

## The Healing Effect of *Hypericum perforatum* Extract on Acetic Acid-Induced Ulcerative Colitis in Rat

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**Background:** Ulcerative colitis (UC) is a form of inflammatory bowel disease (IBD). There are several chemical and herbal drug regimens for treatment of UC.

**Objectives:** The aims of this study were to investigate the effects of *Hypericum perforatum* on histopathological and tissue malondialdehyde (MDA) level of colonic tissue in rat with induced UC.

**Materials and Methods:** Two milliliters of 3% acetic acid was administered into the colon to induce UC. Seventy rats were divided into seven equal groups. Groups I and II received 1 mL of 600 and 300 mg/kg *H. perforatum* extract orally per day respectively; groups III and IV received 1 mL of 20% and 10% intra-colonic gel form of *H. perforatum* extract daily respectively; group V as positive control received 2 mL of intra-colonic asacol; group VI was a negative control receiving 0.5 mL/kg of normal saline after induction of UC; group VII received just intra-colonic gel base. All the animals were evaluated for histological changes and tissue MDA level seven days after the treatment.

**Results:** *H. perforatum* extract in the two forms of trans-rectal and oral administration on the seventh day after the therapy could result in a more healing effect on acetic acid-induced damaged colonic tissue with a reduction in the MDA activity. In trans-rectal administration, the 20% gel form had a better healing response than the 10% gel form and was prominently more effect on the seventh day of the therapy. In oral administration of strawberry extract, the 600 mg/kg dosage had a better healing response than the 300 mg/kg and was significantly more effective on the seventh day of therapy.

**Conclusions:** So *H. perforatum* may be considered as a treatment of choice for UC especially in gel form to broaden the current therapy options of the disease.

**Keywords:** *Hypericum perforatum*; Inflammatory Bowel Disease; Ulcerative Colitis; Malondialdehyde

### 1. Background

Inflammatory bowel disease (IBD) comprises ulcerative colitis (UC) and Crohn's disease (CD) which are defined as relapsing, chronic and remitting inflammatory diseases of the large intestine and are complex and multi-factorial diseases with unknown etiologies, caused by several pathophysiological mechanisms and many clinical manifestations such as abdominal pain, diarrhea, blood in stool and weight loss (1, 2). The incidence of UC has been reported about 10-20 per 1000000 per year with a prevalence of 100-200 per 1000000 in Western countries (3). In addition, a persistent UC can increase the risk of development of colorectal cancer by ~10 folds (4-6). During the past 20 years, various murine models of colitis

have been developed as indispensable tools to decipher the underlying mechanisms of IBD pathogenesis as well as to determine many potential therapeutics (7). These included dextran sodium sulfate, 2,4,6-trinitrobenzene sulfonic acid, oxazolone, acetic acid, and indomethacin. Among them, acetic acid-induced colitis models are identical to human UC in terms of histopathological changes and various herbal drugs have been studied regarding the treatment of experimental models of UC (8). There are several drug regimens for the treatment of UC. The extent and severity of UC and its anatomic location affect the agents used to induce remission in patients with UC, including both oral and topical regimens. Sulfasalazine

and its aminosaliclylate analogues, corticosteroids, immunomodulators, suppressive antimetabolites, antitumor necrosis factor biologics including infliximab, and in some cases antibiotics have been reported as treatments of choice (9). In previous studies, herbal extracts have been used to treat UC in many cases (8-14). *Hypericum perforatum*, known botanically as St. John's wort, is an herbaceous perennial medicinal plant with yellow flowers belonging to the family of Clusiaceae (15). It is used for treatment of anxiety, depression, cuts, burns, cancer, and as an antioxidant, analgesic, and neuroprotective agent (16). Moreover, it has been proposed to have antibacterial and antiviral effects (17) which are partially mediated via inhibition of the transcription factor NF- $\kappa$ B (18) and interfere with some serine/threonine kinases of the protein kinase C (PKC) family (19). *H. perforatum* also possesses remarkable wound healing and anti-inflammatory properties (20). Although, the healing activity of *H. perforatum* in induced oral mucositis in golden hamster was reported recently by our group (16), but the evidences about its efficiency in UC in human and animal models is scarce.

