INTRODUCTION

The walnut plant belongs to the genus Juglans from Juglandaceae family of the order Juglandales. Walnut is a fruit that has been known and cultivated since ancient times. Its plantation is spread over all regions of Turkey (Gedikli, 2006). The seeds, green husks, and leaves of the walnut are a rich source of phenolic compounds such as flavonoids, phenolic acids, and naphthoquinones (Pereira et al., 2008; Zhao et al., 2014). Walnut is a valuable source of nutrients with cholesterol-free contents, substitutes for animal proteins, high concentrations of unsaturated fatty acids, linoleic acid and linolenic acid, and polyunsaturated fatty acids that are essential for a healthy life (Aydın, Gökçe, Yılmaz, 2015). In Iranian folk medicine, walnut is a well-documented remedy for treatment of several diseases such as infections, inflammations, and diabetes (Nasiry, Khalatbary, Ahmadvand, 2017). Several therapeutic effects such as anti-oxidative (Almeida et al., 2008), anti-inflammatory and antinociceptive (Erdemoglu, Küpeli, Yezilada, 2003), anti-carcinogenic (Carvalho et al., 2010), anti-microbial (Rather et al., 2012), and antifungal (Noumi et al., 2010) were reported for this plant. It
was reported that walnut contained high concentrations of α-tocopherol, which have strong anti-oxidant effect preventing the process of lipid oxidation (Amaral et al., 2005; Köksal et al., 2006). In addition, walnut consumption has also been shown to decrease the plasma concentration of C-reactive protein (CRP), interleukin-6 (IL-6), fibrinogen, vascular cell adhesion molecule-1 (VCAM-1) and intracellular adhesion molecule-1 (ICAM-1), (Salas-Salvadó et al., 2008).

Chemical compositions and nutritional of WEW include phenolic compounds (gallic acid, phthalic acid, catechin, vanillin, ethyl gallate, dihydroquercetin, kaempferol, taxifolin-3-O-α-L-arabinofuranoside, quercetin-3-rhamnoside, quercetin-3-O-(4'-O-acetyl)-α-L-rhamnopyranoside, blumenol B, propyl gallate and vanillic acid), fatty acids (octanoic acid, decylic acid, lauric acid, myristic acid, palmitic acid and margaric acid), amino acids (lysine, phenylalanine, threonine, isoleucine, leucine, valine and aspartic acid), monosaccharides (mannose, rhamnose, ribose, glucuronic acid, trehalose and galacturonic acid) and mineral element (k, Na, Ca, Mg, Fe, Cu, Zn, Mn and Se), (Table I), (Hu et al., 2019).

Ulcerative colitis is a chronic disease of the large intestine, which is characterized by acute and chronic inflammation of the mucosa, ulceration of the colon, bloody diarrhea, rectal bleeding, abdominal pain, cramping and weight loss (Shih, Targan, 2008). Etiology and pathogenesis of ulcerative colitis is unidentified and depends on multiple immune, genetic, and environmental factors, reactive oxygen species and gastrointestinal infections (Loftus, 2004). Oxidative stress is an important factor in the pathogenesis of ulcerative colitis disease (Pavlick et al., 2002). It was reported that enhanced oxidative stress in colonic mucosal and decreased antioxidant defense caused tissue damage and inflammation of the colon in the patients with ulcerative colitis (Mehrabani et al., 2011). Most of the current therapies for ulcerative colitis include non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, immunomodulators, and selective cyclooxygenase-2 (COX-2) inhibitors (Strober, Ludviksson, Fuss, 1998). Although many types of treatments have good outcomes, additional therapeutic approaches are needed because they produce adverse effects which have reduced their clinical applications.

Therefore, the present study aimed to evaluate the effect of the woody endocarpium of walnut (WEW) alcoholic extract on the levels of gastric acid, malondialdehyde (MDA), glutathione peroxidase (GPX), superoxide dismutase (SOD) and thiol and colon pathological scores on acetic acid-induced ulcerative colitis in rats.

