11.11.2016 : müracaat tarihi / application date 28.11.2016 : kabul tarihi / acceptance

The Effectivity of Centella Asiatica Extract in Experimental Colitis Model Induced by Dextran Sulfate Sodium in Rats

Deneysel Kolit Modelinde Centella Asiatika Ekstresinin Etkinliği

Mustafa UĞUR *
Tümay ÖZGÜR **
Oğuzhan ÖZCAN ***
Seçkin AKKÜÇÜK *
Erol KILIÇ *
Onur KOYUNCU ****
Cem ORUÇ *
Akın AYDOĞAN *
Ozan Utku ÖZTÜRK *
Sedat MOTOR ***
İbrahim YETİM *
Muhittin TEMİZ *

* Mustafa Kemal Üniversitesi Tıp Fakültesi, Genel Cerrahi AD, Hatay, Türkiye
** Mustafa Kemal Üniversitesi Tıp Fakültesi, Patoloji AD, Hatay, Türkiye
*** Mustafa Kemal Üniversitesi Tıp Fakültesi, Biyokimya AD, Hatay, Türkiye
**** Mustafa Kemal Üniversitesi Tıp Fakültesi, Anestezi ve Reanimasyon AD, Hatay, Türkiye

Yazışma Adresi:

Yrd.Doç.Dr. Mustafa Uğur
Mustafa Kemal Üniversitesi Tıp Fakültesi
Genel Cerrahi Anabilim Dalı Hatay
e-mail: drmustafaugur@gmail.com

Öz

Amaç: İnflamatuvar barsak hastalıkları (İBH) çevresel, genetik ve immün faktörlerin sebep olduğu düşünülen bir grup kronik ve inflamatuvar durum olarak tanımlanmaktadır. Tedavisinde başlıca antiinflamatuvar, immünsupresif ve sitotoksik ilaç kombinasyonları kullanılmaktadır. Fakat bu ajanların ciddi yan etkilerinden dolayı yeni tedavi yöntemleri bulmak için araştırmalar yapılmaktadır. Bu çalışmanın amacı dekstran sülfat sodium (DSS) ile indüklenmiş deneysel kolit modelinde Centella Asiatica (CA) ekstresinin etkinliğini araştırmaktır. **Gereç ve Yöntem:** Çalışmada 24 adet Wistar Albino rat üç eşit gruba ayrıldı. Grup 1' e (kontrol) sadece su verildi. Grup 2' ye (kolit) DSS' li içme suyu ve Grup 3'e (medikasyon grubu) 200mg/kg/gün CA ekstresi ve DSS' li içme suyu verildi. Deney sonunda kolonun histopatolojik incelemesi ve myeloperoksidaz (MPO), malonildialdehit (MDO), tümör nekroz factör alfa (TNF-α) ve interlökin-10 (IL-10) düzeyleri belirlendi. **Bulgular:** CA ekstratı veilen grupta kolit grubu ile kıyaslandığında TNF- α düzeyi daha düşüktü ve İL 10 düzeyi daha yüksekti. MPO ve MDA düzeyleri kolit grubunda diğer gruplarla karşılaştırıldığında anlamlı düzeyde daha yüksek bulundu. ($p<0.05$). Histopatolojik incelemede medikasyon grubunda daha az doku hasarı saptandı. **Sonuç:** CA ekstresi proinflamatuvar ve antiinflamatuvar sitokin düzeyini değiştirerek immünregulatuvar bir etki göstermektedir. Ayrıca serbest oksijen radikalleri oluşumunu önleyerek doku hasarını önlemektedir. Bu etkilerinden dolayı CA ekstresinin İBH tedavisinde faydalı bir ajan olarak kullanılabileceğini düşünmekteyiz.