## 2. Objectives

The aims of this study were to determine the healing effects of administration of *H. perforatum* as two dietary and two gel dosages and compare them with asacol on the tissue histopathological changes and malondialdehyde (MDA) level in male rats with experimentally acetic acid induced ulcerative colitis.

## 3. Materials and Methods

### 3.1. Animal Housing

This study was approved by the Animal Care and Use Committee of Shiraz University of Medical Sciences, Shiraz, Iran. Seventy male Sprague Dawley rats weighing 200-250 g were provided from the Center of Experimental and Comparative Medicine, Shiraz University of Medical Sciences and were randomly allocated into seven equal groups and treated as following:

Group I: received 1 mL of 600 mg/kg *H. perforatum* extract orally per day.

Group II: received 1 mL of 300 mg/kg *H. perforatum* extract orally per day.

Group III: received 1 mL of 20% intracolonic gel form of *H. perforatum* extract daily.

Group IV: received 1 mL of 10% intracolonic gel form of *H. perforatum* extract daily.

Group V: received 2 mL of asacol intracolonic (0.5 mL asacol dissolved in 9.5 mL normal saline) as the positive control group.

Group VI: received 0.5 mL/kg intracolonic normal saline after induction of colitis as the negative control.

Group VII: received only intracolonic gel base.

Each rat was housed individually in a single cage in a 65-

70% relative humidity and an ambient temperature of  $21 \pm 2^\circ\text{C}$ . The animals received a balanced diet and had access to water ad libitum.

### 3.2. Plant Material and Extraction

*H. perforatum* fresh plants were prepared from Shiraz, Iran and its species was determined in the Department of Botany of Shiraz University of Medical Sciences. To prepare the hydroalcoholic extract, the provided plants were dried for five days in room temperature and were then powdered using percolation method, while 1000 g of the powdered form of the plant was transferred to ethanol:water (70:30) solution for 48 hours. The semisolid and gel forms of the extract (37.7% w/w) were provided after filtration and evaporation under reduced pressure in the rotary evaporator (8).

### 3.3. Ulcerative Colitis Induction

All the animals were fasted overnight and their bowels were cleaned before induction of colitis. A polyurethane cannula (2 mm diameter) was applied for the rectal entrance of acetic acid and the tip was inserted up to 8 cm proximal to the anus verge. Two milliliters of 3% acetic acid was administered transrectally into the colon by a cannula during 30 seconds to induce UC under ketamine and xylazine anesthesia.

### 3.4. Histopathological Evaluation

To determine the induction of UC, all the animals were euthanized after seven days. For all the animals, the distal 10 cm portion of the colon was removed for histological evaluation. Tissue processing and section preparation were performed according to previous studies (21-23). The degree of inflammation of the colon was graded as described before (24).

### 3.5. Malondialdehyde Measurement

Tissue specimens were provided and stored at  $-80^\circ\text{C}$  for subsequent evaluation of tissue MDA activity. MDA production was determined in the tissues (25). MDA resulted into formation of a colored complex in the presence of TBA (thiobarbituric acid) and HCl which was later detectable by spectrophotometer measurement at the absorbance of 532 nm (Shimadzu uv-160, Japan). 1,1', 3, 3'-tetraethoxypropane was used as the standard solution and the findings were presented as  $\mu\text{mol/g}$  of tissue.

### 3.6. Statistical Analysis

The results were presented as mean  $\pm$  SD. Differences among the groups were determined using one-way ANOVA and less significant difference (LSD) post-hoc test. All the statistical analyses were performed by SPSS software (version 16, Chicago, IL, USA). Significance was considered at  $P < 0.05$ .

