Int J Acad Med Pharm, 2021; 3 (1); 1-6

International of Academic Medicine and Pharmacy

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

Ersen Eraslan [1*], Ayhan Tanyeli [2], Mehmet Ramazan Bozhüyük [3], Mustafa Can Güler [4], Erdem Toktay [5], Nezahat Kurt [6], Gülay Özkan [7], Esra Çapanoğlu Güven [8]

[1] Department of Physiology, Faculty of Medicine, Yozgat Bozok University, Yozgat, Turkey
[2,4] Department of Physiology, Faculty of Medicine, Atatürk University, Erzurum, Turkey
[3] Department of Horticulture, Faculty of Agriculture, Iğdır University, Iğdır, Turkey
[5] Department of Histology and Embryology, Faculty of Medicine, Kafkas University, Kars, Turkey
[6] Department of Biochemistry, Faculty of Medicine, Erzincan Binali Yıldırım Üniversitesi, Erzincan, Turkey
[7,8] Department of Food Engineering, Chemical and Metallurgical Engineering Faculty, Istanbul Technical University, Istanbul, Turkey

ORCID; 0000-0003-2424-2269, 0000-0002-0095-0917, 0000-0001-5021-6019, 0000-0001-8588-1035, 0000-0002-7447-6023, 0000-0002-1685-5332, 0000-0002-6375-1608, 0000-0003-0335-9433

Article info **Research article**

Received :21.07.2020
Received in revised form :29.09.2020
Accepted :02.11.2020
Available online :05.01.2021

Keywords

Pear
Ethanol
Ulcer
HPLC
NF-kB
Caspase-3
Rat

Abstract; Various natural molecules have been examined against ethanol-induced gastric ulcer up to present. Pear, *Pyrus communis* L. (PYR), includes various antioxidant and anti-inflammatory features. Current study was planned to find out potential preventive properties of PYR extract against gastric ulcer induced by ethanol in rats. 32 rats were assigned to 4 groups (n=8). Group I was sham, group II was ulcer group. Groups III and IV were 4 and 8 ml/kg PYR groups. Group I, normal saline was administered and group II, III and IV were administered ethanol (5 ml/kg) by oral gavage to rats. Phenolic substances in PYR content were detected via high-pressure liquid chromatography (HPLC). CUPRAC, ABTS and FCR amounts of PYR extract were determined. Gastric tissue was evaluated through macroscopic and immunohistochemical methods. NF-kB and caspase-3 expressions increased in ulcer group but PYR treatment reversed these levels. PYR extract performed protective effects against ethanol-induced gastric ulcer by decreasing NF-kB and caspase-3 expressions and preventing gastric mucosal injury. As a result, PYR extract has been shown to have a strong therapeutic effect against gastric ulcer. Therefore, we propose PYR as a potential antiulcer drug.

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

*Corresponding author: Ersen Eraslan, E-mail; ersen.eraslan@bozok.edu.tr, http://dx.doi.org/10.29228/jamp.45434

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-ethylbenzothialozine-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.

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

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

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.

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

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

Trolox equivalent antioxidant capacity (TEAC), Gallic acid equivalents (GAE).

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 [25]. Ethanol-induced gastric ulcer leads to inflammatory response which is characterized with increased neutrophil infiltration, thereby disrupting the oxidant/ antioxidant balance [26]. 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 [27]. Ethanol-induced gastric ulcer in experimental animals is one of the most common ulcer models examining various compounds for determining antiulcer effects [16]. 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 [28-36]. Protective effects of p-coumaric acid [37-39], gallic acid [40] and chlorogenic acid [41,42] were examined in previous studies. In addition, ABTS and CUPRAC values indicate that the PYR extract contains powerful antioxidant compounds.

NF-kB 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 [43,44]. 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 [45]. In this regard, ethanol-induced gastric ulcer enhances caspase-3 expression [16]. 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.