### TABLE I - Chemical compositions and nutritional attributes of WEW

| Phenolic         | Fatty acids    | Amino acids | Monosaccharides | Minerals |
|------------------|---------------|-------------|-----------------|----------|
| Gallic acid      | Octanoic acid | Lysine      | Mannose         | k        |
| Phthalic acid    | Decylic acid  | Phenylalanine| Rhamnose        | Na       |
| Catechin         | Lauric acid   | Threonine   | Ribose          | Ca       |
| Vanillin         | Myristic acid | Isoleucine  | Glucuronic acid | Mg       |
| Ethyl gallate    | Pentadecanoic acid | Leucine | Trehalose     | Fe       |
| Dihydroquercetin | Palmitic acid | Valine      | Galacturonic acid | Cu       |
| Kaempferol       | Margaric acid | Aspartic acid | Xylose       | Zn       |

(continues on the next page...)
**TABLE I** - Chemical compositions and nutritional attributes of WEW

| Phenolic                  | Fatty acids      | Amino acids | Monosaccharides | Minerals |
|---------------------------|------------------|-------------|-----------------|----------|
| Taxifolin-3-O-α-L-        | Stearic acid     | Serine      | Galactose       | Mn       |
| arabinofuranoside         |                  |             |                 |          |
| Quercetin-3-rhamnoside    | Arachidic acid   | Glutamate   | Arabinose       | Se       |
| Quercetin-3-O- (4/-O-acetyl)-α-L-rhamnopyranoside | Heneicosanoic acid | Glycine |                  |          |
| Blumenol B                | Behenic acid     | Alanine     |                 |          |
| Propyl gallate            | Tricosanoic acid | Cystine     |                 |          |
| Vanillic acid             | Lignoceric acid  | Tyrosine    |                 |          |

**MATERIAL AND METHODS**

**Plant collection and extraction**

Walnut was purchased from Bojnurd city, North Khorasan province, Iran, in July 2017 and identified by botanists in the herbarium of Ferdowsi University of Mashhad (Herbarium No. 44540-FUMH). The WEW were grounded to powder (100 g), mixed with 96% ethanol at a ratio of 1:10 (powder to ethanol) and left for 3 days at 37°C with occasional shaking and stirring. The mixture was then filtered and the resulting liquid was concentrated under reduced pressure at 45°C in an Eyela (Heidolph, Germany) rotary evaporator.

**WEW extract characterization**

Phenolic compounds of the ethanolic extract of WEW were identified by liquid chromatography-mass spectrometry (LC-MS) using a Waters Alliance 2695 HPLC-Micromass Quattro micro API Mass Spectrometer. Five polyphenols were identified in this extract, they were Gallic acid, Protochatechuic acid, Epicatechin, Catechin and Vanillic acid (Figure 1).

![FIGURE 1 - The chromatographic separation of bioactive compounds from ethanolic extract of WEW.](image-url)
**Animals**

Experiments were performed using adult male Wistar rats (200-250g) prepared from Animal house, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran. The animals were kept in cages receiving clean filtered air (Maximiser, Thoren Caging System Inc., Hazleton, PA, U.S.A.) under standard condition at 22±2 °C and regular 12 hr/12 hr light/dark cycles. They also had free access to food and water ad libitum during experimental period.

**Induction of ulcerative colitis in rat**

Animals were anesthetized by intraperitoneal injection of thiopental (50mg/kg), and colitis was induced by intra-colonic administration of 4% acetic acid (2 ml) through a lubricated catheter under thiopental anaesthesia (Keshavarzi *et al.*, 2018) and treatment was started post-colitis induction. The instillation site was about 8 cm from the anal verge into the rectum. Rats were maintained in trendelenburg position for 30 seconds to prevent the leakage of the acid. Control group rats received the saline intraperitoneally. Rats were acclimatized to laboratory conditions for 8 days before the start of experimental procedures and maintained in a well-ventilated cage under standard protocols. Study protocol was approved by ethical committee of North Khorasan University of Medical Sciences (Ethics allowance No. 950015) and experiments were performed in compliance with the regulations of the Institute of Laboratory Animals Resources Commission on Life Sciences. On the 8th day, animals were sacrificed and colon samples were collected and stored at -80 °C until analysis.