## 4. Results

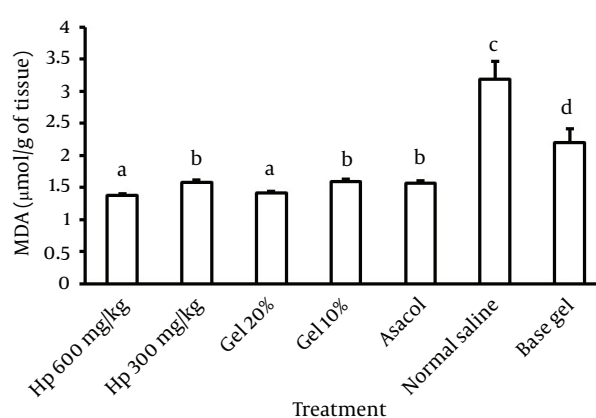
Comparisons of tissue MDA level between seven groups are presented in Figure 1. There were significant differences between groups VI and VII, between each of them with all other five groups, and comparing groups II, IV and V with I and III ( $P < 0.05$ ), seven days after the treatment measurements. However, in all treated groups (I-V), the level of MDA was significantly lower than the negative control (group VI) and the base gel group (VII). The overall histopathology score that included all the inflammatory processes is shown in Figure 2. Regarding scoring of all the inflammatory features, significant decreases were observed in groups I-V when compared with group V ( $P < 0.05$ ). However, this decrease was not significant between groups II-IV and VII ( $P > 0.05$ ). Difference between groups VI and VII was not significant as well ( $P > 0.05$ ) (Figure 2). Regarding the inflammatory features, some significant decreases in ulcer, inflammation, crypt absence, disorganization and destruction and some increases in repair and granulation were observed in groups I-V in comparison to groups VI and VII ( $P < 0.05$ ). Totally, the most healing effects were seen in 600 mg/kg dietary and 20% gel forms according to all the seven inflammation steps evaluations (Figure 3). *H. perforatum* extract had a dose-dependent healing effect while the 20% gel intracolonic administration resulted in a better healing effect in comparison with the 10% gel form. In oral administration, the 600 mg/kg dosage resulted in a better healing effect in comparison with the 300 mg/kg dose of the extract. These healing effects were identical to asacol, especially in 20% intracolonic gel form and 600 mg/kg oral administration, which showed a significant healing effect on acetic acid-induced colitis in rats.

## 5. Discussion

This study compared the healing effects of *H. perforatum* extract in 300 and 600 mg/kg dietaries and 10% and 20% gel forms against acetic acid-induced UC by measuring tissue histopathology and MDA level in rats. Both dietary and gel forms had significant obvious healing effects and these effects were dose dependent. The mainstay of medical therapy for UC focuses on medications that change the host response to decrease mucosal inflammation (9). Between these medical agents, natural products represent a source of chemical structures of varying pharmacological interests. *H. perforatum* possessed remarkable wound healing and anti-inflammatory properties (20). The anti-inflammatory effects of *H. perforatum* can be related in part to its inhibition of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) (15). The most important anti-inflammatory compounds found in this plant are pseudohypericin and hyperforin. These constituents along with flavonoids can exert their effects by inhibiting prostaglandin E2 production (26). Furthermore, hyperoside, another active compound of *H. perforatum*, has an anti-inflammatory action by suppressing

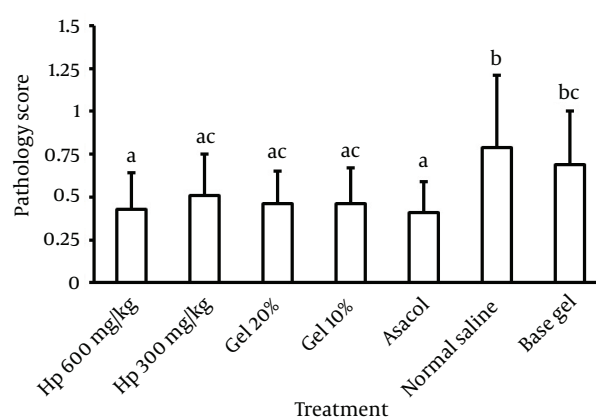
the production of tumor necrosis factor, interleukin 6, and nitric oxide (27). In addition, the Clusiaceae family has been studied extensively. *Hypericum* as a member of this family has been effective in the treatment of burns and has been used for treatment of gastrointestinal diseases (28) and wound healing (29). However, the effect of *H. perforatum* on UC model has been reported by one study performed by Dost et al. In their study, the effects of *H. perforatum* on the inflammatory and immune responses of colonic mucosa in Wistar rat with 2, 4, 6-trinitrobenzene sulfonic acid (TNBS)-induced IBD were evaluated. They concluded that *H. perforatum* had a protective effect on TNBS-induced IBD, probably due to an anti-inflammatory and antioxidant mechanism (30). In our study, compared to the report by Dost et al.