Anahtar Kelimeler: İnflamatuvar barsak hastalığı Centella Asiatika, Dekstran sülfat sodyum, Deneysel Kolit

Abstract

Background: Inflammatory bowel disease (IBD) is a group of chronic and inflammatory conditions that are considered to be caused by environmental, genetic, and immune factors. The treatment of IBD mainly involves combinations of anti-inflammatory, immunosuppressive, and cytotoxic agents. However, due to serious side effects of these agents, research is being performed to find new treatment methods. The aim of this study is to investigate the efficacy of Centella asiatica (CA) in the experimental colitis model. **Materials and Methods:** In this study, twenty-four rats were divided into 3 equal groups; control group (group 1) received only water and standard rat chow; colitis group (group 2) received water with dextran sulfate sodium (DSS); medication group (group 3) received water with DSS, and 200 mg/kg/day CA extract was administered Histopathologic examination of colon, concentrations of myeloperoxidase

(MPO), malondialdehyde (MDA), tumor necrosis factor alpha (TNF-α), and interleukin-10 (IL-10), were determined. **Results:** The results indicated that the TNF-α level was lower and the IL-10 was higher in the rats administered with CA extract compared to the rats in the colitis group ($p<0.05$). The MPO and MDA levels were significantly higher in the colitis group compared to other groups ($p<0.05$). Histopathologic examination revealed less tissue damage in the rats administered with CA extract. **Conclusion:** The CA extract exerts an immunoregulatory effect by altering the levels of anti- and pro-inflammatory cytokines in experimental IBD models. It also prevents tissue damage by inhibiting the formation of free oxygen radicals. Depending on these effects, we consider that the CA extract can be a useful agent in the treatment of IBD.

Keywords: Inflammatory bowel disease, Centella asiatica, Dextran sulfate sodium, experimental colitis

Introduction

Inflammatory bowel disease (IBD) is a group of chronic and inflammatory conditions of the gastrointestinal tract, consisting of Crohn's disease and ulcerative colitis. Although the exact etiopathogenesis of IBD remains unknown, several environmental, genetic, and immune factors are considered to play a role in the development of IBD. In genetically susceptible individuals, an excessive immune response that develops against several antigens or environmental factors is blamed in the etiology of both subtypes of IBD (1). This abnormal response is considered to develop against the luminal bacteria or bacterial products, or against the changes in the barrier function of intestinal mucosa (2).

The treatment of IBD mainly involves combinations of anti-inflammatory and immunosuppressive agents as well as cytotoxic and immune-modulator agents. However, all of these treatment modalities are known to have serious side effects and an extensive research is being carried out to find new treatment methods (3).

Centella asiatica (CA) is a medicinal herb that can reach a height of about 2 meters and is mainly grown in Far Eastern countries including Bangladesh, India, China, and Japan. The leaves of CA are dried and used for medicinal purposes. The CA extract contains three principal ingredients: asiatic acid (AA), asiaticoside (AS), and madecassic acid (MA). In experimental studies, the CA extract has been shown to have antioxidant, antiulcerative, immunomodulatory, antimicrobial, and wound healing properties. In traditional and modern medicine, the CA extract is used in wound healing processes and in the treatment of gastric and duodenal ulcers, leprous skin ulcers, diarrhea, eczema, and various abdominal tumors (4-9).

In this study, we aimed to investigate the healing efficacy and the immunoregulatory effects of the CA extract in experimental colitis model induced by dextran sulfate sodium (DSS) in rats.

Materials And Methods

Prior to the study, an ethical approval was obtained from the Mustafa Kemal University Medical School Local Ethics Committee (Document no: 2013/3-3). All the experimental procedures were performed at the Mustafa Kemal University Medical School Experimental Research Center.

The study included 24 male Wistar Albino rats aged 20 weeks, weighing 220-235 g. Throughout the experiment, the rats were kept under 12h/12h light/dark cycle with standard temperature (23°C) and humidity. All the rats were fed standard rat chow.

The rats were randomly divided into three groups with 8 rats each. In Group I (control group), the rats received drinking water and standard rat chow between the days 1-15, in Group II (colitis group), the rats received drinking water with DSS between the days 1-15, and in Group III (medication group), CA extract was administered and then received drinking water with DSS between the days 1-15. The CA extract was given 200 mg/kg/day in 4 ml of distilled water via orogastric gavage (7).