**Experimental groups**

Animals were randomly divided into the six groups (n=7 in each group) including: (1) control group (group C) without induction of ulcerative colitis; (2) ulcerative colitis group (group UC) which was induced by acetic acid; (3) UC group treated with sulfasalazine 200 mg/kg (group S); and (4-6) UC groups treated with WEW extract 10, 20, and 50 mg/kg (groups WEW). The WEW extract and sulfasalazine were injected intraperitoneally for the 8-day after induction period of the ulcerative colitis.

**Measurement of malondialdehyde (MDA) level**

Malondialdehyde (MDA) level, as an index of lipid peroxidation, was measured. MDA reacts with thiobarbituric acid (TBA) as a thiobarbituric acid reactive substance (TBARS) to produce a red complex with the maximum absorbance at 535 nm. For MDA measurement, 2 mL of TBA/trichloroacetic acid (TCA)/HCl was added to 1 mL of tissue homogenate and the mixture was heated in a water bath for 40 min. Then, the mixture was centrifuged at 1000 g for 10 min. Finally, the absorbance was measured at 535 nm (Eftekhar *et al.*, 2019).

**Measurement of thiol level**

Total thiol concentration was measured using reagent DTNB which reacts with the thiol to produce a yellow coloured complex with a peak absorbance at 412 nm. One ml Trisethylene diamine tetraacetatic acid (EDTA) buffer (pH 8.6) was added to 50 μl serum in 1 ml cuvettes and sample absorbance was read at 412 nm against Tris-EDTA buffer alone (A1). Twenty microlitre DTNB reagents (10 mmol in methanol) were then added to the mixture and keep it in laboratory temperature for 15 min and the sample absorbance was read again (A2). The absorbance of DTNB reagent was also read as a blank (B). Total thiol concentration (mmol) was calculated using the following equation (Shakeri, Boskabady, 2017):

\[ \text{Total thiol concentration (mM)} = \frac{(A2–A1–B)×1.07}{0.05×13.6}. \]

**Measurement of SOD and GPX levels**

Levels of SOD and GPX in colon tissue were measured, using the Randox assay kits, and the content of GPX and SOD were given as U/g protein. Estimation of protein content follows the method of Lowry *et al.* (1951).
Measurement of gastric acid level

Gastric acid concentration was measured as previously described by washout method (Rafsanjani et al., 2007). The animals were completely deprived of food for 24 hours before the test. Then they were laparotomized, and by creating a hole in the duodenum, the cannula was inserted into the duodenum and then pushed into the stomach. To prepare the specimens, first, 1 ml of the physiological saline solution was injected into the stomach, and at the end of 15 minutes, was drawn. The amount of acid was measured immediately after sample collection using a manual titrator at laboratory temperature.

Colon macroscopic damage evaluations

After sacrificing the animals using ether overdose, a segment of the colon, 8 cm in length and 3 cm proximal to the anus was excised, opened longitudinally and washed in saline buffer. The criteria for macroscopic evaluation relied on a previously validated scoring system (0-4). The scores were: 0=no ulcer; 1=mucosal erythema only; 2=mild mucosal edema, slight bleeding or slight erosion; 3=moderate edema, bleeding ulcers or erosions; and 4=severe ulceration (Millar et al., 1996).

Assessment of colon histological damage

For histological examination, colon tissues were separately fixed in 10% formalin, dehydrated, paraffin embedded, processed, sectioned as 4 µm-thick sections, and stained with haematoxylin and eosin (HE), (Shahrokhi et al., 2018).

Statistical Analysis

The data of oxidant and antioxidant biomarkers, level of gastric acid and colon macroscopic damage were quoted as mean ± SEM. Statistical comparisons among and within groups were performed using one-way analysis of variance (ANOVA) with Kruskal- Wallis test. The results were considered statistically significant if the p value was less than 0.05. InStat (GraphPad Software, Inc, La Jolla, USA) was used for data analysis.

