ABSTRACT

The aim of the study was to investigate the protective and therapeutic effects of *Polygonum cognatum* Meissn (PC) in the treatment of colitis. Rats were orogastrically treated with PC (2g/kg) throughout a 10-day interval before and/or after colitis, which was induced by intracolonic administration of acetic acid. Microscopic damage scores and scanning electron microscopy changes of colon segments were analyzed. The levels of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) in serum, malonyldialdehyde (MDA), glutathione (GSH), tissue factor (TF) levels, myeloperoxidase (MPO), superoxide dismutase (SOD), catalase (CAT) and gammaglutamyltransferase (GGT) activities in the colon tissues were measured. PC treatment ameliorated the acetic acid-induced disturbances in colonic tissue. Serum IL-6 and TNF-α levels decreased with PC treated colitis group. Colonic tissue GGT, MPO activities and MDA levels were decreased on the other hand SOD, CAT activities and GSH levels were increased in PC treated colitis group. PC was effective in the treatment of acetic acid-induced colitis in rats. This may be due to the suppression and down regulation of mediators in intestinal inflammatory response and the expression of antioxidant and anti-inflammatory response. In conclusion, PC can play a protective role by downregulating the colonic tissue damage and oxidative stress which may be related to modulating the activities of antioxidant molecules in rat model of colitis.

Keywords: *Polygonum cognatum* Meissn, Oxidative Stress, Colitis, Antioxidant, Anti-inflammatory

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory bowel disease that causes ongoing inflammation and ulcers in the innermost layers of the colon and rectum (1). Although the etiology and pathophysiology of UC is not known with certainty, inflammatory mediators, such as cytokines and eicosanoids, and excessive production of reactive oxygen species (ROS) by the inflamed mucosa have been proposed to contribute significantly to the development of tissue injury (2). Various animal models of experimental colitis have been used to study the effect of inflammation on intestinal area. An acetic acid-induced colitis model is one of them. This model causes a mild acute mucosal inflammation in the distal colon of rats (1). The acetic acid-induced colitis model is an experimental model that has shown morphological similarities to human UC (3). Inflamed colonic mucosa in acetic acid induced colitis is also known to produce excess ROS such as superoxide...
(O$_2^-$) radical, the hydroxyl radical (OH$^-$), hydrogen peroxide (H$_2$O$_2$). A major source of ROS in inflammatory lesions comes from the reduction of oxygen to the O$_2^-$ by neutrophil NADPH oxidase, a multicomponent enzyme system (4) and by myeloperoxydase (MPO) which produces hypochloric acid from hydrogen peroxide (5).

Therapeutic drugs such as 5-aminosalicylic acid, sulfasulfapyridine and glucocorticoids inhibit the inflammatory mediators through different mechanisms (6,7) but their adverse effects can limit their use. Therefore, it is important to develop new therapeutic strategies. On the other hand several traditional drugs tried in the treatment of UC to be quite effective, have exerted severe side-effects. 

**Polygonum cognatum** Meissn (PC), called “Madimak” is an endemic plant from family of Polygonaceae and is widely consumed as a traditional Turkish food in Sivas, Turkey. It is not known whether PC might have an anti-inflammatory effect and a resistance to biologic oxidation and have a therapeutic value in the inflammatory state of UC. However, neither the mechanisms of actions nor pharmacological properties of PC’s potency are not sufficiently clarified yet. No studies regarding the therapeutic use of PC are found in literature. Therefore, in the present study, we used an experimental model of colitis to explore the possible anti-inflammatory and antioxidant effects of PC.

**MATERIALS AND METHODS**

**Plant Material and Preparation of PC Extract**

PC was collected from Sivas (Turkey) and preserved at the Herbarium of the Faculty of Pharmacy, Marmara University, Istanbul, Turkey (MARE.11646 ). Ten grams of wet plant (with leaves and body of plant) were homogenized and mixed with 150 ml saline and were subjected to maceration in 2 hours maintaining temperature of 40°C. After the three parallel maceration procedures, macerates were filtered and combined. Then samples were lyophilised for the removal of solvents and dry powder was made. Major components of PC Extract (PCE) were analysed as sterols, triterpene, tannins, reduced compounds of carbohydrate, polyuronides, saponines carotenoids, alkaloids, flavone aglicon, anthracene aglicon and coumarin (8).

