The protective effect of *Echinacea spp. (Echinacea angustifolia* and *Echinacea purpurea)* in a rat colitis model induced by acetic acid

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Abstract: Ulcerative colitis (UC) is a chronic disease that causes an inflammatory condition in the colon. Several cytokines, including tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β) and transforming growth factor beta (TGF-β) are crucial components of these inflammatory pathways. New therapeutic strategies are needed for improved clinical outcomes in UC and with less adverse effects. That is why alternative therapies such as herbal remedies are increasingly being used with favorable effects in the treatment of UC. Hence, in the present study, we aimed to evaluate the protective effect of *Echinacea spp* in an experimental rat colitis model induced by acetic acid (AA). Acetic acid was given via a rectal route to induce acute colitis in rats. Rats were placed in four groups: control, *Echinacea*, *Echinacea*-colitis and colitis. Tumor necrosis factor alpha, IL-1β and TGF-β levels were measured. Histopathological comparison of the groups was also performed. The disease activity index (DAI) was significantly higher in the colitis group compared to the control, *Echinacea* and *Echinacea*-colitis groups ($p<0.001$). There was no significant difference between the DAI of control, *Echinacea* and *Echinacea*-colitis groups ($p>0.07$). The inflammatory mediators IL-1β and TNF-α were significantly elevated in the colitis group compared to the other groups ($p<0.007$, <0.001 respectively). Therefore, *Echinacea spp.* may likely have some therapeutic favorable effects in the management of UC.

Keywords: *Echinacea spp.*, ulcerative colitis, TNF-α, IL-1β, TGF-β.

INTRODUCTION

Ulcerative colitis is a chronic inflammatory disease of the colon whose etiology remains obscure (Fiocchi, 1998). Genetic and environmental factors have a role in the pathogenesis, and cytokines, including TNF-α, IL-1β and TGF-β, are crucial components of the inflammatory pathways (Papadakis and Targan, 2000; Rogler and Andus, 1998; Ogata and Hibi, 2003).

Although considerable advances in the medical treatment of UC have been seen, the current medicines are not always effective and may cause serious adverse effects (Ferkolji, 2009; Ha and Dassopoulos, 2010). Recently, a popular biologic agent, anti-tumor necrosis factor alpha (anti-TNF-α) antibody (infliximab), has been in clinical use and shown to be effective in maintenance or remission induction treatment of UC. However, several serious side effects, including increased susceptibility to infection, an obscure risk of mutagenesis, hypersensitivity and anti-antibody reactions have been attributed to the anti-TNF-α drug (Peltz *et al.,* 2012; Ardizzone and Porro, 2005; Reddy and Loftus, 2006). Therefore, new treatment options might be necessary in the management of UC. That is why alternative therapies such as herbal remedies are increasingly being used for the treatment of UC.

Echinacea spp., one of the oldest and most popular herbal species in the world, belonging to the family *Asteraceae*, is found abundantly throughout the world. It is useful in several inflammatory diseases and wound healing due to its immunomodulatory effects. The polysaccharide components in the structure of *Echinacea* have been found to reduce inflammation and accelerate tissue regeneration. Another way *Echinacea* has an anti-inflammatory effect is by increasing the secretion of adrenal cortex hormones such as cortisone, which can reduce inflammation. Interestingly, both the alkylamide and polysaccharide portions of the *Echinacea* plant play a role in the latter mechanism (Borchers *et al.,* 2000; Zhai *et al.,* 2009). *Echinacea* may have a favorable effect in fighting various viral and bacterial infections. Inulin, a different component in *Echinacea* root, has a particularly neutralizing effect on viruses and a bactericidal effect on bacteria. This action of *Echinacea* against bacteria is mostly attributed to the plant component echinacoside of the angustifolia species in the *Echinacea* plant (Sharma *et al.,* 2009; Plescha *et al.,* 2009). The anti-inflammatory and immunomodulatory properties therefore form a good basis for its use in UC. Hence, in the present study, we wanted to evaluate the protective effect of *Echinacea spp* in an experimental colitis model induced by AA in Wistar albino rats.

