosal integrity. The main cause of mucosal damage is the disruption of the balance between mucous protective and aggressive mechanisms. Ethanol-induced gastric ulcer leads to inflammatory response which is characterized with increased neutrophil infiltration, thereby disrupting the oxidant/antioxidant balance. Ethanol injury begins with microvascular damage including edema formation, surface epithelium disruption and necrotic lesions which penetrate deep into the mucosa. It can also lead to vascular permeability and even cell lysis. Ethanol-induced gastric ulcer in experimental animals is one of the most common ulcer models examining various compounds for determining antiulcer effects. The current study revealed that PYR has preventive activity against ethanol-induced gastric ulcer. We detected several phenolic compounds from PYR via HPLC measurements. Direct and indirect effects of some of these molecules on gastric ulcer have been investigated. Gallic acid, arbutin, isorhamnetin 3-o-rutinoside, p-coumaric acid, caffeic acid, chlorogenic acid, routine and catechin molecules have been reported to mediate antiulcer mechanisms in experimental ulcer models. Protective effects of p-coumaric acid, gallic acid and chlorogenic acid were examined in previous studies. In addition, ABTS and CUPRAC values indicate that the PYR extract contains powerful antioxidant compounds.

NF-κB is an important transcription factor involved in the inflammatory response process and production of several cytokines. NF-κB is activated in gastric ulcer, promoting the production of a number of pro-inflammatory cytokines. Suppression of the NF-κB pathway is considered a target for gastric ulcer treatment. The reduction of NF-κB
immunoreactivity by PYR administration may show that it has gastroprotective effects by decreasing cytokine production.

Apoptosis cascade is a significant pathway mediating ethanol-induced gastric ulcer, which is also associated with inflammatory response and oxidative stress. In this regard, ethanol-induced gastric ulcer enhances caspase-3 expression. Caspase-3 inhibitory effects of PYR were presented in current data. Although the decline in caspase-3 expression can be attributed to PYR, the main responsible molecules for this decrease are phenolic compounds in the extract content.

As a result, especially 8ml/kg PYR extract contributes more to the preservation of mucosal integrity, decreases NF-κB and caspase-3 expression, and exerts antioxidant effects in ethanol-induced gastric ulcer and exhibits gastroprotective effect. Thus, new studies will be necessary to evaluate PYR as an anti-ulcer drug.

Conflict of interest
The authors declare that they have no conflict of interest.

Figure 1. Macroscopic and histopathological images of stomach tissues A) sham, B) ulcer C) PYR 4 ml/kg and D) PYR 8 ml/kg.
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