The original CA extract used in the experiment was granted by Bayer Turk (Istanbul, Turkey) (Lot: 634A, Code: 81104043). DSS was purchased from Sigma Aldrich (St Louis, USA) (molecular weight: 40,000). A DSS concentration of 3% (w/v) was used for the study. Ketamine hydrochloride (Ketalar 50 mg/ml) was obtained from Pfizer (Turkey) and xylazine hydrochloride (Rompun) was obtained from Bayer (Turkey).

Sample Collection

On the morning of day 15, body weight of the rats was measured following the intraperitoneal administration of ketamine 80 mg/kg + 10 mg/kg xylazine. A midline incision was made using sterile techniques. Blood samples were drawn from the cardiac ventricle and the serum was separated and stored in a freezer at -80°C. The entire colon (from the colocecal junction to the anus) was rapidly removed, opened longitudinally along the mesenteric margin, rinsed with ice-cold phosphate-buffered saline (PBS), and cleared of its fatty and mesenteric content. A

10-cm long segment of the distal colon (proximal to the anus) was dissected proximally and distally. The distal segment was placed in formalin solution for histopathologic examination and the proximal segment was used for biochemical analysis and the assessment of cytokines.

Histopathologic Examination

The degree of tissue damage was evaluated by assigning a numerical score to the colon tissue sections obtained from each animal modified from the method of Stucchi et al. (10). After the removal of the colon specimens, the specimens were immediately fixed in 10% neutral-buffered formalin for 24 h and three different areas were sampled. Colon tissues were serially sectioned at 4 µm, and full-thickness sections were stained with hematoxylin and eosin (H&E). Every sample in each group was evaluated by an Olympus BX53 microscope (Olympus, Tokyo, Japan) and graded by a pathologist (TO) blinded to the experiment groups formed according to the scoring system in Table 1.

Biochemical Analysis

The large bowel tissues were removed after sacrificing and frozen immediately at -80°C for biochemical analysis. The tissues were homogenized in ice-cold PBS at pH 7.4 (10% w/v), centrifuged at 10.000 x g for 10 min at 4°C, and the supernatants were removed and portioned for analysis.

The myeloperoxidase (MPO) level was assessed using an enzyme-linked immunosorbent assay (ELISA) kit (Boster, Pleasanton, USA) and the IL-10 and TNF-α levels were also determined using an ELISA kit (ASSAYPRO LLC, St. Charles; BIOSOURCE, Invitrogen Immunoassay kit, California, USA). The IL-10 and TNF-α levels were expressed as picogram (pg)/mg protein and the MPO levels as ng/mg protein. The total protein concentration was assessed by the Lowry method (11). The malondialdehyde (MDA) levels in the tissue homogenates were measured by the double heating method developed by Draper and Hadley (12). This method is based on spectrophotometric measurement of the end product of lipid peroxidation with thiobarbituric acid at 450 nm. The calibration curve was constructed by using commercially available MDA equivalents (Eastbiopharm; Hangzhou, China) and the MDA results were expressed as nmol/mg-protein. Hemoglobine, hematocrit ve lökocyte count were measured in serum analysis.

Statistical Analysis

Data were analyzed using SPSS for Windows 15.0 (Chicago, IL, USA). Data were expressed as mean ± standard deviation (SD). The significance of the differences among the quantitative data of the groups was determined by One-Way ANOVA, followed by post hoc Tukey. The Kruskal-Wallis test was used for the evaluation of the significance among the microscopic colitis scores of the experimental groups, followed by the Mann-Whitney U test. The body weights of the rats both before and after the induction of colitis were compared using the paired t test. A p value of <0.05 was considered significant.

Table 1 Microscopic colitis score

Mucosal epithelium	Crypts	Lamina propria	Submucosal
Ulceration	Mitotic activity	Plasmacytoid infiltrate	Neutrophilic infiltrate
Fibrin deposition	Neutrophilic infiltrate	Neutrophilic infiltrate	Edema
	Mucus depletion	Vascularity	

Scale: 0, none; 1, mild; 2, moderate; 3; severe; maximum score: 30

Results

The hemoglobin and hematocrit levels were significantly decreased both in the colitis and the medication groups compared to the control group ($p<0.05$). The decrease was greater in the colitis group and a significant difference was observed between the colitis and medication groups ($p<0.05$). The leukocyte count was significantly increased in the colitis and medication groups and the highest increase was in the colitis group. Moreover, the increase in the colitis group established a significant difference with that of the medication group ($p<0.05$) (Table 2).