**Animals, Colitis Induction and PCE Administration**

All experimental protocols were approved by the Marmara University Animal Care and Use Committee (16.04.2009). Sprague-Dawley rats of both sexes (200-250 g) supplied by the Marmara University (MU) Animal Center (DEHAMEP) were randomly divided into six groups and each group consisted of 7 rats. Rats were kept in a light-and temperature-controlled room with 12/12-hour light/dark cycles, where the temperature (22±0.5°C) and relative humidity (65–70%) were kept constant. The animals were fed a standard pellet, and food was withdrawn overnight before colitis induction. Access to water was allowed ad libitum. After an overnight fasting under ether anesthesia application of acute experimental colitis was performed by intracolonic administration of 1 ml of acetic acid (4% vol/vol in 0.9% NaCl), diluted through a polyethylene catheter (PE-60) which was put into the colon extending a distance of eight centimeters beyond the anus. After a 30-second period of exposure, excess fluid was withdrawn and the colon was then flushed with saline. In the control animals isotonic saline was infused into the colon through this catheter, held in place for 30 s, and then was withdrawn.

PCE, 2g/kg was orally administered once a day only as a pre-treatment for 10 days before the induction of colitis, only as a post-treatment after colitis induction or as pre-treatment followed by post-treatment for 10-days (Table 1). Similary saline was given once a day as a pre-treatment or as treatment regimen in the colitis groups for 10 days. Control group was orally administered only isotonic saline. On the 10th day of intracolonic saline or acetic acid administration, blood samples for TNF-α and IL-6 levels as a marker of inflammation were collected by cardiac puncture under light ether anesthesia and after rats were decapitated. Until they were decapitated, the groups were checked for body weight changes daily during the course of the experiment. The distal 8 cm of the colon obtained from
each animal was stored at \(-80^\circ\text{C}\) until for the detection of myeloperoxidase (MPO), catalase (CAT), superoxide dismutase (SOD), gamma-glutamyl transferase (GGT), tissue factor (TF) activity, malondialdehyde (MDA) and glutathione (GSH) levels.

**Light-microscopic analysis**

For light-microscopic analysis, samples from distal colon were fixed in 10% buffered formalin for 48 h, dehydrated in ascending alcohol series, and embedded in paraffin wax. Approximately 7\(\mu\)m thick sections were stained with hematoxylin and eosin (H&E) for general morphology analysis. Stained sections were observed under an Olympus BX50 photomicroscope (Tokyo, Japan). Assessment of colonic injury was performed by using the previously described criteria: damage/necrosis, submucosal edema, inflammatory cell infiltration, glandular degeneration, epithelial degeneration as 0, none; 1, localized; 2, moderate; 3, severe (9). All tissue samples were evaluated in blinded fashion by an experienced histologist.

**Scanning electron microscopy**

For scanning electron microscopy (SEM) the samples were fixed in glutaraldehyde (0.13 M and pH 7.4) phosphate buffered for 4 h and postfixed with 1% OsO\(_4\) for 1 h and dehydrated in alcohol series, put into amyl acetate series, dried with liquid CO\(_2\) under pressure with critical point drier (Bio-Rad E 3000, Hertfordshire, UK), covered with gold particles (Bio-Rad SC502, Hertfordshire, UK) and examined under Jeol JSM SEM (Tokyo, Japan).

**Biochemical Analyses**

**Measurement of Serum TNF-\(\alpha\) and IL-6 Levels**

Serum TNF-\(\alpha\), and IL-6 levels were quantified using enzyme-linked immunosorbent assay kits specific for the previously mentioned rat cytokines according to the manufacturer’s instructions and guidelines (Invitrogen, USA). These particular assay kits were selected because of their high degree of sensitivity, specificity, inter- and intra-assay precision, and small amount of plasma sample required to conduct the assay.