**Figure 1.** Malondialdehyde Level in Colitis-Induced Colonic Tissue of Rats After Seven Days



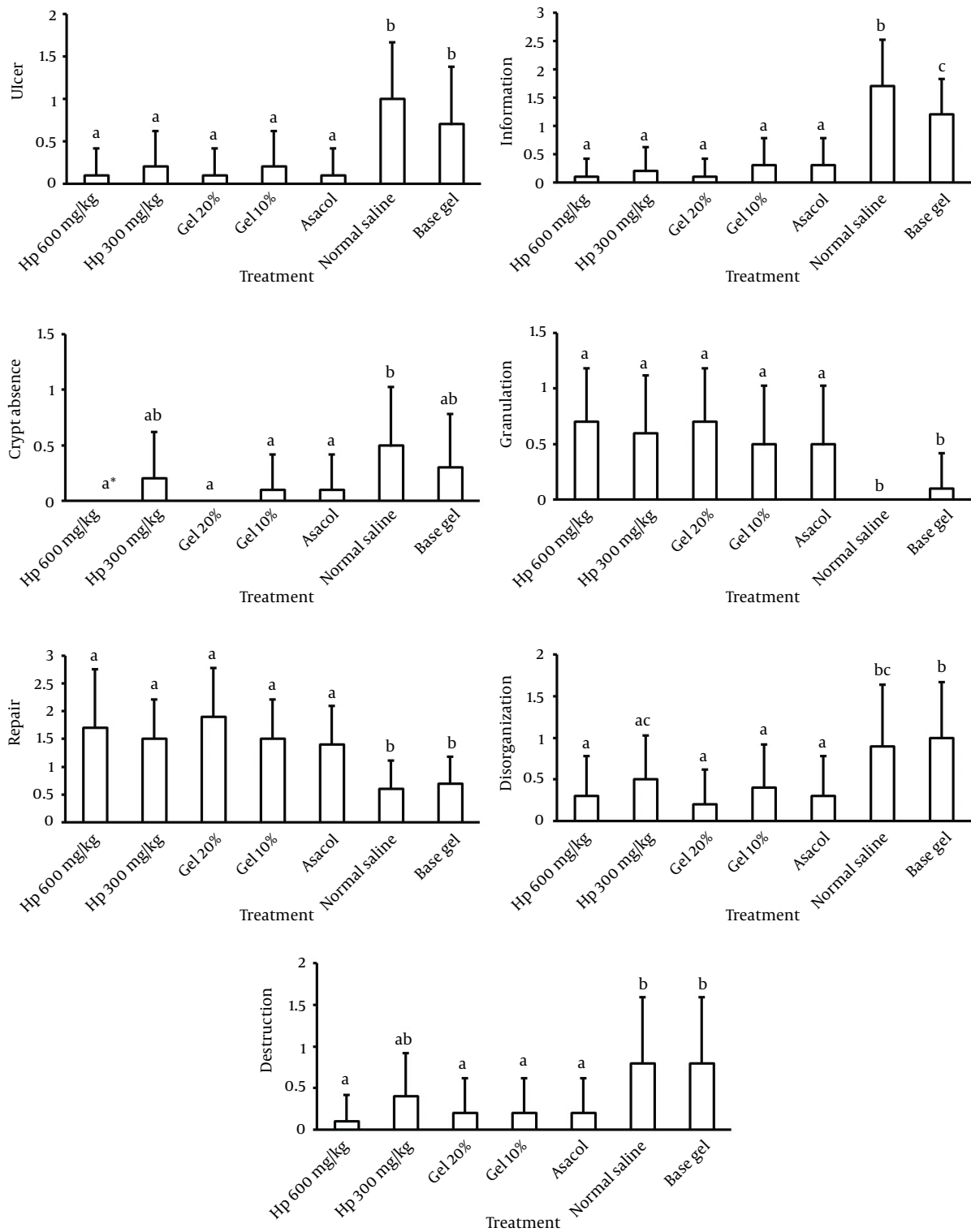
Different superscript letters show significant differences between different groups ( $P < 0.05$ ).