The comparison of pre- and post-experiment measurements of body weights revealed no significant weight loss in the control group but indicated a slight weight loss in the experimental groups. Although no difference was found between the colitis and medication groups, the weight loss was greater in the colitis group than in the medication group ($p>0.05$) (Table 2).

The TNF-α level was significantly increased in the experimental groups compared to the control group and a significant difference was observed among the groups. The highest increase was seen in the colitis group and a significant difference was found between the colitis and medication groups ($p<0.05$), (Table 2).

The IL-10 level was significantly lower in the colitis group compared to other groups. No significant difference was found between the control and medication groups ($p>0.05$) but a significant difference was established between the medication and colitis groups ($p<0.05$) (Table 2).

The MDA and MPO levels were higher in the colitis group compared to other groups. Although no significant difference was found between the control and medication groups ($p>0.05$), a significant difference was observed between the control and colitis groups ($p<0.05$) and between the colitis and medication groups ($p<0.05$) (Table 2).

Histopathologic examination revealed a significant difference among the three groups with regards to the microscopic colitis scores ($p<0.05$). The lowest microscopic colitis scores were seen in the control group and the highest in the colitis groups (Table 2). Normal colon tissue was observed in the control group (Figure 1), whereas diffuse infiltration of acute and chronic inflammatory cells and diffuse mucosal and submucosal injury were found in the colitis group (Figure 2) and both the inflammatory cell

Table 2 Mean values of the blood, tissue, and histopathologic parameters used in the experiment (TNF-α: Tumor necrosis factor, MPO: Myeloperoxidase, MDA: Malondialdehyde, IL-10: Interleukin-10)

	Group 1	Group 2	Group 3
Parameter (Mean ± SD)	n=8	n=8	n=8
Hemoglobin (g/dl)	14.39±0.12	11.08±0.59^{a*}	12.20±0.38^{a,b*}
Hematocrit (vol%)	40.70±1.26	32.79±1.31^{a*}	34.99±1.24^{a,b*}
Leukocyte (x10^3/ul)	9.09±0.37	16.95±1.41^{a*}	14.40±1.41^{a,b*}
Pre-experimental body weight (gr)	225.79±4.44	226.06±3.95	227.05±4.43
Post-experimental body weight (gr)	225.19±4.70	215.00±4.74	219.41±4.38
TNF-□ (pg/mg protein)	56.56±2.29	69.66±4.72^{a*}	60.25±2.36^{a,b*}
MPO (ng/mg protein)	25.63±1.84	44.53±5.10^{a*}	26.97±1.53^{b*}
MDA (mmol/gr protein)	13.57±0.40	15.69±0.40^{a*}	13.88±0.44^{b*}
IL-10 (pg/mg protein)	0.0623±0.007	0.0461±0.003^{a,b*}	0.0590±0.004^{a*}
Microscopic colitis score	1.88±1.73	17.25±2.66^{a*}	10.00±1.30 ^{a,b*}

a: vs control **b:** vs colitis *: $p<0.05$

infiltration and the mucosal and submucosal injury were decreased in the medication group (Figure 3).

Discussion

Inflammatory bowel disease (IBD) is a group of idiopathic, inflammatory, and ulcerogenic diseases of the gastrointestinal tract. Available evidence indicates that an abnormal immune response against the defective mucosal immune system is responsible for the development of IBD. This abnormal response is considered to develop against the luminal bacteria or bacterial products, or against the changes in the barrier function of intestinal mucosa (2,13). Several experimental animal studies have investigated the pathogenesis of IBD by inducing intestinal inflammation and have also analyzed the effectivity of novel therapeutic agents. Literature shows that most of the colitis models have been induced by a 1.5%-5% DSS solution. The colitis induced by DSS has been shown to resemble ulcerative colitis both histologically and clinically. DSS exerts its functions by impairing the barrier function of epithelial cells. As a result, the luminal bacteria gain access to the lamina propria and thereby initiate the inflammation. Several animal experiments have also reported that the administration of DSS in drinking water leads to weight loss, inflammatory changes such as histopathologic erosion in crypts, and impairment in the antioxidant mechanism (14-18). In our study, DSS was administered at a concentration of 3% (w/v) and was found to cause mild-moderate colitis.