RESULTS

The effect of extract on oxidant and antioxidant biomarkers

The levels of MDA were significantly increased while SOD, thiol and GPX levels decreased in ulcerative colitis animals compared to the control group (p<0.05 to p<0.001; Figures 2 and 3). MDA levels in ulcerative colitis animals treated with all concentrations of WEW extract, and thiol level in the group treated with the highest concentration were significantly improved compared to those of untreated ulcerative colitis group (p<0.001 for both cases; Figures 2b and 3a). Sulfasalazine treatment also significantly reduced MDA but increased SOD, thiol and GPX levels compared to the ulcerative colitis group (p<0.05 to p<0.001; Figures 2 and 3). GPX and thiol levels in the groups treated with the two lower concentrations of WEW extract were significantly different with those of the control group (p<0.05 to p<0.001; Figures 2b and 3b). However, the effects of the highest concentration of WEW extract on thiol level were significantly higher than that of sulfasalazine (p<0.05; Figure 2b).

The effect of high concentration of WEW extract (50 mg/kg) on thiol and GPX levels were significantly higher than the low concentration (10 mg/kg), (p<0.01 to p<0.001; Figures 2b and 3b). In addition, there was a significant difference between the effects of high (50 mg/kg) and medium (20 mg/kg) concentrations of WEW extract on thiol level (p<0.001; Figure 2b).
The effect of extract on gastric acid level

The level of gastric acid was significantly increased in the ulcerative colitis animals compared to those of the control group (p<0.05; Figure 4a). Treatment with the two higher concentrations of WEW extract and sulfasalazine were significantly improved level of gastric acid compared to those of untreated ulcerative colitis group (p<0.01 to p<0.001; Figure 4a). No significant
difference was seen between treated groups and control group on gastric acid level.

The effect of the lowest concentration of WEW extract on gastric acid level was significantly lower than those of sulfasalazine (p<0.05; Figure 3a). The effect of the high (50 mg/kg) concentration of WEW extract on gastric acid level was significantly higher than the low (10 mg/kg) concentration (p<0.001; Figure 4a).

**FIGURE 4** - Acid level (a) and pathological (b) scores in the colon tissue of control (C), ulcerative colitis group (UC), ulcerative colitis rats treated with sulfasalazine (UC+S) and woody endocarpium of walnut extract (UC+WEW), (n=7 in each group). Data are presented as mean ± SEM values. * p<0.05 and *** p<0.001 shows significant differences compared to group C. + p<0.05, ++ p<0.01 and +++ p<0.001 show significant differences compared to group UC. # p<0.05 shows significant differences compared to group UC+S. x p<0.05 and xx p<0.01 show significant differences between the three concentrations WEW. Statistical analyses were performed using ANOVA with Tukey-Kramer’s post-test.

The effect of extract on pathological scores and histopathological

The scores of pathological changes in ulcerative colitis group were significantly increased compared to the control group (p<0.001; Figure 4b). Pathological scores in ulcerative colitis group treated with the highest concentration of WEW extract were significantly reduced compared to the untreated ulcerative colitis group (p<0.05; Figure 4b).

Sulfasalazine treatment was reduced pathological scores compared to the ulcerative colitis group. However, this change was not statistically significant. Pathological scores in groups treated with the two lower concentrations of WEW extract and sulfasalazine were significantly different with those of the control group (p<0.05 to p<0.001 Figure 4b). There was no significant difference in pathological scores among sulfasalazine-treated group and groups treated with the three concentrations of WEW extract.

The effect of the high (50 mg/kg) concentration of WEW extract on pathological scores was significantly higher than the low (10 mg/kg) concentration (p<0.05; Figure 4b).