MATERIALS AND METHODS

Echinacea extract (*Echinacea angustifolia and Echinacea purpurea)* was supplied in the form of its

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original preparation by General Nutrition Center (GNC), Pittsburgh, USA. The original form of *Echinacea* extract contains 100mg of *Echinacea angustifolia* and 400mg of *Echinacea purpurea*.

Animals

Twenty male Wistar-Albino rats weighting from 200 to 250gm were obtained from the Ankara Education and Research Hospital, Experimental Research Laboratory (Ankara, Turkey). Rats were housed in the animal room on a 12h dark and 12 h light cycle at 21-22◦C. Free access to food and water ad libitum was given to all rats. This study was approved by the Ethical Committee of the Ankara Education and Research Hospital. The ethical number for the study is 159.

Acute colitis induction

One ml of a previously prepared solution of 4% AA was given via a rectal route to induce acute colitis in rats. After ketamin anesthesia, AA was carefully dispensed into the colon using a soft 6F pediatric catheter placed into the anus for 6cm. Before removing the catheter from the anus, 2 ml of air was inflated into the colon to spread the AA completely in the colon. All rats were kept in a head down position for 30 seconds to prevent AA leakage from the colon to the outside. *Echinacea* 50 mg/kg/day (equivalent to human dosage) was given into the stomach using a soft 6F pediatric catheter. On the eighth day, rats were given *Echinacea* two hours after induction of colitis and were kept from free access to food and water ad libitum. Rats were randomly divided into four groups of five animals each, as follows:

Group 1 (control; *n*=5) was given only food and water ad libitum for 14 days.

Group 2 (*Echinacea*; *n*=5) was given food, water ad libitum and 50 mg/kg/day *Echinacea* for 14 days. On the eighth day, 2 ml saline was administered rectally.

Group 3 (*Echinacea*-colitis; *n*=5) was given food, water ad libitum and 50 mg/kg/day *Echinacea* for 14 days. On the eighth day, 1ml saline and 1ml 4% AA was administered rectally.

Group 4 (colitis; *n*=5) was given food and water ad libitum for 14 days. On the eighth day, 1ml saline and 1 ml 4% AA was administered rectally.

Disease activity index

The DAI during the period for 7 days after inducing colitis was scored as follows : 0-No rectal hemorrhage, weight loss or abnormal stool consistency; 1- Little weight loss (1-5%) and normal stool consistency without rectal hemorrhage; 2- Weight loss (5-10%), flacid stool without rectal hemorrhage; 3- Weight loss (10-20%) and normal stool consistency without rectal hemorrhage; and 4- Weight loss (>20%) and watery stool with gross rectal hemorrhage (Behera *et al.,* 2012).

Evaluation of colonic damage

Cervical decapitation was performed on all rats under deep general anesthesia on the fifteenth day. All rats then underwent surgical operation to open the abdomen and remove the colon. The distal 8 cm of the colon was carefully separated and this section of the colon was exposed by a longitudinal incision to see macroscopic changes of the mucosa. After the colon segments were scored macroscopically, the distal 4 cm of colon was preserved with 10% formalin for microscopic evaluation. The proximal 4 cm of the colon was used for biochemical analysis (TGF-β, IL-1β and TNF-α). After the mucosa of the colon was cleaned with saline solution, mucosal damage was determined macroscopically using a grading scale as follows: normal colonic mucosa (0); limited hyperemia without ulcers (1); linear ulcers without marked inflammation (2); Linear ulcer with inflammation at one site of the colonic mucosa (3); Ulceration and inflammation at two or more sites of the mucosa (4); Ulceration and inflammation at two or more sites of the mucosa or inflammation and ulceration that spread >1 cm length along the colon (5) (Morris *et al.,* 1989). Later, the distal 4 cm of colon samples were preserved in 10% formalin and stained with hematoxylin-eosin for microscopic evaluation. Mucosal damage of the colon was graded histopathologically from 0 to 11 according to these suggested criteria: (a) Loss of mucosal architecture scored between 0 and 3, (b) Inflammatory cell infiltration scored between 0 and 3, (c) Increase in the thickness of the muscle scored between 0 and 3 (d) Crypt abscess formation scored between 0 and 1 and (e) Decreased goblet cells scored between 0 and 1 (Appleyard and Wallace, 1995).