**Tissue MPO Activity, MDA and GSH Assays**

Analysis of MPO, MDA and GSH was performed by a previously described method (10,11). The activity of tissue-associated MPO, a natural constituent of primary granules of neutrophils, was determined in the colonic samples homogenized in 50 mM potassium phosphate buffer. Colonic tissue samples were homogenized with ice-cold 150 mM KCl for determination of MDA and GSH levels. MPO activity was defined as the quantity of enzyme degrading 1 \(\mu\)mol H\(_2\)O\(_2\) min\(^{-1}\), expressed as U/ mg proteins at 460 nm and 37\(^\circ\)C. MDA levels were measured by the method of Ledwozyw for products of lipid peroxidation. Results are expressed as MDA nmol/mg protein. GSH was determined by the spectrophotometric method using Ellman’s reagent and the results were expressed as mg GSH/mg protein.

**Tissue SOD, CAT and GGT Activity**

SOD activity in the colon samples was measured according to the previously described method at 460 nm (11,12). The result was expressed in SOD activity U/mg protein. Measurement of CAT activity is based on the catalytic activity of the enzyme which catalyses the decomposition reaction of H\(_2\)O\(_2\) to give H\(_2\)O and O\(_2\) at 240 nm for about 1 min. GGT activity using gamma-glutamyl p-nitroanalide as a substrate was determined by the spectrophotometric method (13). The p-nitroaniline released was measured at 405 nm. A unit of enzyme activity as the amount of enzyme catalyzing the conversion of 1 mM nitroaniline/min.

**Tissue Factor Activity**

Tissue Factor (TF) is a component of cell membrane and its activity has been measured by prothrombin time test. TF activity of colon tissues was evaluated according to Quick’s one-stage method using normal plasma (14). TF activity was expressed as seconds. Shortened clot formation time indicates increased TF activity.

**Statistical analysis**

Statistical analysis was carried out using SPSS 11.5 statistical software (SPSS, Chicago, IL). Each group consisted of 7 animals. All data were expressed as mean± SD (standard deviation). Groups of data were compared with an analysis of variance (ANOVA) followed by Tukey’s multiple comparison tests. For the evaluation the difference among the day after treatment, repeated ANOVA was used. Values of P < 0.05 were regarded as significant.

**RESULTS**

Despite a slight fall following colitis induction, there was no difference among the body weight changes of the
Cevik et al. P. cognatum protects colitis

Marmara Pharm J 18: 126-134, 2014

Experimental groups. PCE phytochemical analysis of the bioactive compounds of the PCE was shown in Table 2 and were found as positive saponines, sterols and triterpenes, tannins, reduced compounds of carbohydrate and polyuronides.

### Microscopic evaluation

In light microscopic evaluation of the saline-treated colitis group when compared with the normal appearance of the control (Fig.1A) and sham group (Fig.1B) demonstrated severe necrotic degeneration with accumulation of inflammatory cells dominantly polymorphonuclear leukocytes (Fig.1F). Besides inflammatory cells, there was prominent congestion of blood vessels in lamina propria accompanied with dense edema. PCE-treated colitis groups showed remarkable regression in the density of inflammation along with a decrease in both edema and congestion of blood vessels.

The comparison of pre-treatment and post-treatment groups was observed as significant considering the depth of glandular degeneration in all lamina propria in the post-treatment groups (Fig.1E). Considering the depth of glandular degeneration in lamina propria, there was a significant difference between the PCE-pre-treated and post-treated colitis groups, while both PCE-pre-treated (Fig.1C) and pre-plus-post PCE-treated groups (Fig.1D) demonstrated better morphology regarding the invasion of inflammation. Histologic semi-quantitative scoring of saline-treated colitis and PCE-treated colitis groups was in accordance with the morphology (Fig.4F).

SEM demonstrated that the treatment with PCE showed an ameliorating effect on colitis. The control (Fig.2A) and sham groups (Fig.2B) demonstrated regular morphology with cryptic and epithelial structures. PCE-pre-treated colitis group (Fig.2C) revealed minimal regenerative changes on severely desquamated colonic epithelium. On the other hand, pre-plus-post PCE-treated colitis group (Fig.2D) demonstrated prominent regeneration in the morphology. The post-colitis PCE-treated group (Fig.2E) showed a better morphology than the pre-treated group. Both the epithelial and cryptic structures had severe desquamation in the saline-treated colitis group (Fig.2F).