Pro- and anti-inflammatory cytokines play a key role in the modulation of mucosal immune system and the resultant intestinal inflammation. In IBD, this balance is altered, resulting in chronic inflammation. Studies have reported that colon biopsies in IBD indicate an elevation in the levels of proinflammatory cytokines including IL-1, IL-2, IL-6, IL-8, IFN- γ, and TNF-α and a reduction in the levels of anti-inflammatory cytokines (19,20).

TNF-α is a proinflammatory cytokine and is synthesized by activated macrophages. It plays a role in the formation of both acute and chronic inflammation, and the TNF-α antibodies are used in the treatment of IBD. IL-10 is

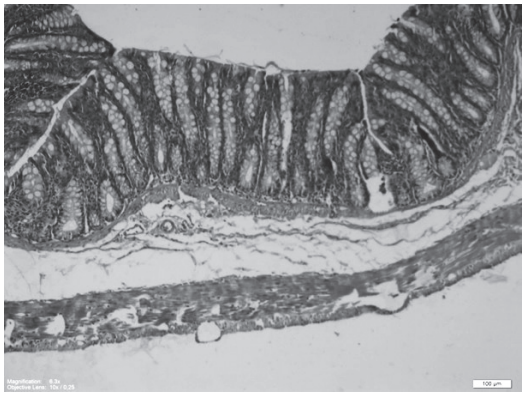

Figure 1: Control group; preserved morphology in colon sections (Hematoxylin&Eosinx100).

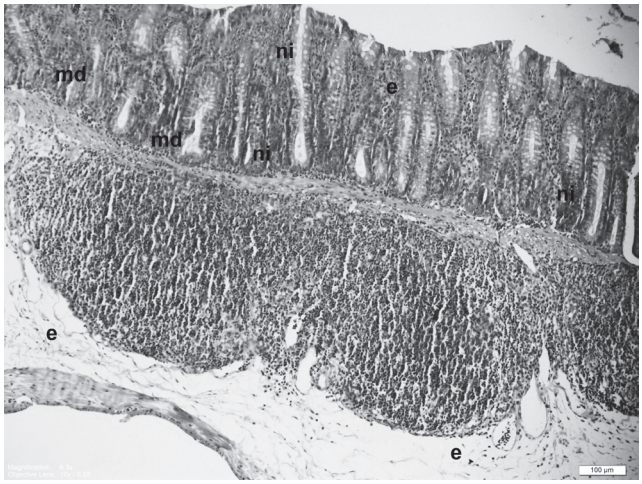

Figure 2: Colitis group; acute and chronic inflammation cells with gland involvement and destruction with mucous depletion in mucosa and submucosa (Hematoxylin&Eosinx100).

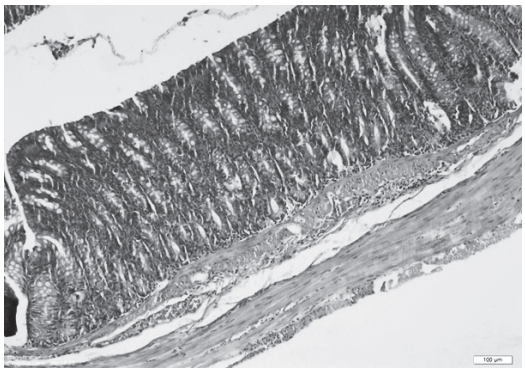

Figure 3: Medication group; partial reduction in acute and chronic inflammation findings in the lamina propria and the glands (Hematoxylin&Eosinx100).

produced by T-cells, B cells, and monocytes activated by lipopolysaccharides. During inflammation, IL-10 inhibits proinflammatory cytokines produced by activated macrophages. IL-10 also plays a role in the modulation of T-cell activation and the downregulation of the acute inflammatory response. Recently, IL-10 has been shown to have a role in the immune modulation of the gastrointestinal tract by alleviating enterocolitis in IL-10-knockout rats (1,21,22).

