www.biodicon.com Biological Diversity and Conservation

ISSN 1308-8084 Online; ISSN 1308-5301 Print 5/2 (2012) 59-65

*Research article/Araştırma makalesi*

# Protective effects of *Echinacea purpurea* in an acetic acid induced rat model of colitis

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**Abstract**

In order to investigate putative protective effects of Echinacea purpurea on colonic inflammation, we determined Adenosine deaminase (ADA) and superoxide dismutase (SOD) activities, malondialdehyde (MDA) levels and protein carbonyl contents were determined in colonic tissues of rats in the colitis and colitis plus Echinacea purpurea (at different doses 50,100 mg/kg) given rats. Besides, histopathology of colon tissues were evaluated. In the Echinacea treatment groups (50 and 100 mg/kg doses) ADA activities were significantly decreased as compared to acetic acid group, but there was no statistically significant between two treatment groups ($p>0.05$). Echinacea treatment also was decreased MDA levels at two doses according to colitis group ($p<0.05$). There was no statistically significant between two treatment groups ($p>0.05$). Slightly decreased protein carbonyl contents and elevated SOD activities were shown in the two Echinacea therapy groups as compared to acetic acid induced colitis group. But these were not statistically significant ($p>0.05$). Similar observations were recorded for these parameters in the extracts of colonic tissue specimens. The results revealed that pretreatment to colitic rats with Echinacea purpurea at doses of 50 and 100 mg/kg, partially attenuated the colonic damage induced by acetic acid. In conclusion, the preventive effects of Echinacea purpurea in the acetic acid model of rat colitis is probably related to its antioxidant properties, due to its flavonoid content.

**Abbreviations:** ADA=Adenosine deaminase, SOD= superoxide dismutase, MDA= malondialdehyde, IBD= Inflammatory bowel diseases, UC= ulcerative colitis, ROS= reactive oxygen species, NO= nitric oxide, US= United States, NBT= nitrobluetetrazolium, TBA= thiobarbituric acid, SPSS=Statistical Package for Social Sciences.

**Key words:** *Echinacea purpurea*, Colitis, Superoxide dismutase, Malondialdehyde, Adenosine deaminase

## 1. Introduction

Inflammatory bowel diseases (IBD) including ulcerative colitis (UC) and Crohn's disease are among the most challenging human illness (Hagar et al., 2007). Ulcerative colitis affects mainly the colon, where inflammatory changes are limited to the mucosa (Soliman et al., 2010). Although the etiology and pathophysiology still remains unclear, clinical observations have found that increased platelet number and platelet activation are notable characteristics of UC. These activated neutrophils produce and release several toxic reagents, such as reactive oxygen species and protease, which can cause tissue damage. Among them, reactive oxygen species (ROS), such as the superoxide anion, hydroxyl radicals and nitric oxide (NO), play an important role (Xing et al., 2012). Evidence indicates that ROS are not merely by products of the inflammatory process, but they are actually involved in its pathogenesis (Seguı et al., 2004). Under normal physiological conditions, chemical and antioxidant defenses protect tissues from the damaging effects of ROS. The toxic oxidants can cause tissue injury if the rate of their production exceeds the capacity of endogenous antioxidant defense mechanisms. Oxidant defense mechanisms in the human colon are relatively deficient so this suggests that colonic inflammation may produce high levels of oxidant products that probably exceed this relatively low antioxidant capacity and lead to oxidative stress and epithelial cell disruption (Yavuz et al., 1999). To regulate overall ROS levels,

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 BioDiCon. 234-0212

the intestinal mucosa possesses a complex of antioxidant systems, of which the superoxide dismutases (SOD) are the initial enzymes (Seguı et al., 2004).

Superoxide dismutases (SOD, EC1.15.1.1) are enzymes that catalyze the dismutation of superoxide into $O_2$ and $H_2O_2$. They are an important antioxidant defense in all cells exposed to $O_2$ (Fukaia et al., 2002). Adenosine deaminase (ADA, EC 3.5.4.4) is an enzyme involved in the catabolism of purine bases, capable of catalyzing the deamination of adenosine, forming inosine in the process. Its main physiologic activity is related to lymphocytic proliferation and differentiation. As a marker of cellular immunity, its plasma activity is found to be elevated in diseases in which there is a cell-mediated immune response (C. Kumar and Kalaivani, 2011). This enzyme is considered as an ecto-enzyme (Franco et al., 1998), since it is found on the surface of many cells. Malondialdehyde (MDA), a carbonile group produced during lipid peroxidation, is used widely in determining oxidative stress (Soydinç et al., 2007). Pratibha *et al* found elevated MDA and ADA levels in acute infective hepatitis and suggested that lipid peroxidation is followed by loss of structural integrity of plasma membranes (Pratibha et al., 2004). Reactive oxygen species are known to convert amino groups of proteins into carbonyl moieties (Parihar et al., 2003). Measurement of protein carbonyl content can be used as a marker for protein damage induced by oxidative stress (Dalle-Donne et al., 2003).