**Figure 2.** Total Histopathology Score of Colon Tissue of Acetic Acid-Induced Ulcerative Colitis in Rats After Seven Days



Different superscript letters show significant differences between different groups ( $P < 0.05$ ).

**Figure 3.** Histopathological Evaluation of Colon Tissue of Acetic Acid-Induced Ulcerative Colitis in Rats After Seven Days



Different superscript letters show significant differences between different groups ( $P < 0.05$ ). \*, Some bars lacked due to the zero value.

(30) UC was induced by different agents (acetic acid vs. TNBS) and the *H. perforatum* extract was administered at different doses and different drug forms. However, time of evaluation and overall findings were similar and demonstrated the potential beneficial healing effects of *H. perforatum* on UC. *H. perforatum* can reduce the area of the surgical wound and increase tissue regeneration (29). The wound-healing effect of *H. perforatum* extract seems to be mainly due to increased stimulation of fibroblast collagen production and activation of fibroblast cells in polygonal shape, which play a role in wound repair by closing the damaged area (30). In addition, there are several reports in which the MDA levels increased in UC, but decreased with treatment by chemical agents (31-33) or medicinal plant extracts (8, 13, 14). MDA is frequently used in measurement of lipid peroxide levels and, exhibiting good correlation with degree of lipid peroxidation. In the present study, the MDA levels decreased in *H. perforatum*-treated groups at the end. This decrease may in fact support the protective effect of *H. perforatum* against lipid peroxidation in UC. Such effect against lipid peroxidation had been reported previously in oral mucositis animal model (16).

In conclusion, this study demonstrated that daily application of *H. perforatum* extract in gel form or by oral administration can relieve the ulcerative colitis induced by acetic acid in rat colon. Results for tissue MDA and histopathological evaluations indicated a reduction in inflammation. The healing, anti-inflammatory, and antimicrobial properties of *H. perforatum* can make it an appropriate drug choice for the treatment of ulcerative colitis. Further studies are required to confirm its clinical effectiveness in humans.

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## Authors' Contributions

Nader Tanideh: study concept and design. Seyede Leila Nematollahi: acquisition of data. Seyed Vahid Hosseini: study supervision. Masood Hosseinzadeh: study concept and design. Davood Mehrabani: writing the manuscript, acquisition of data. Alireza Safarpour: analysis and interpretation of data, statistical analysis. Masood Sepehrimanesh and Omid Koochi-Hosseiniabadi: writing the manuscript. Asma Najibi: acquisition of data.