Biochemical Analysis

The proximal 4cm of colonic tissue was homogenised with an Ultra Turrax homogenizer (T25-B, IKA, Labortechnic, Germany) in Tris/Tween buffer (Hawinkels *et al.,* 2007). The homogenate solution was then centrifuged at 8000 g at 4◦C. The supernatant was taken and TGF-ß1, TNF-α and IL-1β protein concentrations were measured with the Lowry method (Lowry *et al.,* 1951). The level of cytokines was measured as pg/mL. Tissue TGF-ß1 levels were meausured by competitive enzyme immunoassay using ELISA kits (eBioscience, Vienna, Austria) following the manufacturer's protocol. The intra-and inter-assay CV were less than 6.9% and 12% for TGF-ß1. Tissue TNF-α levels were measured by competitive enzyme immunoassay using ELISA kits (eBioscience, Vienna, Austria) following the manufacturer's protocol. The intra- and inter-assay CV were less than 5.0% and 10% for TNF-α. Tissue IL-1β levels were measured by competitive enzyme immunoassay using ELISA kits (eBioscience, Vienna, Austria) following the manufacturer's protocol. The intra- and inter-assay CV were less than 10% and 10% for IL-1β.

STATISTICAL ANALYSIS

Data are given as mean ± SE. After one-way ANOVA was performed on study results, Tukey's multiple comparison test was used for levels of IL-1β, TGF-β and TNF-α, and Kruskal-Wallis test was used for disease activity index, macroscopic and microscopic score using SPSS 16.0 (Statistical Package for Social Sciences version 16.0, Chicago, USA). $P<0.05$ was considered statistically significant.

RESULTS

The disease activity index was significantly higher in the colitis group (group 4) compared to the control (group1), *Echinacea* (group 2) and *Echinacea*-colitis (group 3) groups ($p<0.001$). No significant difference was seen between the DAI of control, *Echinacea* and *Echinacea*-colitis groups ($p>0.07$) (table 1).

The inflammatory mediators IL-1β and TNF-α were elevated in the colitis group compared to the other groups ($p<0.007$, <0.001 respectively). On the other hand, TGF-β was found to be significantly lower in the colitis group compared to the other groups ($p<0.031$). TNF-α ($p>0.16$), IL-1β ($p>0.37$) and TGF-β ($p>0.85$) levels did not show a significant difference between the control, *Echinacea* and *Echinacea*-colitis groups (table 1).

In the colitis group (group 4), typical changes related to ulcerative colitis, e.g. multiple ulcers and diffuse inflammation, were noticed (fig. 4a). The biopsies revealed inflammatory cells in the mucosa and around the crypts, which consisted of polymorphonuclear leukocytes and lymphocytes. Multiple ulcerations were also noticed, indicating the presence of a crypt abscess. Glandular destruction and goblet cell depletion were also seen, denoting UC. In the submucosa, multifocal areas of inflammation and ulceration were present and it was diffusely edematous. Infiltration of polymorphonuclear leukocytes, eosinophils and lymphocytes was extensive (400x, H and E) (fig. 4b). In contrast, macroscopic and microscopic evalutaion showed mild changes in Echinacea, Echinacea-colitis and control groups (fig. 2a, 2b, 3a, 3b, 1a, 1b). Therefore, regarding macroscopic and microscopic features, a significant difference was found between the colitis group and the Echinacea-colitis group ($p<0.001$).

DISCUSSION

Regarding UC induction, there are several reports on different chemicals used to induce experimental colitis, such as acetic acid, 2,4,6-trinitrobenzene sulfonic acid, dextran sodium sulfate, oxazolone and indomethacin (Bauer *et al.,* 2012; Strober *et al.,* 2002; Kawada *et al.,* 2007; Heller *et al.,* 2002; Standnyk *et al.,* 2002). In this study, 4% AA was used for induction of UC. Acetic acid is easily available and is an inexpensive chemical agent. Our findings confirmed induction of UC macroscopically and histologically. Acetic acid causes accumulation of colonic mucosa by acute inflammatory cells, including neutrophils and macrophages. In addition, the production of inflammatory mediators is stimulated by AA in the experimental colitis model. As a mechanism for colitis induced by AA, it is suggested that AA triggers an acute inflammatory response following colonic damage resulting in hemorrhage, release of inflammatory mediators and development of the colonic lesions (Fabia *et al.,* 1992).