Given the chronic nature of IBD, patients generally require lifelong treatment, and this has remained the corner stone of IBD management, since surgery has been relegated to treatment of refractory disease or specific complications. Thus, the limitations in both efficiency and safety encountered with the current medical approaches for IBD continue to drive the search for better therapeutic options. For this reason, the evaluation of plant drugs which are traditionally used in different inflammatory conditions is an important approach for the development of future drug treatments in IBD (Galvez et al., 2006). Animal studies have shown that dietary phytochemical antioxidants are capable of removing free radicals which is result inflammation (Fang et al., 2002). Echinacea purpurea is one of the most popular medicinal plants because is commercially important source of phytopharmaceuticals and other medicinal preparations (dietary supplement in the United States (US), natural health product in Canada, herbal drug or herbal medicinal product in many European countries) (Najafzadeh and Mahabady, 2009). The chemical composition of Echinacea extracts is complex. Unsaturated lipophilic compound (alkamides and ketoalkenes /alkynes), glycoproteins, caffeic acid derivatives and polysaccharides are believed to be responsible for the observed immune stimulatory mechanism of pharmacological activity. Other biologically active compounds of Echinacea extracts: flavonoids, volatile oils, polyacetylenes, alkaloids and terpenoids have also been isolated from Echinacea species ( Mrozikiewicz et al., 2010). Echinacea purpurea are used most widely in prevention or treatment for the common cold, coughs, bronchitis, influenza, inflammation and has anti-oxidative and free-radical scavenging activity (Najafzadeh and Mahabady, 2009). Pellati *et al.* shown that extracts of E. purpurea roots have greater radical scavenging capacity than other Echinacea species (Pellati et al., 2004).

There is no report about the antioxidative effects of Echinacea of ulcerative colitis induced by acetic acid in rats. Among various animal models of intestinal inflammation, acetic acid-induced colitis is one of the widely used models, which uses intrarectal administration of dilute solutions of acetic acid to produce a diffuse colitis in rats and the resulting colonic inflammation is characterized by increased leukocyte infiltration, edema and ulceration. For this aim, the present study was to assess the possible protective effects Echinacea at the doses of 50 and 100 mg/kg body weight against oxidative stress induced by acetic acid in rats.

## 2. Materials and methods

### *2.1. Animals and Experimental Design*

Eighteen adult female Sprague-Dawley rats weighting 160-195g were obtained from TICAM (Medical and Surgical Experimental Research Center, Eskisehir-TURKEY) and housed in polycarbonate cages in a room with controlled temperature (22±2DC), humidity (50±5%), and a 12 hour cycle of light and dark (07:00 AM-07:00 PM light). The experiments were conducted in accordance with the ethical guidelines for investigations in laboratory animals and were approved by the Ethical Committee for Animal Experimentation of the National Center for Scientific Research. The animals were fed a standard pellet and food was withdrawn overnight before colitis induction. Access to water was allowed add libitum. The rats were randomized into 3 groups, each consisting of 6 animals. Group (I) Acetic acid group (n=6): received saline at a dose of 2 ml, Colitis was induced by intracolonic injection of 1 ml of 4% acetic acid. Group (II and III) Echinacea-treated group, rats were given Echinacea at a dose of 50 and 100 mg/kg/day, respectively.

### *2.2. Induction of colitis and treatments*

Colitis was induced in accordance with the method previously described Fabia *et al.* (Fabia et al., 1992). Diffuse colitis was induced with acetic acid between 9.00 a.m. and 10.00 a.m. Briefly, the animals were lightly anesthetized with ether, and 1 ml of 4% v/v acetic acid (pH 2.3) was slowly administered rectally into the lumen of the colon via a 7 cm polyethylene tubing (PE-240) fitted onto a 1-ml syringe. After a 30-s period of exposure, excess fluid in the lumen was withdrawn, and 1.5 ml of phosphate-buffered saline was introduced to flush the colon.