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## References

1. Safarpour AR, Hosseini SV, Mehrabani D. Epidemiology of inflam-

2. matory bowel diseases in Iran and Asia; a mini review. *Iran J Med Sci.* 2013;**38**(2 Suppl):140-9.
3. Taghavi SA, Safarpour AR, Hosseini SV, Noroozi H, Safarpour M, Rahimikazerooni S. Epidemiology of Inflammatory Bowel Diseases (IBD) in Iran: A review of 740 patients in Fars province, Southern Iran. *Ann Colorectal Res.* 2013;**1**(1):17-22.
4. Loftus EV, Jr. Clinical epidemiology of inflammatory bowel disease: Incidence, prevalence, and environmental influences. *Gastroenterology.* 2004;**126**(6):1504-17.
5. Mehrabani D, Almasi-Hashiani A, Moshfeghi K, Khedmati E. Survival Rate and its Predictors in Colorectal Cancer Patients, Southern Iran. *Middle East J Sci Res.* 2012;**12**(8):1072-7.
6. Mehrabani D, Tabei SZ, Heydari ST, Shamsina SJ, Shokrpour N, Amini M, et al. Cancer occurrence in Fars Province, Southern Iran. *Iran Red Crescent Med J.* 2008;**10**(4):314-22.
7. Mehrabani D, Almasi-Hashiani A. Evaluation of the 5-year survival rate and demographic factors in colorectal cancer patients. *Zanjan Univ Med Sci J.* 2012;**20**(82):12-9.
8. Chassaing B, Aitken JD, Malleshappa M, Vijay-Kumar M. Dextran sulfate sodium (DSS)-induced colitis in mice. *Curr Protoc Immunol.* 2014;**104**:Unit 15 25.
9. Tanideh N, Afaridi E, Mehrabani D, Azarpira N, Hosseinzadeh M, Amini M, et al. The Healing Effect of Berberis vulgaris in Acetic Acid-Induced Ulcerative Colitis in Rat. *Middle East J Sci Res.* 2014;**21**(8):1288-94.
10. Mehrabani D, Bahrami F, Hosseini SV, Ashraf MJ, Tanideh N, Rezaianzadeh A, et al. The Healing Effect of Teucrium polium in Acetic Acid-Induced Ulcerative Colitis in the Dog as an Animal Model. *Middle East J Dig Dis.* 2012;**4**(1):40-7.
11. Mehrabani D, Ziaei M, Hosseini SV, Ghahramani L, Bananzadeh AM, Ashraf MJ, 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):884-90.
12. Zargari Samadnejad A, Mehrvarz S, Mohammadi Samani S, Alizadeh-Naeini M, Tanideh N. Healing effect of Licorice extract in acetic acid-induced ulcerative colitis in rat. *Res Pharmaceut Sci.* 2012;**7**(5):S837.
13. Mehrabani D, Nasibi M, Izadpanah A, Tanideh N, Amini M, Hosseini SV. The Comparison of two methods of steroid therapy via appendicostomy and enema in experimental acetic acid induced ulcerative colitis in dog. *World Appl Sci J.* 2013;**26**(12):1538-45.
14. Tanideh N, Baseri FA, Jamshidzadeh A, Ashraf MJ, Kuchi O, Mehrabani D. The Healing Effect of Strawberry Extract on Acetic Acid-Induced Ulcerative Colitis in Rat. *World Appl Sci J.* 2014;**31**(3):281-8.
15. Tanideh N, Masoumi S, Hosseinzadeh M, Safarpour AR, Erjaee H, Koochi-Hosseiniabadi O, et al. Healing effect of pistacia atlantica fruit oil extract in acetic Acid-induced colitis in rats. *Iran J Med Sci.* 2014;**39**(6):522-8.
16. Raso GM, Pacilio M, Di Carlo G, Esposito E, Pinto L, Meli R. In-vivo and in-vitro anti-inflammatory effect of Echinacea purpurea and Hypericum perforatum. *J Pharm Pharmacol.* 2002;**54**(10):1379-83.
17. Tanideh N, Namazi F, Andisheh Tadbir A, Ebrahimi H, Koochi-Hosseiniabadi O. Comparative assessment of the therapeutic effects of the topical and systemic forms of Hypericum perforatum extract on induced oral mucositis in golden hamsters. *Int J Oral Maxillofac Surg.* 2014;**43**(10):1286-92.
18. Barnes J, Anderson LA, Phillipson JD. St John's wort (Hypericum perforatum L.): a review of its chemistry, pharmacology and clinical properties. *J Pharm Pharmacol.* 2001;**53**(5):583-600.
19. Bork PM, Bacher S, Schmitz ML, Kaspers U, Heinrich M. Hypericin as a non-antioxidant inhibitor of NF-kappa B. *Planta Med.* 1999;**65**(4):297-300.
20. Agostinis P, Vandenbogaerde A, Donella-Deana A, Pinna LA, Lee KT, Goris J, et al. Photosensitized inhibition of growth factor-regulated protein kinases by hypericin. *Biochem Pharmacol.* 1995;**49**(11):1615-22.
21. Suntar IP, Akkol EK, Yilmazer D, Baykal T, Kirmizibekmez H, Alper M, et al. Investigations on the in vivo wound healing potential of Hypericum perforatum L. *J Ethnopharmacol.* 2010;**127**(2):468-77.
22. Sepehrimanesh M, Azarpira N, Saeb M, Nazifi S, Kazempour N, Koochi O. Pathological changes associated with experimental 900-MHz electromagnetic wave exposure in rats. *Comp Clin Path.*