Although the real pathogenesis that plays a major role in UC is still unknown, increasing data supports the hypothesis that an increase in levels of proinflammatory mediators, including IL-1β and TNF-α, in the colonic spaces plays a main role in the mechanism of UC (Andoh *et al.,* 2008). In previous studies, serum IL-1β and TNF-α concentrations and colonic IL-1β and TNF-α mRNA expression levels are increased significantly in rats with UC in correlation with the severity of disease. This indicates that IL-1β and TNF-α contribute to the immune abnormalities and inflammatory responses in UC (Autenrieth *et al.,* 1997). A different cytokine, transforming growth factor-beta (TGF-β), may also be involved in the pathogenesis of inflammatory pathways of UC. However, a significant association between serum

Table 1: Effects of *Echinacea spp* on DAI, macroscopy, microscopy and cytokines

	Control (Group 1) (n=5)	*Echinacea* (Group 2) (n=5)	*Echinacea*-Colitis (Group 3) (n=5)	Colitis (Group 4) (n=5)	p*	p**
DAI	0±0	0±0	0,8±0,37	2,8±0,2	<0,001	>0,07
Macroscopy	0±0	0±0	0,8±0,33	4,4±0,4	<0,001	>0,2
Microscopy	0,4±0,24	0,4±0,25	1,6±0,4	4,8±0,58	<0,001	>0,17
IL1β	259,2±25,73	288,4±52,68	358,6±34,47	589,4±50,8	<0,007	>0,37
TNFα	269,6±20,30	289,6±19,61	373,8±16,12	747,4±43,1	<0,001	>0,16
TGFβ	275,4±35	246,6±36,63	239,2±38,55	98±7,59	<0,031	>0,85

DAI: Disease activity index. IL1β: İnterleukin 1 beta. TNFα: Tumor necrosis factor alpha. TGFβ: Transforming growth factor beta. Values expressed as mean ±SE. P* value: Colitis group compared with other groups. P** value: Groups of control, *Echinacea*, *Echinacea*-colitis compared with each other. Cytokines were measured as pg/mL.