Echinacea purpurea (purchase from local drugstore, SOLGAR) was dissolved in saline. Echinacea in a volume of 2 ml were given once daily by using an oral zonde daily for seven days before induction of colitis. The animals were fasted for 24 h and were sacrificed by ether anesthesia 24 h after induction of colitis. The colon was excised, opened by longitudinal incision, rinsed with saline, immediately snap-frozen in liquid nitrogen, and stored at -80°C.

*2.3. Biochemical Analysis:*

*2.3.1. Tissue Protein Carbonyl Content*

Protein carbonyl content was determined according to the method of Reznick and Packer. (Reznick and Packer, 1994). Based on the reaction of 2, 4-dinitrophenylhydrazine with protein carbonyl groups to form hydrazones which are detectable by spectrophotometry. Absorbances were measured at 370 nm and results were expressed as nmol per mg of protein.

*2.3.2.Tissue SOD Activity*

Superoxide dismutase activity was measured according to Beaucham and Fridovich's method which was modified by Winterborn *et al*. (Winterbourn et al., 1975). The principle of this method is the inhibiting rate of SOD on the reaction in which superoxide anion reduces nitrobluetetrazolium (NBT). Absorbances were measured at 560 nm. Tissue SOD activities were expressed as units per mg of protein.

*2.3.3.Tissue Adenozin Deaminase Activity*

Adenosine deaminase activity was determined from the cell free extract by measuring the change in absorbance 265 nm resulting from the deamination of adenosine. Enzyme activity was assayed by spectrophotometry, following the decrease in absorbance at 265 nm due to the conversion of adenosine to inosine based on the Kaplan method (Kaplan, 1955). Tissue ADA activities were expressed as units per mg of protein.

*2.3.4.Serum MDA Measurement*

Lipid peroxidation was determined by the measurement of malondialdehyde reacted with thiobarbituric acid (TBA), according to Ohkawa *et al* (Ohkawa et al., 1979). Absorbances were measured at 532 nm. Plasma MDA levels were expressed as nmol/dl.

*2.3.5.Protein determinations of colon tissues*

Protein levels of colon tissues were determined by the biuret method (Layne, 1957).

*2.4. Histology*

Colon tissues were fixed in neutral-buffered formalin for histopathological examination in light microscopy. Then tissue blocks were processed by routine techniques. Furthermore. Furthermore, serial sections 5 μm were prepared for each block. Moreover sections were stained with hematoxylin-eosin H&E. Finally digital images were obtained by Olympus PM10 ADS photomicroscope with DP70 digital camera.

*2.5. Statistical Analysis*

SSPP (Statistical Package for Social Sciences) for Windows 11.5 package program was used to evaluate results. Independent sample groups' one-way analysis of variance Anova, Tukey multiple-range tests and correlations were used for comparing the biochemical values for 3 groups. Results were accepted as significant for $p<0,05$. Results were presented as mean ± standart deviation.

**3. Results**

*3.1. Biochemical Examination*

Intestinal superoxide dismutase and adenosine deaminase activities, protein carbonyl content and serum MDA levels are shown in Table 1. Adenosine enzyme activities in the Echinacea therapy groups 50 and 100 mg/kg doses respectivelly were significantly reduced as compared to acetic acid colitis group ($p<0.05$). But, there was no statistically significant between two therapy groups ($p>0.05$). Echinacea treatment was decreased MDA levels at two doses according to colitis group ($p<0.05$). There was no statistically significant between two treatment groups ($p>0.05$). Elevated SOD activities were shown in the two Echinacea therapy groups as compared to acetic acid colitis group. But, these were not statistically significant ($p>0.05$). Slightly decreased protein carbonyl contents in the two Echinacea therapy groups as compared to acetic acid induced colitis group. But, there was no statistically significant ($p>0.05$).