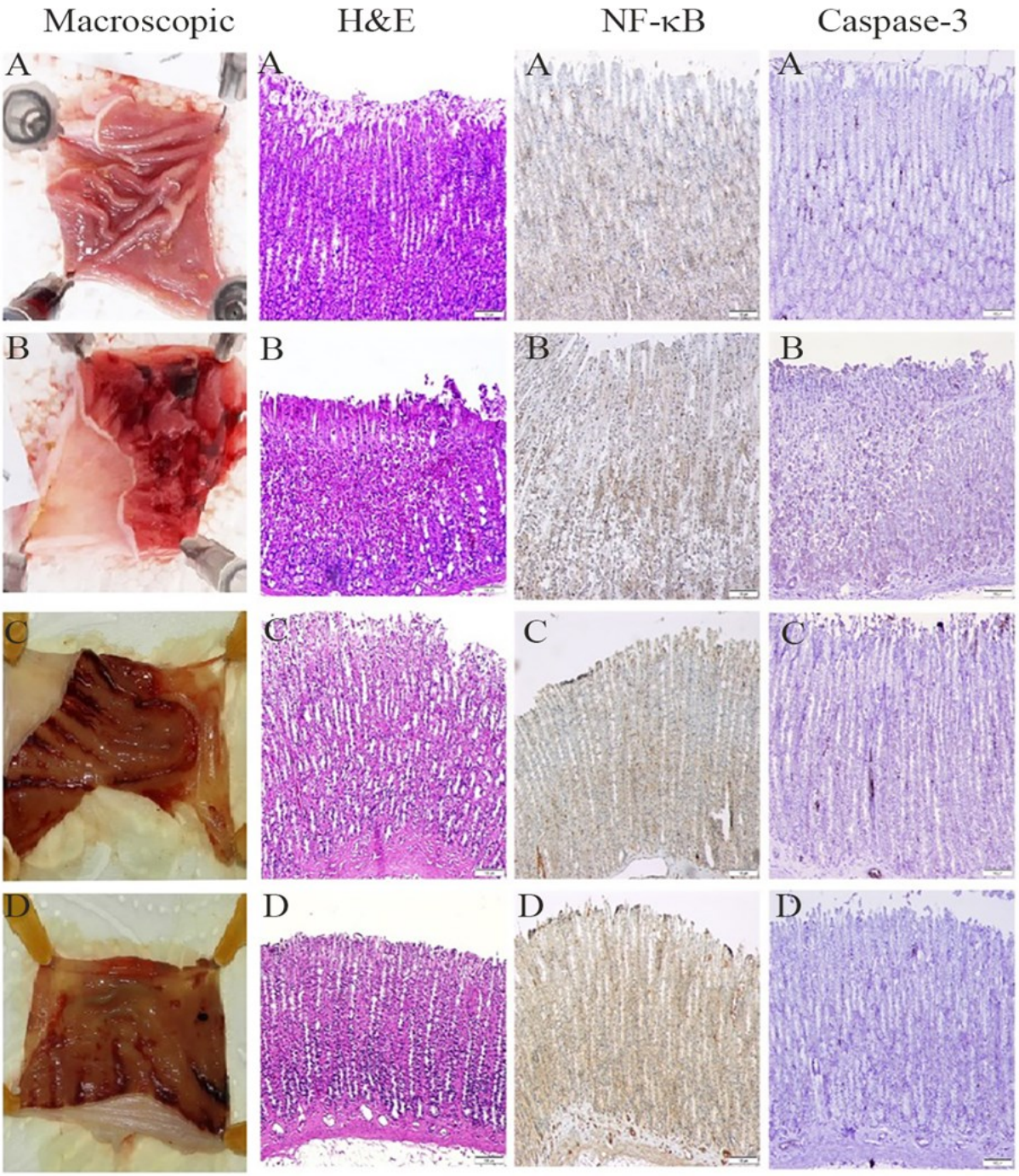

Figure 1. Macroscopic and histopathological images of stomach tissues **A)** sham**, B)** ulcer **C)** PYR 4 ml/kg and **D)** PYR 8 ml/kg.