### Table 2. Bioactive compounds in PCE

<table>
<thead>
<tr>
<th>Bioactive Compounds</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saponines</td>
<td>Positive</td>
</tr>
<tr>
<td>Sterols and triterpenes</td>
<td>Positive</td>
</tr>
<tr>
<td>Tannins</td>
<td>Positive</td>
</tr>
<tr>
<td>Reduced compounds of carbohydrate</td>
<td>Positive</td>
</tr>
<tr>
<td>Polyuronides</td>
<td>Positive</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>Negative</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>Negative</td>
</tr>
<tr>
<td>Flavone aglycone</td>
<td>Negative</td>
</tr>
<tr>
<td>Anthracene aglycone</td>
<td>Negative</td>
</tr>
<tr>
<td>Coumarin</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Figure 1. Microscopic Evaluation A) Control group, colon tissue with regular surface epithelium (arrow) and crypts (*), and B) Sham group, note the alignment of epithelium (arrows) similar to that of control group, C) Pre-treatment group, edema (**) in lamina propria and degeneration in glands (arrow), detachment of epithelium (arrowheads) note the regression of glandular dystrophy D) Pre-post treatment group, prominent regression of edema in lamina propria (**), re-formed glandular structures (arrows) some leukocytes in lamina propria (inset-arrowhead E) Post-treatment group, moderate glandular degeneration (arrows) with leukocyte accumulation (***) and necrosis (arrowheads) in lamina propria, not the regeneration of the epithelium (curved arrow), F) Colitis group, severe inflammation and necrosis (**) throughout the layers, loss of glandular structures (arrow), cellular necrosis in glandular epithelium (inset-arrowheads). HE X 200, insets X 400.
Serum Pro-inflammatory Cytokine Levels

Serum TNF-α and IL-6 levels were not different among the sham and the control groups, while they were significantly increased in the saline-treated colitis group (P<0.001) (Fig.3A,3B). TNF-α levels were slightly decreased in the PCE-pre-treated colitis group but the reduction was no statistically significant. However, TNF-α levels were significantly decreased in the post-colitis PCE-treated and pre-plus-post PCE-treated colitis groups as compared with saline-treated colitis group (p<0.001). In all three treatment groups (pre, post, pre-plus-post) with PCE, IL-6 levels were
significantly decreased when compared with saline-treated colitis group (p<0.01-0.001).

**Colonic Tissue Parameters**

The levels of MDA, which is a major degradation product of lipid peroxidation, were significantly increased in the saline-treated colitis group with respect to control group (p<0.001) while PCE treatment given either before and/or after colitis induction caused marked decreases in the MDA levels of the colonic tissue (p<0.05-0.001) (Fig.3C). As shown in Fig.3D, MPO activity, an indicator of tissue neutrophil infiltration, in the saline-treated colitis group was significantly increased when compared with that of the control group (p<0.001). However, in the colitis group, treated with PCE ( pre, post and pre-plus-post), colitis-induced increases in MPO activity were significantly inhibited (p<0.05-0.001).

On the other hand, colitis induction depleted colonic GSH level (p<0.01) to a significantly lower level than that of the control group, while PCE administration prevented the reduction in GSH level (Fig.4A). The GSH levels were significantly increased in all three colitis groups with PCE treatment (p<0.05- 0.001). When compared with the control group, GGT activity was significantly increased in the saline-treated colitis group (p<0.001). In PCE-treated groups, GGT levels were decreased, but this decrease was significant in the post-colitis treated and pre-plus-post PCE-treated groups (p<0.001) (Fig. 4B). As shown in Fig.4C and 4D, SOD (p<0.01) and CAT activities (p<0.001) were significantly decreased in the saline-treated colitis group, indicating depressed antioxidant capacity of the colonic tissue. SOD activity was significantly increased only when PCE treatment was given both pre- and post-colitis induction (p<0.01) while in the post-colitis treated and pre-plus-post colitis groups PCE treatment increased CAT activity (p<0.001). However, SOD and CAT activities were not significantly changed in the pre-colitis PCE-treated group.