Figure 5 shows a specimen of colon photograph of each studied group. Colonic tissue histopathological evaluation from the ulcerative colitis group showed severe submucosal edema and crypt loss, while the control group showed a preserved mucosal architecture. The morphological characteristics of the ulcerative colitis group treated with the highest concentration of the WEW extract (50mg/kg) revealed significant amelioration of colonic tissue injury.
DISCUSSION

This study evaluated the healing effects of the WEW ethanolic extract against acetic acid-induced ulcerative colitis by measuring tissue histopathology, gastric acid and MDA, SOD, GPX and thiol level in rats. This study found that treatment with the WEW ethanolic extract could lead to enhancement in colonic antioxidant capacity and a decrease in inflammation and acute colonic injury induced by acetic acid, which is dose-dependent. The results were confirmed by histopathological examinations. Concentration-dependent effects of the WEW ethanolic extract could be another reason indicating the preventive effect of plant in colon inflammation in the ulcerative colitis rats. Sulfasalazine is mainly used for treatment of inflammatory bowel disease (IBD), including UC and Crohn’s disease (Karaca et al., 2010). Sulphasalazine is composed of sulphapyridine, which has antibacterial activity and 5-aminosalicylate (5-ASA), which has anti-inflammatory potency (Klotz, 1985). We used sulfasalazine as a reference drug, and found that the 50 mg/kg dosage of the extract in most parameters was more effective than sulfasalazine.

The model of acetic acid induced colitis shares many of the histologic features of the ulcerative colitis in human beings including mucosal edema and submucosal ulceration (Sharon, Stenson, 1985). The destruction of colon structure and mucosa barrier is due the chemical stimulation, enhanced vessel permeability, increased inflammatory mediators, and reactive oxygen species (ROS), (Carty et al., 2000).

There is strong evidence that oxidative stress plays a vital role in the IBD initiation and continuance (Kruidenier, Verspaget, 2002). Increased production of oxidants in ulcerative colitis subjects has been reported by human studies (Babbs, 1992). Imbalance between oxidant and antioxidant parameters in ulcerative colitis induced oxidative damage (Sahebari et al., 2015), which is a characteristic feature of colitis (Droge, 2002).

In the current experiment, MDA level as indicator of oxidative stress were significantly increased but SOD, GPX and thiol levels decreased in the ulcerative colitis.
The effect of woody endocarpium of walnut alcoholic extract on acetic acid-induced ulcerative colitis in rats

animals compared to the control group. Similarly, previous evidences also indicated an imbalance in oxidant/antioxidant enzymes balance towards oxidative conditions in ulcerative colitis animals (Balmus et al., 2016; Lih-Brody et al., 1996). GPX is involved in different mechanisms including the synthesis and repair of DNA, recycling of vitamins C and E, prevention of free radicals-induced damage, improvement of the antioxidant activity of vitamin C, and facilitation of the transport of amino acids and plays a principle role in detoxification (Chavan et al., 2005). SOD, levels are reduced in the inflamed intestinal tissues as free radicals affect the intestinal epithelium (Kandhare et al., 2013). Oxidative damage of the colitis imbalances the catalytic activity resulting in the inflammation and oxidative damage to the colonic mucosa (Circu, Aw, 2012). Findings of this study showed that treatment of ulcerative colitis animals with the extract of WEW resulted in a significant reduction in MDA but increased SOD, GPX and thiol levels. Consistently, previous studies on the effect of the hydro-ethanolic extract of walnut on MDA and SOD levels in CCl4-induced liver damage in the rat had comparable results with those of the present study (Aydın, Gökçe, Yılmaz, 2015; Eidi et al., 2013). A recent study has demonstrated that dietary administration of walnut (6% and 9% w/w) significantly increased SOD, catalase and GPX in transgenic mouse model of alzheimer disease, which confirms the findings of our study (Pandareesh, Chauhan, Chauhan, 2018). Increased level of thiol has been observed in rat that feeded with the walnut extract (10% w/w), (Obabiyi, Oboh, Adefegha, 2017) which supports the results of the present study.

Extensive studies have shown that many extra-intestinal tissues including skin, intestines, kidneys, and stomach are also affected by ulcerative colitis (Greenstein, Janowitz, Sachar, 1976). Also, inflammation of the duodenum, gastric and esophageal ulcers is also seen in children suffering from the ulcerative colitis, which indicates a link between these gastric and colon disorders (Ruuska et al., 1994). Therefore, due to the fact that ulcerative colitis is a complex disorder that can affect many tissues, including the stomach. In the present study, the effect of walnut extract on the gastric secretory response and histopathological changes in ulcerative colitis model induced by acetic acid was designed in male rats.