- 2014;**23**(5):1629-31.
22. Khodakaram Tafti A, Nazifi S, Rajaian H, Sepehrimanesh M, Poorbaghi SL, Mohtarami S. Pathological changes associated with experimental salinomycin toxicosis in sheep. *Comp Clin Pathol*. 2008;**17**(4):255-8.
  23. Tamadon A, Nikahval B, Sepehrimanesh M, Mansourian M, Naeini AT, Nazifi S. Epididymis ligation: a minimally invasive technique for preparation of teaser rams. *Vet Surg*. 2010;**39**(1):121-7.
  24. Shamsa F, Ahmadiani A, Khosrokhavar R. Antihistaminic and anticholinergic activity of barberry fruit (*Berberis vulgaris*) in the guinea-pig ileum. *J Ethnopharmacol*. 1999;**64**(2):161-6.
  25. Hajhashemi V, Ghannadi A, Hajiloo M. Analgesic and Anti-inflammatory Effects of *Rosa damascena* Hydroalcoholic Extract and its Essential Oil in Animal Models. *Iran J Pharm Res*. 2010;**9**(2):163-8.
  26. Hammer KD, Hillwig ML, Solco AK, Dixon PM, Delate K, Murphy PA, et al. Inhibition of prostaglandin E(2) production by anti-inflammatory hypericum perforatum extracts and constituents in RAW264.7 Mouse Macrophage Cells. *J Agric Food Chem*. 2007;**55**(18):7323-31.
  27. Kim SJ, Um JY, Lee JY. Anti-inflammatory activity of hyperoside through the suppression of nuclear factor-kappaB activation in mouse peritoneal macrophages. *Am J Chin Med*. 2011;**39**(1):171-81.
  28. Conforti F, Statti GA, Tundis R, Menichini F, Houghton P. Antioxidant activity of methanolic extract of *Hypericum triquetrifolium* Turra aerial part. *Fitoterapia*. 2002;**73**(6):479-83.
  29. Lavagna SM, Secci D, Chimenti P, Bonsignore L, Ottaviani A, Bizzarri B. Efficacy of *Hypericum* and *Calendula* oils in the epithelial reconstruction of surgical wounds in childbirth with caesarean section. *Farmacol*. 2001;**56**(5-7):451-3.
  30. Dost T, Ozkayran H, Gokalp F, Yenisey C, Birincioglu M. The effect of *Hypericum perforatum* (St. John's Wort) on experimental colitis in rat. *Dig Dis Sci*. 2009;**54**(6):1214-21.
  31. Cetinkaya A, Bulbuloglu E, Kurutas EB, Ciralik H, Kantarceken B, Buyukbese MA. Beneficial effects of N-acetylcysteine on acetic acid-induced colitis in rats. *Tohoku J Exp Med*. 2005;**206**(2):131-9.
  32. Cetinkaya A, Bulbuloglu E, Kantarceken B, Ciralik H, Kurutas EB, Buyukbese MA, et al. Effects of L-carnitine on oxidant/antioxidant status in acetic acid-induced colitis. *Dig Dis Sci*. 2006;**51**(3):488-94.
  33. Necefli A, Tulumoglu B, Giris M, Barbaros U, Gunduz M, Olgac V, et al. The effect of melatonin on TNBS-induced colitis. *Dig Dis Sci*. 2006;**51**(9):1538-45.