TF activity was increased in the saline-treated colitis group as compared with the control group (p<0.05) (Fig.4E). PCE treatment given as a post-treatment or a pre-plus-post treatment abolished this reduction in TF activity (p<0.001 or 0.05), but pre-treatment only had no effect on colitis-reduced TF activity.

**DISCUSSION**

In accordance with previous reports, our findings indicated the presence of inflammation and oxidative injury in the colonic tissue of acetic acid-induced colitis (3). Moreover, elevated serum TNF-α and IL-6 levels were also reduced by PCE treatment. The PCE treatment showed potent anti-inflammatory and some protective effects against oxidative injury. The ability of PCE to protect against colitis was observed with significant reductions in tissue MDA levels, MPO, GGT and increases in GSH levels, SOD, CAT and TF activities.

UC is an inflammatory bowel diseases (IBD) that is characterized by cycles of acute inflammation, ulceration, and bleeding of colonic mucosa (15). One common characteristic of this disease is excessive production of proinflammatory mediators and excessive production of ROS (16, 17,18). Oxidative stress can result from excess production of ROS by inappropriate activation of phagocytic cells in UC (19). Consequently, lipid peroxidation could aggravate free radicals chain reactions, disrupt the integrity of intestinal mucosal barrier, and activate inflammatory mediators. A complementary aspect to the elevation of ROS in inflammation is the observation that UC mucosa is relatively depleted of antioxidant defenses, thereby rendering it susceptible to oxidation injury and ROS can cause direct epithelial cell damage in UC (20). A lower level of antioxidant as a consequence of a past situation of oxidative stress will correspond to a higher membrane lipid peroxide. Both are products of the lipid peroxidation process and presumably the result of ROS action (21,22). In the present study, MDA, a good indicator of the degree of lipid peroxidation, was increased in the colonic tissues of rats with acetic acid induction, indicating the presence of oxidative damage (23). Increased MDA content might have resulted from an increase in ROS as a result of oxidative stress in the rats after acetic acid administration (24). Several studies have reported on the antioxidant and antiradical activities of tannins. Experimental studies have shown that containing tannin compounds increased SOD, GPx, GSH levels and decreased MDA, MPO, nitric oxide (NO), inducible nitric oxide synthase (iNOS), TNF-α levels (25,26). Tannins have also been considered as “health-promoting” components in plant derived foods and beverages (27). Tannins have the ability to chelate metal ions such as Fe (II) and interfere with one of the reaction steps in the Fenton reaction and thereby retard oxidation (28). The inhibition of lipid peroxidation by tannin constituents can act via the inhibition of cyclooxygenase (29). Thus reduction in MDA levels by PCE treatment shows that tannins are effective inhibiting lipid peroxidation.

Since proinflammatory cytokines play a key role in the pathophysiology of IBD (30) anti-TNF-α antibodies (31)
and anti-inflammatory compounds are therapeutically used in some severe forms of IBD (32). Some approaches for UC treatment include inhibition of ROS producing enzymes, direct scavenging of ROS or improvement of cellular antioxidant pools. As a possible mechanism herbal treatment could scavenge oxidative-free radicals, down-regulate some of the inflammatory mediators involved in the intestinal immune and inflammatory responses, including TNF-α, NF-κBp65 and IL-6 resulting in the improvement of UC (33). In our study, sterols, triterpenes, tanins, polyuronides, and saponines have been observed in the contents of the PC. These bioactive compounds were shown to have anti-inflammatory effects. On the other hand, having compounds as of triterpenes (34) and tanins (35) in the composition was shown to exert anti-inflammatory effects by reducing NF-κB activation. The observed anti-inflammatory effect of PCE may be due to these bioactive compounds. Many plant extracts have also been occasionally used in Turkey as an herbal medicine for the treatment of IBD. Several mechanisms of action of herbals have been discussed. Including the suppression of cytokine production via an inhibition of NF-κB activation by extract (36). Among these herbals, it was proposed that nettle extract has broad in vitro anti-inflammatory activities and the synergistic interactions of bioactive compounds in the nettle extract may be responsible in addressing multiple steps in the pro-inflammatory cascade (37).