Our results also showed increased pathological scores and gastric acid level in the ulcerative colitis animals. The same results were observed in ulcerative colitis model induced by acetic acid in rats (Keshavarzi et al., 2018), which confirms the induction of ulcerative colitis in animals. Treatment with the various concentrations of WEW extract resulted in a significant protection against the most ulcerative colitis-related colon pathological damages and gastric acid level. Similar to our study, this condition was reversed in another study using ethanolic extract of the walnut (10 and 20 mg/kg, intraperitoneal) in acetic acid-induced experimental colitis in the rat (Keshavarzi et al., 2019). Moreover, the administration of walnuts (0, 3.5, 7 and 14% g/kg, orally) reduced overall colitis scores in a rat model of dextran sulfate sodium-induced acute colitis in the mice (Nakanishi et al., 2019). These differences may be due to some limitations of the present study, such as the number of animals and short term period of the treatment protocol.

All the above-described studies, in line with the current experiment, support the preventive therapeutic effect of the WEW extract on the ulcerative colitis.

The results of the present study showed that WEW extract improves the oxidative stress and colon inflammation in the ulcerative colitis rats. These results suggest a therapeutic effect for the WEW extract against the IBD through both antioxidant activities and preventive effects on the colon inflammation. However, further studies are needed to evaluate the effects of the plant and its constituents on animal models of the ulcerative colitis as well as human studies.

In conclusion, the results of the present study indicated a preventive effect for WEW extract on oxidative markers and colon pathological damages in ulcerative colitis rats, which were comparable but more specific, to the effect of sulfasalazine at used concentrations.

ACKNOWLEDGMENT

This study was financially supported by a grant from Research Council of North Khorasan University of Medical Sciences.
CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Almeida IF, Fernandes E, Lima JL, Costa PC, Bahia MF. Walnut (Juglans regia) leaf extracts are strong scavengers of pro-oxidant reactive species. Food Chem. 2008;106(3):1014-20.

Amaral JS, Alves MR, Seabra RM, Oliveira BP. Vitamin E composition of walnuts (Juglans regia L.): a 3-year comparative study of different cultivars. J Agric Food Chem. 2005;53(13):5467-72.

Aydın S, Gökçe Z, Yılmaz Ö. The effects of Juglans regia L.(walnut) extract on certain biochemical parameters and in the prevention of tissue damage in brain, kidney, and liver in CCl4 applied Wistar rats. Turk J Biochem. 2015;40(3):241-50.

Babbs CF. Oxygen radicals in ulcerative colitis. Free Radic Biol Med. 1992;13(2):169-81.

Balmus IM, Ciobica A, Trifan A, Stanciu C. The implications of oxidative stress and antioxidant therapies in inflammatory bowel disease: clinical aspects and animal models. Saudi J Gastroenterol. 2016;22(1):3-17.

Carty E, De Brabander M, Feakins R, Rampton D. Measurement of in vivo rectal mucosal cytokine and eicosanoid production in ulcerative colitis using filter paper. Gut. 2000;46(4):487-92.

Carvalho M, Ferreira PJ, Mendes VS, Silva R, Pereira JA, Jerónimo C, et al. Human cancer cell antiproliferative and antioxidant activities of Juglans regia L. Food Chem Toxicol. 2010;48(1):441-7.

Chavan S, Sava L, Saxena V, Pillai S, Sontakke A, Ingole D. Reduced glutathione: importance of specimen collection. Indian J Clin Biochem. 2005;20(1):150-2.

Circu ML, Aw TY. Intestinal redox biology and oxidative stress. Semin Cell Dev Biol. 2012;23(7):729-37.

Droge W. Free radicals in the physiological control of cell function. Physiol Rev. 2002;82(1):47-95.

Eidi A, Moghadam JZ, Mortazavi P, Rezazadeh S, Olamafar S. Hepatoprotective effects of Juglans regia extract against CCl4-induced oxidative damage in rats. Pharm Biol. 2013;51(5):558-65.