In our study, the TNF-α level was found to be significantly increased in the colitis group compared to the medication group. Moreover, the IL-10 level was significantly decreased in the colitis group. A slight decrease was seen in the medication group but no significant difference was established. However, a significant difference was found between the colitis and medication groups. These findings indicate that the CA extract exerted an anti-inflammatory effect by inhibiting further secretion of TNF-α by preventing the decrease in IL-10 concentration.

In IBD, the neutrophils and macrophages activated during inflammation lead to increased production of free oxygen and nitrogen radicals including superoxide, nitric oxide (NO), and hydroxyl radical. Excessive production of these reactive oxygen products exceed the capacity of intestinal antioxidant defense systems, and thus oxidative injury results in increased mucosal injury. The level of free oxygen radicals can be determined by the measurement of the level of MDA, which is the end product of lipid peroxidation (23-25). MPO is a major granule constituent and converts hydrogen peroxide to hypochlorous acid in the presence of chloride ion. Hypochlorous acid is a potent oxidant and antimicrobial agent. During inflammation, the MPO levels are increased and lead to increased tissue damage (26).

Literature shows that there have been numerous studies reporting on the effectivity of CA extract. In several experimental studies, the CA extract has been shown to cause increased fibroblast proliferation and collagen synthesis. The role of the CA extract in wound healing both in vivo and in vitro has also been demonstrated and the CA extract has been reported to increase epithelization. Several other studies reported that the administration of CA extract at a dose of 100 mg/kg exerted an anti-inflammatory effect by decreasing the cytokine level and this effect was equivalent to the effect exerted by the standard Ibuprofen. The CA extract has also been demonstrated to increase the levels of antioxidant enzymes including superoxide dismutase, catalase, and glutathione peroxidase and to exert antioxidant activity by decreasing the level of MDA, which is an end product of lipid peroxidation. Additionally, in an experimental IBD model, the CA extract was found to decrease the colonic MPO level and to decrease the severity of the disease by preventing weight loss (4, 27,28). In a recent study, CA had been shown to reduce reactive oxygen species leading to an amelioration in the experimantal colitis (29).

In our study, no significant difference was observed between the MDA and MPO levels in the control and medication groups. However, the MDA and MPO levels in the colitis group were significantly higher compared to those in other groups. These findings suggest that the CA extract exerted antioxidant activity in the presence of colitis both by decreasing the secretion of MPO through the inhibition of neutrophil infiltration into the colon and by preventing the formation of free oxygen radicals.

Experimental studies indicate that IBD patients may be presented with decreased hemoglobin (Hgb) and hematocrit (Hct) levels and increased leukocyte count (30, 31). Weight loss is another clinical finding presented by IBD patients and is used as a parameter for the determination of the severity of the disease. Weight loss of more than 10% of body weight is accepted as an indicator of the severity of the disease (32,33). In our study, depending on the Hgb and Htc levels, leukocyte count, and the amount of weight loss, we consider that the administration of the CA extract led to a significant decrease in the severity of the disease and provided effective outcomes. Especially decreased blood loss due to CA treatment may explain the higher Hgb and Htc levels in Group III.

In the histopathologic examination of IBD, a large number of activated neutrophils and macrophages are observed in the intestinal mucosa during inflammation. In addition, mucosal injury, mucus depletion in the crypts, increased vascularity in the lamina propria, fibrin deposition, and submucosal edema may also be detected. The levels of these parameters are used for the calculation of the microscopic colitis score (10,34).

In our study, the microscopic colitis score was increased in the experimental groups compared to the control group and the highest score was seen in the colitis group. It was also found that the administration of the CA extract led to a significant decrease in the microscopic colitis score in the medication group compared to the colitis group. We consider that the CA extract exerted this effect due to its anti-inflammatory properties and performed the effect by inhibiting neutrophil and macrophage infiltration into the intestinal mucosa. We also consider that the antioxidant

properties of the CA extract prevented further tissue damage.