Colonic depleted GSH and decreased SOD activity in experimental colitis was reversed by PCE administration showing the antioxidant impact of PCE on the colonic tissue. Since the antioxidant properties of PCE have not been reported in the literature before, our finding related to its enhancing effect on the antioxidant defense is a novel finding suggesting that PCE has immunomodulatory and antioxidant bioactive compounds. The turnover of GSH depends on activities of enzymes involved in its metabolism. In the present study, restoration of GSH levels by PCE may be attributed to its free radical scavenging ability. Similarly, it was shown in other experimental colitis models that colonic oxidative damage increases the activity of antioxidant enzymes along with a decrease in GSH levels, and this is associated with increases in the activity of GGT (15). It is also reported that reduced levels of intracellular GSH may induce the activity of GGT (38). On the other hand GGT activity during the catabolism of GSH in the presence of chelated metals leads to free radical generation. Recent research indicates that increased GGT activity could be used as a marker for increased oxidative stress in human (39). Our findings showed that administration of PCE increased GSH levels and suppressed GGT activity showing the antioxidant action of PCE treatment. Our results suggest that increase in SOD and GSH levels in the colonic tissue of PCE-treated groups may be associated with saponins in the PC extract. Saponins were reported to carry antimicrobial, antiinflammatory, antitumoral, antiviral ve antioxidant properties (40), which are effective in GSH, GPx, CAT and SOD metabolism (41,42). Otherwise polyuronides have long-term and powerful antioxidant features (43) and polyuronides in the contents of PCE may be effective in the treatment of colitis. Human and animal diseases for which oxygen radical scavenging therapy is being recommended continues to grow, based primarily on inferential evidence suggesting a potential role for oxygen-derived free radicals in various types of pathophysiology (44).

Increased MPO activity was seen in conjunction with considerable neutrophil accumulation in intestinal mucosal lesions of patients with IBD. Results from animal models further support the contention that neutrophils contribute to tissue injury in IBD (45). In our observation, elevated MPO levels in colonic tissues indicate that neutrophil accumulation contributes to the colitis-induced oxidative injury and PCE appears to have a preventive effect through the inhibition of neutrophil infiltration. It was shown that saponins inhibited neutrophil infiltration and decreased MPO activity in cerebral ischemia reperfusion rats (46). In this study we have also shown that saponins in the structure of PCE on the effect of neutrophil infiltration in colitis decreased MPO activity in PCE-treated groups.

In support of the histopathological findings, acetic acid-induced colitis has increased TF activity of colonic tissue, which can be attributed to the inflammation of the colonic membrane. TF activity can easily be changed by the alterations in membrane composition, heating, changing in pH, or the lipid peroxidation of membrane due to oxidative stress (47,48) as TF is not a stable protein. It can also promote the activation and/or release of many pro-inflammatory and pro-coagulant mediators, whereas inflammatory cytokines and other factors can stimulate TF production (49). PCE administration decreased TF activity in colonic tissue. It can be assumed that the anti-colitis effect of PCE was also reflected to TF activity in colonic tissue.

In conclusion, the results of the current study suggest that extract of Polygonum cognatum Meissn ameliorates colonic inflammation through its anti-inflammatory and antioxidant actions and merits consideration as a potential agent for further clinical experimental studies.
Acknowledgements
The authors would like to thank Prof. Dr. Ertan Tuzlaci (Department of Pharmaceutical Botany, Faculty of Pharmacy, Marmara University, Istanbul, Turkey) for his help for identification of the plant material.

References


42. Li YG, Ji DF, Zhong S, Shi LG, Hu GY, Chen S. Saponins from Panax japonicus protect against alcohol-induced hepatic injury in mice by up-regulating the expression of GPX3, SOD1 and SOD3. Alcohol Alcohol 2010; 45:320-31.