Eftekhar N, Moghimi A, Boskabady MH, Kaveh M, Shakeri F. Ocimum basilicum affects tracheal responsiveness, lung inflammatory cells and oxidant–antioxidant biomarkers in sensitized rats. Drug Chem Toxicol. 2019;42(3):286-94.

Erdemoglu N, Küpeli E, Yeşilada E. Anti-inflammatory and antinociceptive activity assessment of plants used as remedy in Turkish folk medicine. J Ethnopharmacol. 2003;89(1):123-9.

Gedikli F. Investigation of walnut (Juglans regia), black mulberry (Morus nigra), barberry (Berberis crataegina), madder (Rubia tinctorum) and alder (Alnus glutonosa) as a protein dye in polyacrylamide gel electrophoresis. Masters Thesis, Gaziosmanpaşa University, Graduate School of Natural and Applied Science Department of Chemistry, Tokat, 2006.

Greenstein AJ, Janowitz HD, Sachar DB. The extra-intestinal complications of Crohn's disease and ulcerative colitis: a study of 700 patients. Medicine (Baltimore). 1976;55(5):401-12.

Hu Q, Liu J, Li J, Liu H, Dong N, Geng YY, et al. Phenolic composition and nutritional attributes of diaphragma juglandis fructus and shell of walnut (Juglans regia L.). Food Sci Biotechnol. 2019; 29(2):187-196.

Kandhare AD, Ghosh P, Ghule AE, Zambare GN, Bodhankar SL. Protective effect of Phyllanthus amarus by modulation of endogenous biomarkers and DNA damage in acetic acid induced ulcerative colitis: Role of phyllanthin and hypophyllanthin. Apollo Med. 2013;10(1):87-97.

Karaca T, Bayiroglu F, Yoruk M, Kaya MS, Uslu S, Comba B, et al. Effect of royal jelly on experimental colitis Induced by acetic acid and alteration of mast cell distribution in the colon of rats. Eur J Histochem. 2010;54(4):193-6.

Keshavarzi Z, Nazari M, Razmi Z, Behnamfar M. The Modulatory effects of aqueous extract of the plant biebersteinia multifida on the gastric acid level and intestinal cytokines in ulcerative colitis model. J Babol Univ Med Sci. 2018;20(9):7-13.

Keshavarzi Z, Nurmohammadi F, Majlesi S, Maghool F. Protective effects of walnut extract against oxidative damage in acetic acid-induced experimental colitis rats. Physiol Pharmacol. 2019;23:51-8.

Klotz U. Clinical pharmacokinetics of sulphasalazine, its metabolites and other prodrugs of 5-aminosalicylic acid. Clin Pharmacokinet. 1985;10(4):285-302.

Köksal Aİ, Artik N, Şimşek A, Güneş N. Nutrient composition of hazelnut (Corylus avellana L.) varieties cultivated in Turkey. Food Chem. 2006;99(3):509-15.

Kruidenier La, Verspaget H. oxidative stress as a pathogenic factor in inflammatory bowel disease—radicals or ridiculous? Aliment Pharmacol Ther. 2002;16(12):1997-2015.
The effect of woody endocarpium of walnut alcoholic extract on acetic acid-induced ulcerative colitis in rats

Lih-Brody L, Powell SR, Collier KP, Reddy GM, Cerchia R, Kahn E, et al. Increased oxidative stress and decreased antioxidant defenses in mucosa of inflammatory bowel disease. Dig Dis Sci. 1996;41(10):2078-86.

Loftus Jr EV. Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences. Gastroenterology. 2004;126(6):1504-17.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem. 1951;193(1):265-75.

Mehrabani D, Ziae M, Hosseini S, Ghahramani L, Bananzadeh A, Ashraf M, et al. The effect of *Calendula officinalis* in therapy of acetic acid induced ulcerative colitis in dog as an animal model. Iran Red Crescent Med J. 2011;13(12):884.