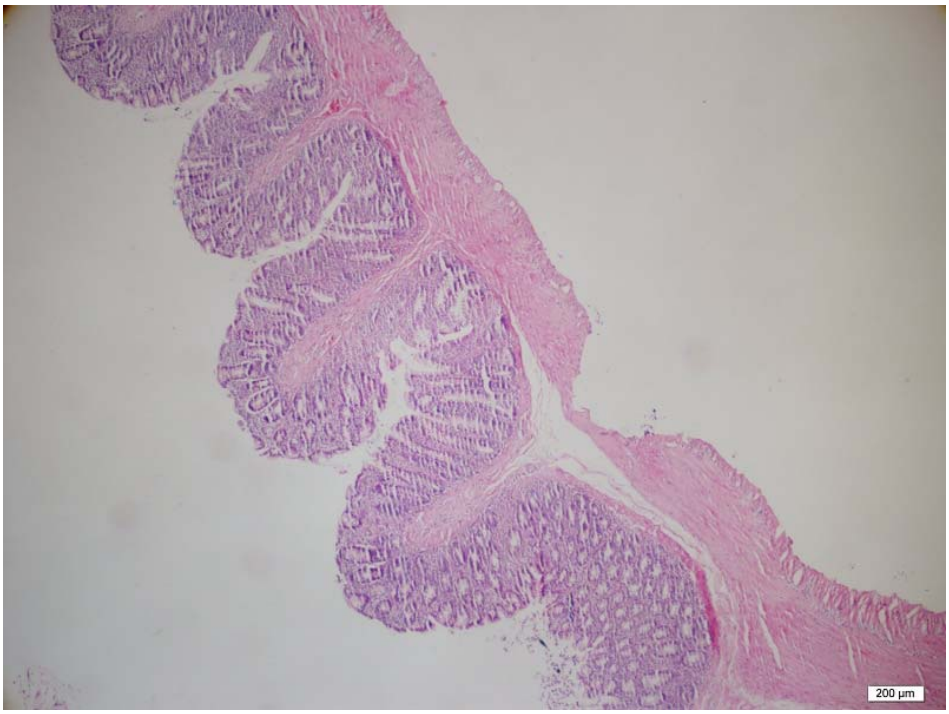

Fig. 1a: Normal macroscopic appearance of colon in control group

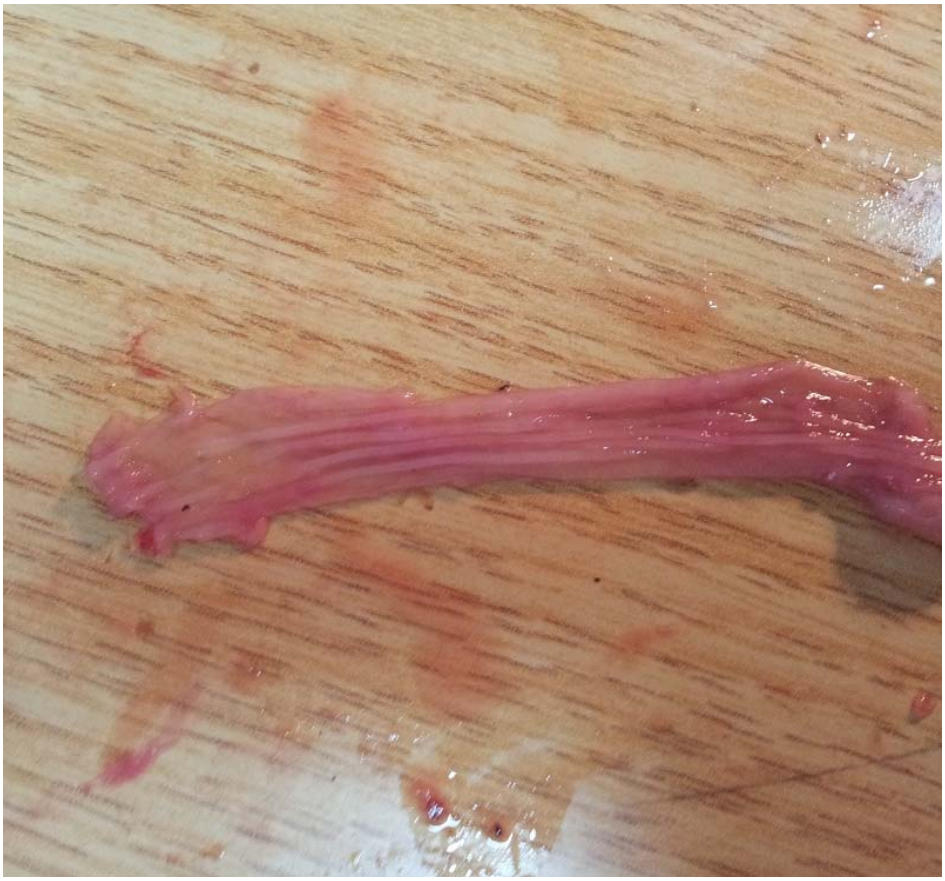

Fig. 1b: Normal colonic tissue architecture of colon in control group

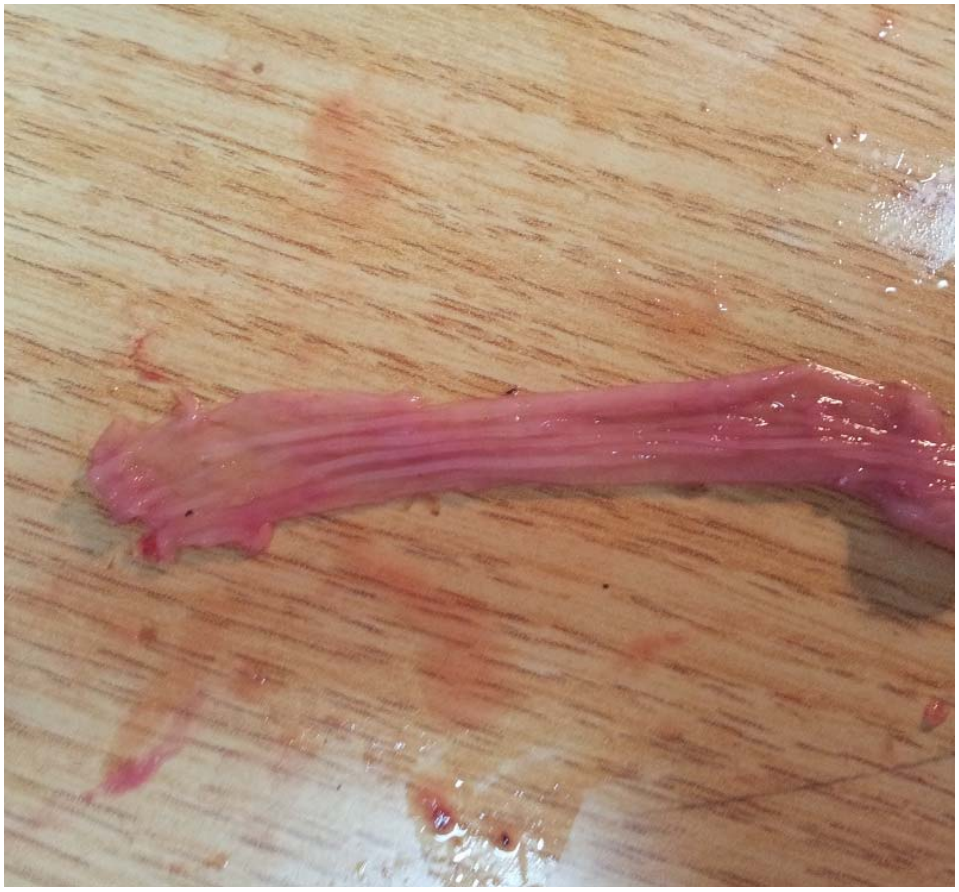