In experimental IBD, CA extract prevents the secretion of TNF alpha which is a pro-inflammatory cytokine. Furthermore, CA extract keeps the IL 10 levels in the range which can produce an anti-inflammatory effect. In addition to that, it reduces the infiltration of neutrophiles and macrophages into intestine, thus prevents tissue damage by reducing the inflammation and preventing SOR production. We believe that CA extract application in the treatment of IBD patients should be further investigated by clinical studies.

This study was financially supported by the Mustafa Kemal University Scientific Research Projects Unit.
We thank Bayer Turk for granting us the Centella Asiatica extract to be used for scientific experiments.

References

1. Podolsky DK. Inflammatory bowel disease. N Engl J Med. 2002;8:347:417-29.
2. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature. 2007; 26;448:427-34.
3. Kuhbacher T, Fölsch UR. Practical guidelines for the treatment of inflammatory bowel disease. World J Gastroenterol. 2007:28;13:1149-55.
4. Roy DC, Barman SK, Shaik MM. Current updates on Centella asiatica: Phytochemistry, pharmacology and traditional uses. Medicinal Plant Research 2013;3: 20-36
5. Won JH, Shin JS, Park HJ, Jung HJ, Koh DJ, Jo BG et al. Anti-inflammatory effects of madecassic acid via the suppression of NF-kappaB pathway in LPS-induced RAW 264.7 macrophage cells. Planta Med. 2010;76:251-7.
6. Jayashree G, Kurup Muraleedhara G, Sudarslal S, Jacob VB. Anti-oxidant activity of Centella asiatica on lymphoma-bearing mice. Fitoterapia. 2003;74:431-4.
7. Sairam K, Rao CV, Goel RK. Effect of Centella asiatica Linn on physical and chemical factors induced gastric ulceration and secretion in rats. Indian J Exp Biol. 2001;39:137-42.
8. Punturee K, Wild CP, Kasinrerk W, Vinitketkumnuen U. Immunomodulatory activities of Centella asiatica and Rhinacanthus nasutus extracts. Asian Pac J Cancer Prev. 2005;6:396-400.
9. Sunilkumar, Parameshwaraiah S, Shivakumar HG. Evaluation of topical formulations of aqueous extract of Centella asiatica on open wounds in rats. Indian J Exp Biol. 1998;36:569-72.
10. Stucchi AF, Shofer S, Leeman S, Materne O, Beer E, McClung J et al. NK-1 antagonist reduces colonic inflammation and oxidative stress in dextran sulfate-induced colitis in rats. Am J Physiol Gastrointest Liver Physiol. 2000;279:1298-306.
11. Lowry O, Rosenbrough N, Farr L. Protein measurement with the folin phenol reagent. J Biol Chem. 1951;193;265-275.
12. Draper HH, Hadley M. Methods.Enzymol. Malondialdehyde determination as index of lipid peroxidation.1990;186:421-31.
13. Malik TA. Inflammatory Bowel Disease: Historical Perspective, Epidemiology, and Risk Factors. Surg Clin North Am. 2015;95:1105-22.
14. Okayasu I, Hatakeyama S, Yamada M, Ohkusa T, Inagaki Y, Nakaya R. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. Gastroenterology. 1990;98:694-702.
15. Dieleman LA, Palmen MJ, Akol H, Bloemena E, Peña AS, Meuwissen SG et al. Chronic experimental colitis induced by dextran sulphate sodium (DSS) is characterized by Th1 and Th2 cytokines. Clin Exp Immunol. 1998;114:385-91.
16. Chen LL, Wang XH, Cui Y, Lian GH, Zhang J, Ouyang CH et al. Therapeutic effects of four strains of probiotics on experimental colitis in mice. World J Gastroenterol. 2009;15:321-7.
17. Jungbeck M, Daller B, Federhofer J, Wege AK, Wimmer N, Männel DN et al. Neutralization of LIGHT ameliorates acute dextran sodium sulphate-induced intestinal inflammation. Immunology. 2009;128:451-8.
18. Cooper HS, Murthy SN, Shah RS, Sedergran DJ. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. Lab Invest. 1993; 69:238-49.
19. Harries AD, Beeching NJ, Rogerson SJ, Nye FJ. The platelet count as a simple measure to distinguish inflammatory bowel disease from infective diarrhoea. J Infect. 1991;22:247-50.
20. Herías MV, Koninkx JF, Vos JG, Huis in't Veld JH, van Dijk JE. Probiotic effects of Lactobacillus casei on DSS-induced ulcerative colitis in mice. Int J Food Microbiol. 2005;103:143-55.
21. Stange EF, Travis SP, Vermeire S, Reinisch W, Geboes K, Barakauskiene A et al. European Crohn's and Colitis Organisation (ECCO). European evidence-based Consensus on the diagnosis and management of ulcerative colitis: Definitions and diagnosis. J Crohns Colitis. 2008;2:1-23.
22. Camuesco D, Comalada M, Rodríguez-Cabezas ME, Nieto A, Lorente MD, Concha A et al. The intestinal anti inflammatory effect of quercitrin is associated with an inhibition in iNOS expression. Br J Pharmacol. 2004;143:908-18.
23. Obermeier F, Kojouharoff G, Hans W, Schölmerich J, Gross V, Falk W. Interferon-gamma (IFN-gamma)- and tumour necrosis factor (TNF)-induced nitric oxide as toxic effector molecule in chronic dextran sulphate sodium (DSS)-induced colitis in mice. Clin Exp Immunol. 1999;116:238-45.