Table 1: Changes of ADA and SOD activities, MDA levels and protein carbonyl content in rat colon

| | Acetic acid induced colitis group | 50 mg/kg Echinacea group | 100 mg/kg Echinacea group |
|---|---|---|---|
| ADA (U/mg protein) | 0.094 ± 0.006 [a] | 0.074 ± 0.005 | 0.074 ± 0.004 |
| MDA (nmol/dL) | 24.88 ± 2.88 [a] | 14.18 ± 0.70 | 15.66 ± 1.89 |
| SOD (U/mg protein) | 1.18 ± 0.08 | 1.41 ± 0.09 | 1.36 ± 0.09 |
| Protein carbonyl content (U/mg protein) | 4.28 ± 0.60 | 3.86 ± 0.38 | 3.81 ± 0.15 |

Results were presented as mean ± standart deviation.
[a] Significantly different from Echinacea treatment groups, $p<0.05$.

*3.2. Histological Examination*

When the specimens of colitis group is investigated hyperemia at lamina propria, polymorphonuclear leukocytes PMNL infitration and damage and blurring at the criptas had been observed Figure 1.,2. Even though damage at criptas, hyperemia and PMNL infiltration had been evaluated at the specimens of Echinacea groups, it is observed less when compared to colitis group Figure 3.,4.,5.,6. Also no significant difference had been observed due to dose between Echinacea groups.

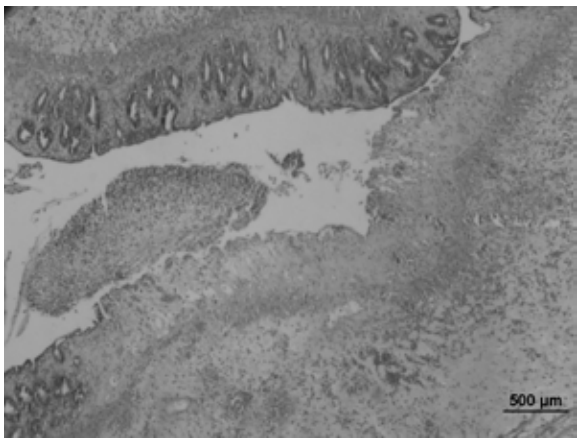


Figure 1. Hyperemia, PMNL infiltration and wide damage at cyriptas seen at the colitis group specimens. HXE. Scale bar 500µm

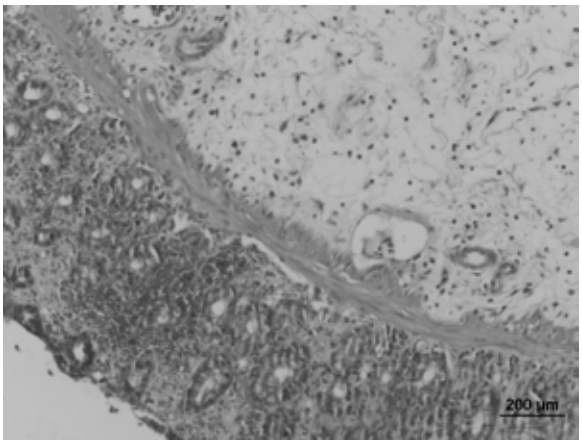


Figure 2. Hyperemia and PMNL infiltration seen at the colitis group specimen. HXE. Scale bar 200µm

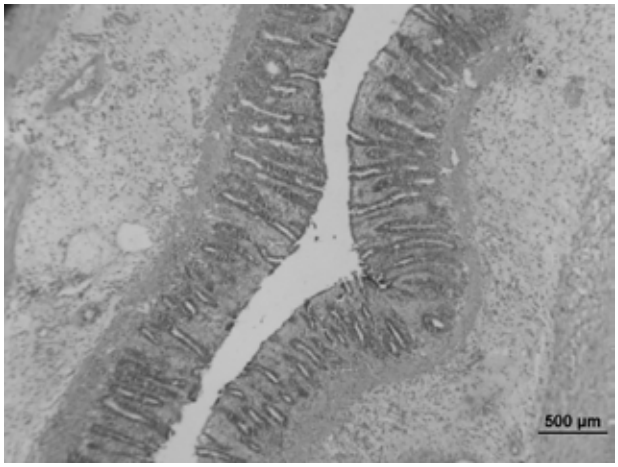


Figure 3. Hyperemia, PMNL infiltration and partially saved cyriptas seen at Echinacea 50mg/kg group. HXE. Scale bar 500µm