REFERENCES

1. Repetto MG, Llesuy SF. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. *Brazilian Journal of Medical and Biological Research*. 2002;35(5):523-34.
2. Syam AF, Sadikin M, Wanandi SI, Rani AA. Molecular mechanism on healing process of peptic ulcer. *Acta Medica Indonesiana*. 2009;41(2):95-8.
3. Choi YJ, Kim N, Lee JY, Nam RH, Chang H, Seo JH, et al. Protective effects of garlic extract, PMK-S005, against nonsteroidal anti-inflammatory drugs-induced acute gastric damage in rats. *Digestive Diseases and Sciences* 2014;59(12):2927-34.
4. Paulrayer A, Adithan A, Lee JH, Moon KH, Kim DG, Im SY, et al. Aronia melanocarpa (Black Chokeberry) reduces ethanol-induced gastric damage via regulation of HSP-70, NF-kappaB, and MCP-1 signaling. *International Journal of Molecular Sciences.* 2017;18(6).
5. Antonisamy PAA, Jeong HL, Kwang HM, Dae GK, So YI, Chang-Won K, Nam S K and Jong-Hoon K. Experimental study on gastroprotective efficacy and mechanisms of luteolin-7-O-glucoside isolated from Ophiorrhiza mungos Linn. in different experimental models. *Journal of Functional Foods*. 2016;25:302-13.
6. Sid B, Verrax J, Calderon PB. Role of oxidative stress in the pathogenesis of alcohol-induced liver disease. *Free Radical Research*. 2013;47(11):894-904.
7. Choi JI, Raghavendran HR, Sung NY, Kim JH, Chun BS, Ahn DH, et al. Effect of fucoidan on aspirin-induced stomach ulceration in rats. *Chemico-Biological Interactions*. 2010;183(1):249-54.
8. Chen T, Mou Y, Tan J, Wei L, Qiao Y, Wei T, et al. The protective effect of CDDO-Me on lipopolysaccharide-induced acute lung injury in mice. *International Immunopharmacology*. 2015;25 (1):55-64.
9. Eraslan E, Tanyeli A, Güler MC, Kurt N, Yetim Z. Agomelatine prevents indomethacin-induced gastric ulcer in rats. *Pharmacological Reports*. 2020.
10. Hahashi AAD, S. Abdollahi, H. Kermani, M. J. Comparing vacuum agroinoculation in two pear (Pyrus communis L.) cultivars “Bartlett” and “Harrow Delight”. *Annals Biology*. 2012;3:3200−7.
11. Davis PH. Flora of Turkey and the East Aegean Islands. Edinburgh, Edinburgh University Press. 1972;4:135-6/62-63.
12. Kiymet G, Yucel E, Cetinta F. Antimicrobial activities of fruits of Crataegus and Pyrus species. *Pharmaceutical Biology*. 2006;4:79-83.
13. Hamauzu Y Forest F, Hiramatsu K, Sugimoto M. Effect of pear (Pyrus communis L.) procyanidins on gastric lesions induced by HCl/ethanol in rats. *Food Chemistry*. 2007;100:255–63.
14. Albaayit SF, Abba Y, Abdullah R, Abdullah N. Prophylactic effects of Clausena excavata Burum. f. leaf extract in ethanol-induced gastric ulcers. Drug Design, *Development and Therapy.* 2016;10:1973-86.
15. De Sales IRP, Formiga RO, Machado FDF, Nascimento RF, Pessoa MMB, Barros M. Cytoprotective, antioxidant and anti-inflammatory mechanism related to antiulcer activity of Cissampelos sympodialis Eichl. in animal models. *Journal of Ethnopharmacology*. 2018;222:190-200.
16. Tanyeli A, Eraslan E, Polat E, Bal T. Protective effect of salusin-alpha and salusin-beta against ethanol-induced gastric ulcer in rats. *Journal of Basic and Clinical Physiology and Pharmacology*. 2017;28(6):623-30.
17. Colak AM, Kupe M, Bozhuyuk MR, Ercisli S, Gundogdu M. Identification of some fruit characteristics in Wild Bilberry (Vaccinium myrtillus L.) Accessions from Eastern Anatolia. *Gesunde Pflanzen*. 2018;70(1):31-8.
18. Mehmet RB, Mücahit P, Tuncay K, Berna D. Organic acid composition of selected Mulberry Genotypes from Aras Valley. *Atatürk University Journal of Agricultural Faculty*. 2015;46(2):69-74.
19. Capanoglu E, de Vos RC, Hall RD, Boyacioglu D, Beekwilder J. Changes in polyphenol content during production of grape juice concentrate. *Food Chemistry*. 2013;139(1-4):521-6.
20. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*. 1999;26 (9-10):1231-7.
21. Celik SE, Ozyurek M, Guclu K, Apak R. Determination of antioxidants by a novel on-line HPLC-cupric reducing antioxidant capacity (CUPRAC) assay with post-column detection. *Analytica Chimica Acta.* 2010;674(1):79-88.
22. Folin OC, Polin O. On tyrosine and tryptophane determinations in proteins. *Journal of Biological Chemistry*. 1927;73:627-50.
23. Singleton VL, Joseph AR. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Society for Enology and Viticulture*. 1965(16):144-158.
24. Farzaei MH, Rahimi R, Abbasabadi Z, Abdollahi M. An Evidence-based review on medicinal plants used for the treatment of peptic ulcer in traditional Iranian medicine. *International Journal of Pharmacology*. 2013;9(2):108-24.
25. Szabo S, Trier JS, Frankel PW. Sulfhydryl compounds may mediate gastric cytoprotection. *Science*. 1981;214(4517):200-2.
26. Abdelwahab SI, Mohan S, Abdulla MA, Sukari MA, Abdul AB, Taha MM, et al. The methanolic extract of Boesenbergia rotunda (L.) Mansf. and its major compound pinostrobin induces anti-ulcerogenic property in vivo: possible involvement of indirect antioxidant action. *Journal of Ethnopharmacology*. 2011;137(2):963-70.
27. Taha MME, Salga MS, Ali HM, Abdulla MA, Abdelwahab SI, Hadi AHA. Gastroprotective activities of Turnera diffusa Willd. ex Schult. revisited: Role of arbutin. *Journal of Ethnopharmacology*. 2012;141(1):273-81.
28. Galati EM, Mondello MR, Giuffrida D, Dugo G, Miceli N, Pergolizzi S, et al. Chemical characterization and biological effects of Sicilian Opuntia ficus indica (L.) Mill. fruit juice: Antioxidant and antiulcerogenic activity. *Journal of Agricultural and Food Chemistry*. 2003;51(17):4903-8.
29. Kim S, Hong I, Woo S, Jang H, Pak S, Han S. Isolation of abscisic acid from Korean acacia honey with anti-helicobacter pylori activity. *Pharmacognosy Magazine*. 2017;13(50):S170-S3.
30. Panda V, Suresh S. Gastro-protective effects of the phenolic acids of Macrotyloma uniflorum (horse gram) on experimental gastric ulcer models in rats. *Food Bioscience*. 2015;12:34-46.
31. Souza MO, Fernando PB, Gushiken LFS, Pellizzon CH. Evaluation of the gastroprotective and antioxidant effects of caffeine and caffeic acid on ethanol induced gastric ulcer. *JSM Hepatitis*. 2017;2(1):1008-13.
32. Abdel-Raheem IT. Gastroprotective effect of rutin against indomethacin-induced ulcers in rats. *Basic & Clinical Pharmacology & Toxicology*. 2010;107(3):742-50.
33. Siddaraju MN, Dharmesh SM. Inhibition of gastric H+, K+-ATPase and helicobacter pylori growth by phenolic antioxidants of zingiber officinale. *Molecular Nutrition & Food Research*. 2007;51(3):324-32.
34. Zhou D, Yang Q, Tian T, Chang Y, Li Y, Duan LR, et al. Gastroprotective effect of gallic acid against ethanol-induced gastric ulcer in rats: Involvement of the Nrf2/HO-1 signaling and anti-apoptosis role. *Biomedicine & Pharmacotherapy* 2020;126:110075.
35. Hamaishi K, Kojima R, Ito M. Anti-ulcer effect of tea catechin in rats. *Biological and Pharmaceutical Bulletin*. 2006;29(11):2206-13.
36. Tanyeli A, Ekinci AFN, Eraslan E, Güler MC, Özbek SS, Gülçin İ. Role of p-Coumaric acid in alleviating of the intestinal ischemia/reperfusion injury. *Kocaeli Medical Journal*. 2020;9(1):166-73.
37. Erdoğan Güzel D, Tanyeli A. Protective Effect of p-Coumaric acid as free oxygen radical scavenger in experimental renal ischemia-reperfusion model. *Sakarya Medical Journal*. 2018;8(3):625-31.