24. Rogler G, Andus T. Cytokines in inflammatory bowel disease. World J Surg. 1998;22:382-9.
25. Cui HH, Chen CL, Wang JD, Yang YJ, Cun Y, Wu JB et al. Effects of probiotic on intestinal mucosa of patients with ulcerative colitis. World J Gastroenterol. 2004;10:1521-5.
26. Mosmann TR Cytokine secretion patterns and cross-regulation of T cell subsets. Immunol Res. 1991;10:183-8.
27. Shukla A, Rasik AM, Jain GK, Shankar R, Kulshrestha DK, Dhawan BN. In vitro and in vivo wound healing activity of asiaticoside isolated from Centella asiatica. J Ethnopharmacol. 1999;65:1-11.
28. di Paola R, Esposito E, Mazzon E, Caminiti R, Toso RD, Pressi G et al. 3,5-Dicaffeoyl-4-malonylquinic acid reduced oxidative stress and inflammation in a experimental model of inflammatory bowel disease. Free Radic Res. 2010;44:74-89.
29. Guo W, Liu W, Jin B, Geng J, Li J, Ding H, et al. Asiatic acid ameliorates dextran sulfate sodium-induced murine experimental colitis via suppressing mitochondria-mediated NLRP3 inflammasome activation. Int Immunopharmacol. 2015;24:232-8.
30. Kühn R, Löhler J, Rennick D, Rajewsky K, Müller W. Interleukin-10-deficient mice develop chronic enterocolitis. Cell. 1993;75:263-74.
31. Kitahora T, Suzuki K, Asakura H, Yoshida T, Suematsu M, Watanabe M et al. Active oxygen species generated by monocytes and polymorphonuclear cells in Crohn's disease. Dig Dis Sci. 1988;33:951-5.
32. Mahida YR, Wu KC, Jewell DP. Respiratory burst activity of intestinal macrophages in normal and inflammatory bowel disease. Gut. 1989;30:1362-70.
33. Grisham MB, Granger DN. Neutrophil mediated mucosal injury. Role of reactive oxygen metabolites. Dig Dis Sci. 1988;33: 6-15.
34. Korenaga D, Takesue F, Kido K, Yasuda M, Inutsuka S, Honda M et al. Impaired antioxidant defense system of colonic tissue and cancer development in dextran sulfate sodium-induced colitis in mice. J Surg Res. 2002;102:144-9.