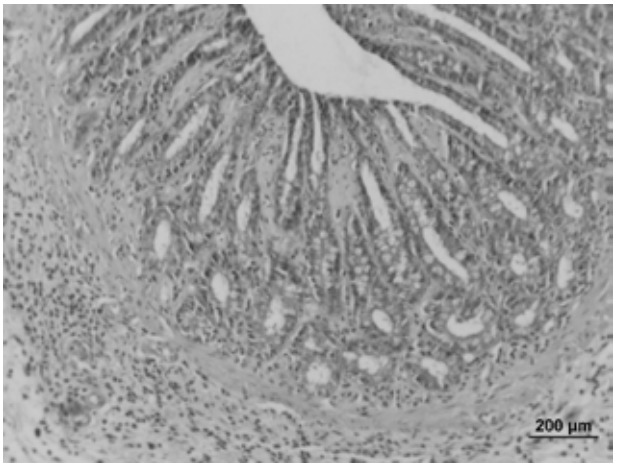


Figure 4. PMNL infiltration and partially saved cyriptas seen at Echinacea 50mg/kg group. HXE. Scale bar 200µm

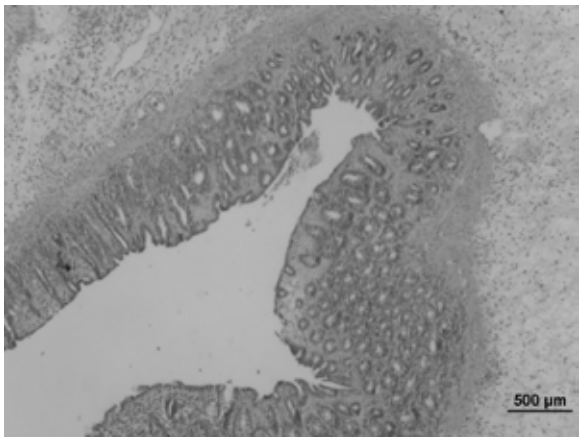


Figure 5. Hyperamia, PMNL infiltration and partially saved intestinal glands seen at Echinacea 100mg/kg group HXE Scale bar 500µm

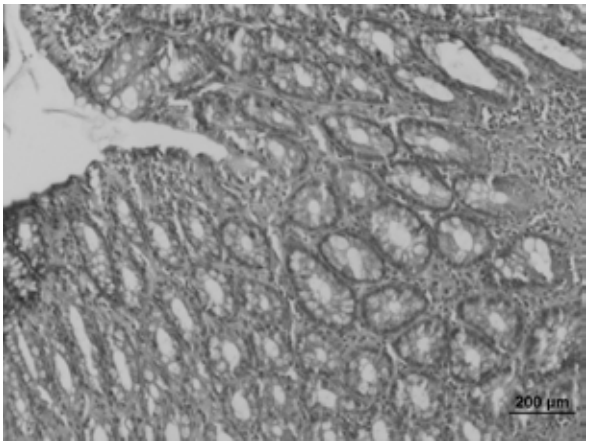


Figure 6. Partially saved cyriptas seen at Echinacea 100mg/kg group. HXE Scale bar 200µm

## 4. Conclusions

An increasing number of both clinical and laboratory findings support the importance of oxidative stress in the pathogenesis of inflammatory bowel diseases (Ghafari et al., 2006; Forrest et al., 2003). Also increased oxidative stress and decreased antioxidant defense system have been shown by mucosal biopsies in IBD (Brody et al., 1996). A positive correlation between the severity of IBD and the intestinal level of ROS has been reported. Thus, antioxidant molecules (radical scavengers) have attracted considerable attention as therapeutic candidates for the treatment of IBD (Ishihara et al., 2009). It has been found that Echinacea preparations have antioxidative and radical scavenging properties (Hu and Kitts et al., 2000). The rich content of polysaccharides and phytosterols in Echinacea are what make it a immune system modulator. Echinacea root extracts are widely used as immune modulator and therapeutic agents for infection in the upper respiratory tract (Baranauskas et al., 2005;Pettinari et al., 2006).

SOD is an enzymatic scavenger, which can convert superoxide anion to hydrogen peroxide. Thus, it can exert defensive effects against oxidative damage. Many experiments proved beneficial effects of SOD treatment against experimental colitis (Xing et al., 2012). For example, some reports have described a beneficial effect of SOD treatment in the prevention of experimental colitis and of a SOD mimetic in the treatment of established colitis, whereas in studies using transgenic mice overexpressing SOD, a more severe colitis or a reduction in neutrophil infiltration without affecting the clinical or histological severity of colitis has been reported. Therefore, further investigation about the effects of SOD on IBD seems warranted, especially elucidating the value of this therapeutic approach in established colitis (Seguı et al., 2004). SOD or SOD derivatives, administered subcutaneously or intraperitoneally, have been found to reduce the severity of colonic inflammation induced by TNBS or acetic acid (Seguı et al., 2004). The data in our study showed that Echinacea increased SOD activity in acetic acid-induced colitis rats. Therefore, Echinacea can strengthen enzymatic defensive system and reduce free radicals, and then alleviate inflammation.