38. Erdoğan GD, Tanyeli A. p-Coumaric acid reduces renal ischemia reperfusion-induced acute lung injury. *Sakarya Medical Journal*. 2018;8(3):644-9.
39. Ekinci AFN, Yildirim S, Kandemir FM, Tanyeli A, Küçükler S, Bahaeddin DM. Protective effects of gallic acid on doxorubicin-induced cardiotoxicity; an experimantal study. *Archives of Physiology and Biochemistry*. 2019:1-8.
40. Tanyeli A, Erdoğan GD. Investigation of chlorogenic acid (Cga) as an antioxidant in renal ischemia-reperfusion injury: An experimental study. *Sakarya Medical Journal*. 2018;8(2):410-5.
41. Erdoğan Güzel D, Tanyeli A. p-Coumaric acid reduces renal ischemia reperfusion-induced acute lung injury. *Sakarya Medical Journal.* 2018;8(3):644-9.
42. Li WF, Huang HM, Niu XF, Fan T, Mu QL, Li HN. Protective effect of tetrahydrocoptisine against ethanol-induced gastric ulcer in mice. *Toxicology and Applied Pharmacology*. 2013;272(1):21-9.
43. Tanyeli A, Ekinci AFN, Eraslan E, Guler MC, Nacar T. Anti-oxidant and anti-inflamatuar effectiveness of caftaric acid on gastric ulcer induced by indomethacin in rats. *General Physiology and Biophysics*. 2019;38(2):175-81.
44. Al Moutaery M, Al Rayes H, Al Swailam R, Elfaki I, Khan HA, Arshaduddin M. Protective effect of a cysteine prodrug and antioxidant, L-2-oxothiazolidine-4-carboxylate, against ethanol-induced gastric lesions in rats. *Experimental and Toxicologic Pathology*. 2012;64(3):233-7.