Fig. 2a: Normal macroscopic appearance of colon in *Echinacea* group

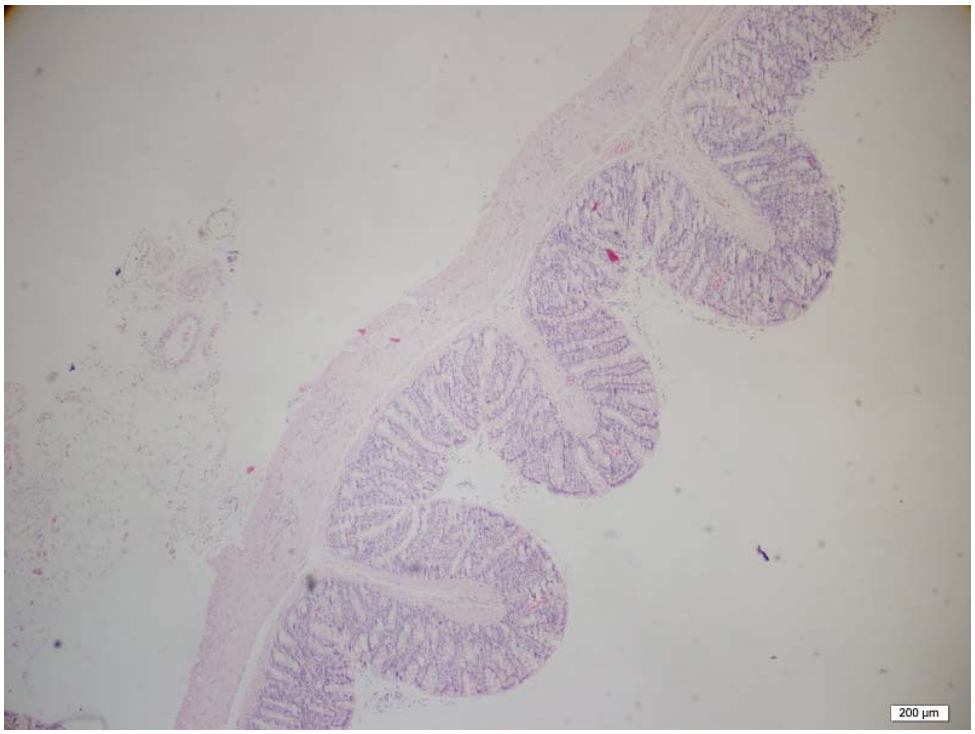

Fig. 2b: Normal colonic tissue architecture of colon in *Echinacea* group

Fig. 3a: Minimal macroscopic changes in *Echinacea*-colitis group