Millar AD, Rampton DS, Chander CL, Claxson AWD, Blades S, Coumbe A, et al. Evaluating the antioxidant potential of new treatments for inflammatory bowel disease in a rat model of colitis. Gut. 1996;39(3):407-15.

Nakanishi M, Matz A, Klemashevich C, Rosenberg DW. Dietary walnut supplementation alters mucosal metabolite profiles during DSS-induced colonic ulceration. Nutrients. 2019;11(5):1-13.

Nasiry D, Khalatbary AR, Ahmadvand H. Therapeutic potential of *Juglans regia* L. leaf extract against diabetic retinopathy in rat. Iran J Basic Med Sci. 2017;20(11):1275-1281.

Noumi E, Snoussi M, Hajlaoui H, Valentin E, Bakhrouf A. Antifungal properties of *Salvadora persica* and *Juglans regia* L. extracts against oral Candida strains. Eur J Clin Microbiol Infect Dis. 2010;29(1):81.

Olabiyi AA, Oboh G, Adefegha SA. Effect of dietary supplementation of tiger nut (*Cyperus esculentus* L.) and walnut (*Tetracarpidium conophorum* mull. Arg.) on sexual behavior, hormonal level, and antioxidant status in male rats. J Food Biochem. 2017;41(3):1235-1240.

Pandareesh MD, Chauhan V, Chauhan A. Walnut Supplementation in the Diet Reduces Oxidative Damage and Improves Antioxidant Status in Transgenic Mouse Model of Alzheimer's Disease. J Alzheimer's Dis. 2018;64(4):1295-1305.

Pavlick KP, Laroux FS, Fuseler J, Wolf RE, Gray L, Hoffman J, et al. Role of reactive metabolites of oxygen and nitrogen in inflammatory bowel disease1, 2. Free Radical Biol Med. 2002;33(3):311-22.

Pereira JA, Oliveira I, Sousa A, Ferreira IC, Bento A, Estevinho L. Bioactive properties and chemical composition of six walnut (*Juglans regia* L.) cultivars. Food Chem Toxicol. 2008;46(6):2103-11.

Rafsanjani FN, Shahrani M, Ardakani ZV, Ardakani MV. Marjoram increases basal gastric acid and pepsin secretions in rat. Phytother Res. 2007;21(11):1036-8.

Rather MA, Dar BA, Dar MY, Wani BA, Shah WA, Bhat BA, et al. Chemical composition, antioxidant and antibacterial activities of the leaf essential oil of *Juglans regia* L. and its constituents. Phytotherapy. 2012;19(13):1185-90.

Ruuska T, Vaajalahti P, Arajärvi P, Mäki M. Prospective evaluation of upper gastrointestinal mucosal lesions in children with ulcerative colitis and Crohn's disease. J Pediatr Gastroenterol Nutr. 1994;19(2):181-6.

Salas-Salvadó J, Casas-Agustench P, Murphy MM, López-Uriarte P, Bulló M. The effect of nuts on inflammation. Asia Pac J Clin Nutr. 2008;17(5):333-6.

Shakeri F, Boskabady MH. Anti-inflammatory, antioxidant, and immunomodulatory effects of curcumin in ovalbumin-sensitized rat. BioFactors. 2017;43(4):567-76.

Sharon P, Stenson WF. Metabolism of arachidonic acid in acetic acid colitis in rats. Similarity to human inflammatory bowel disease. Gastroenterology. 1985;88(1):55-63.

Shih DQ, Targan SR. Immunopathogenesis of inflammatory bowel disease. World J Gastroenterol. 2008;14(3):390-400.

Strober W, Ludviksson BR, Fuss IJ. The pathogenesis of mucosal inflammation in murine models of inflammatory bowel disease and Crohn disease. Ann Intern Med. 1998;128(10):848-56.

Zhao MH, Jiang ZT, Liu T, Li R. Flavonoids in *Juglans regia* L. leaves and evaluation of in vitro antioxidant activity via intracellular and chemical methods. J Sci World J. 2014;2014:1-10.

Received for publication on 27th July 2019
Accepted for publication on 29th September 2021