Malondialdehyde MDA is one of end-product of lipid peroxidation. In the most studies, oxidative stress has been evaluated by parameters of lipid peroxidation such as MDA in ulcerative colitis (Barbosa et al., 2003; Cetinkaya et al., 2006). Many studies have found that toxic colitis injury can increase MDA levels in rats, and conversely that various agents used in the treatment of the disease can decrease its level. Our results demonstrated that Echinacea could markedly decrease the MDA level with acetic acid-induced colitis (Xing et al., 2012). In the present study MDA levels were found to be significantly decreased in both Echinacea treatment groups compared to acetic acid group. Recent experimental studies showed protective effects of Echinacea against lipid peroxidation. Raman *et al*. reported that, Echinacea extracts substantially decreased lipid peroxidation assessed by fluorescence spectroscopy (Raman, et al., 2008).

Carbonyl groups aldehydes and ketones are produced on protein side chains when they are oxidized. Protein carbonyl content is the most general indicator and most commonly used marker of protein oxidation (Chevion et al., 2000). Also, Lih-Brody *et al.* showed accumulation of protein carbonyl content in ulcerative colitis according to oxidative stress (Brody et al., 1996). Comparison of our findings with prior data is limited because few studies evaluate effects of Echinacea on protein oxidation but in our opinion antioxidative properties of Echinacea responsible this preventive effect. There are a lot of studies about phenolic components also found in Echinacea which have antioxidant capacity to reduce lipid and protein oxidation.

Adenosine deaminase an enzyme in the purine catabolic pathway, catalyzes the conversion of deoxyadenosine to deoxyinosine and adenosine to inosine. Siegmund *et al*. indicate that adenosine help to maintain tissue integrity by reducing energy demand, increasing nutrient availability and modulating the immune system ( Siegmund, et al., 2001). Also, Hasko and Cronstein point out that, adenosine can play a benefical role as an immune modulator (Hasko and

Cronstein, 2004). In spite of these beneficial effects, therapeutic application of adenosine and its analogs limited by its short half-life. Therefore, enzyme inhibitor drugs which prevents adenosine catabolism, represent another therapeutic approach. For this purpose, Antonioli *et al.* used adenosine deaminase inhibitors in experimental colitis model and they showed that these drugs attenuates inflammation in colitis (Antonioli et al., 2007). Recent studies showed that Echinacea extracts have anti-inflammatory properties in some cases like sinusitis and arthritis (Turner et al., 2000). Ineffectiveness of Echinacea for prevention of experimental rhinovirus colds (Block, 2003). Schoop *et al*. also demonstrated that Echinacea have anti-inflammatory and immune modulatory effects in virus induced inflammatory (Schoop, 2006). In our study, adenosine deaminase enzyme activities significantly decreased in Echinacea treatment groups compared to acetic acid induced colitis group. In the literature limited data present about the influences of Echinacea on the activity of adenosine deaminase enzyme. Therefore, we could not compared our findings to other experimental studies. But, histological analysis in our study, we shown reduced the damage, decreased hyperemia and PMNL infiltration in the Echinacea treatment groups. These histological findings also confirm anti-inflammatory effects of *Echinacea purpurea*.

Our findings have antioxidant properties of Echinacea become significant besides its anti-inflammatuar effects at an inflammatory disease as colitis. Besides free radical scavenging properties of its compounds and the inhibiting effect of lipid and protein oxidation that's had been observed in our study, activating SOD enzyme activity explains its positive effects on oxidative stress in inflammation. Besides all, decrease we observed in PMNL infiltration and hyperemia in histological specimens at Echinacea treatment groups and its effect as inhibiting inflammatory enzyme adenosine deaminase, seem to be the proof of its anti-inflammatuar effect. Due to biochemical parameters and histological investigations, no significant difference observed between Echinacea treatment groups in our study, this can provide additional information about extra treatment strategies addition to conventional treatment.

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*(Received for publication 15 January 2012; The date of publication 15 August 2012)*