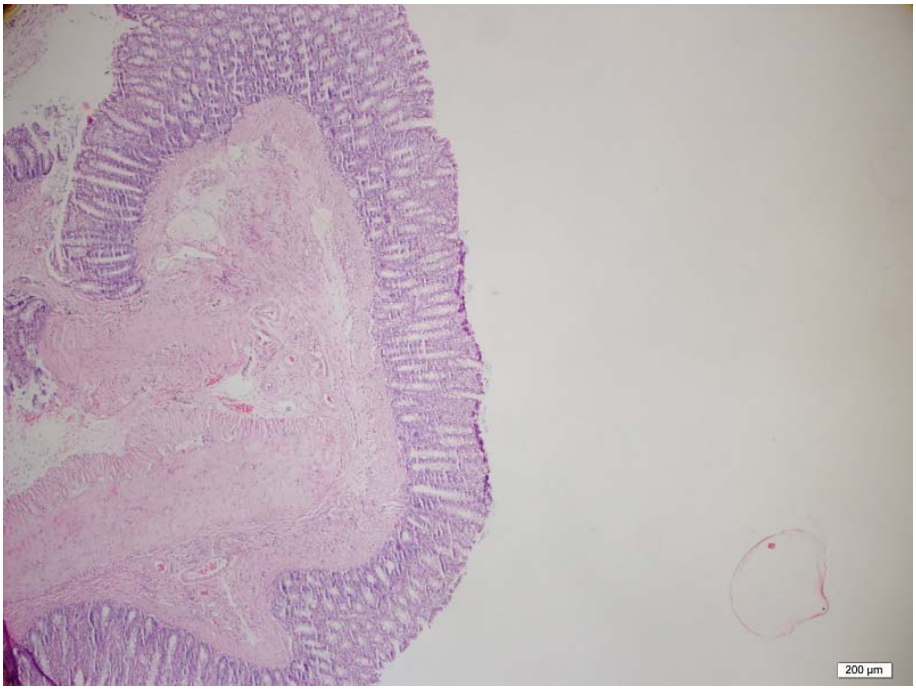

Fig. 3b: Minimal changes in tissue architecture and cell infiltration in *Echinacea*-colitis group

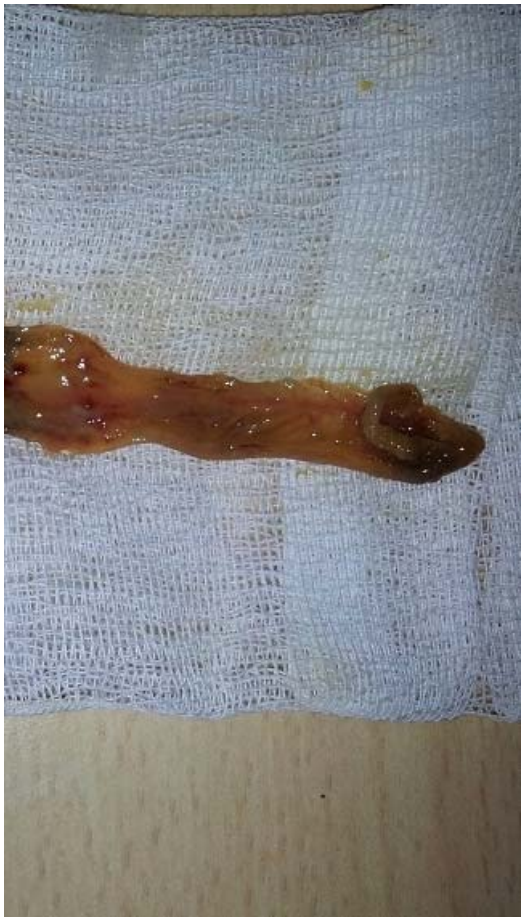

Fig. 4a: Detrimental effect of AA on colon tissue with necrosis and ulceration in colitis group

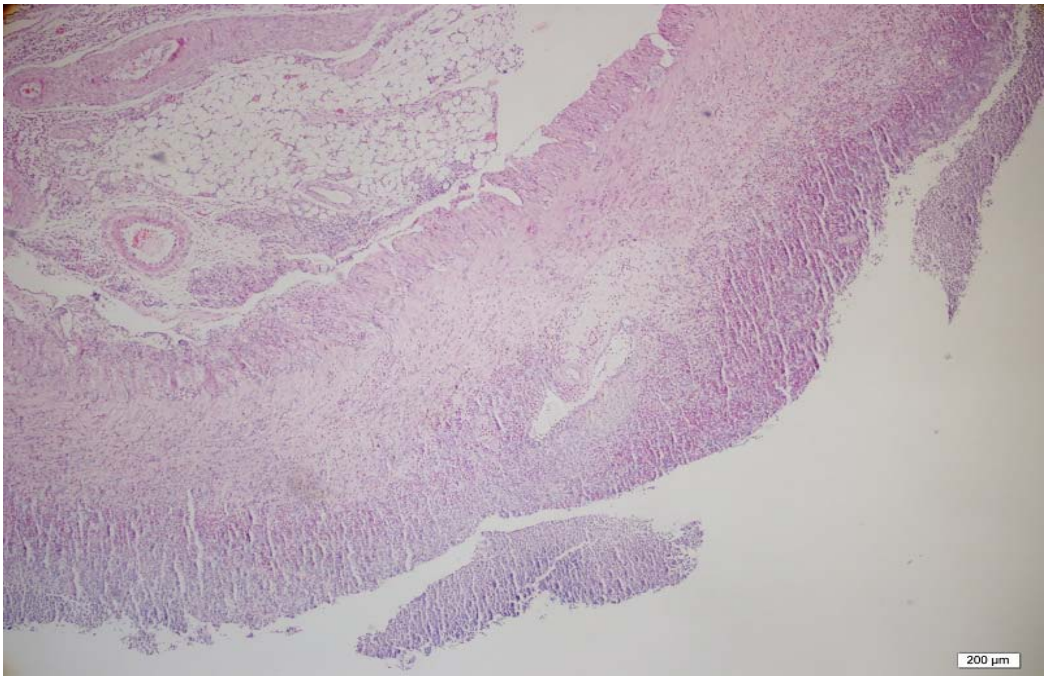

Fig. 4b: Loss of epithelial cells with ulceration and goblet cell depletion with inflammatory cell infiltration in colitis group.

TGF-β level and UC activity was not found in previous studies (Ebert *et al.,* 2009). Significantly elevated TNF-α and IL-1β levels and decreased levels of TGF-β in group 4 (colitis group) are consistent with the AA colitis model. Histological examination in colitis was in accordance with the elevated cytokine levels.

Interestingly, TNF-α, IL-1β and TGF-β levels were not different between group 3 (*Echinacea*-Colitis) and the control group for both serum and colonic tissue. Significantly lower TNF-α and IL-1β levels and mild histological changes in-group 3 show the protective role of *Echinacea* in colitis.

In a few clinical trials, *Echinacea* was found to have favorable effects on several types of infections, such as colds and upper respiratory infection diseases. When *Echinacea* was taken early in the prodromal phase of these infections, a relationship was found showing milder and shorter duration of these infectious diseases (Linde *et al.,* 2006; Thomas, 2001). TNF-α, IL-1β and TGF-β levels in-group 2 were not different from the control group in our study, meaning that *Echinacea* had no hazardous effect on the normal colon.

The protective effect of *Echinacea* on the colonic mucosa is probably due to its anti-inflammatory effect. If the present study is supported by clinical research, *Echinacea* could likely be used for favorable effects in man.

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