Biochemical and Histopathological Evidences for Beneficial Effects of Satureja Khuzestanica Jamzad Essential Oil on the Mouse Model of Inflammatory Bowel Diseases

Ghazal Ghazanfari
Laboratory of Toxicology, Department of Toxicology & Pharmacology, Faculty of Pharmacy, and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran

Bagher Minaie
Laboratory of Histopathology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Narges Yasa
Laboratory of Pharmacognosy, Faculty of Pharmacy, and Medicinal Plants Research Center, Tehran University of Medical Sciences, Tehran, Iran

Leila Ashtaral Nakhai, Azadeh Mohammadirad, Shekoufeh Nikfar, Gholamreza Dehghan, Vahid Shetab Boushehri, Hamidreza Jamshidi, Reza Khorasani, Alinazar Salehnia, and Mohammad Abdollahi
Laboratory of Toxicology, Department of Toxicology & Pharmacology, Faculty of Pharmacy, and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran

The essential oil from Satureja Khuzestanica Jamzad (SKEO), an endemic plant from Iran, was evaluated for its activity against inflammatory bowel disease (IBD). SKEO was examined on the experimental mouse model of inflammatory bowel disease, which is acetic acid-induced colitis. Prednisolone was used as the standard drug for comparison. Biochemical, macroscopic, and microscopic examinations of colon were performed. Lipid peroxidation significantly increased in acetic acid-treated mice in comparison to the normal group (4.88 vs. 3.02 µmol/g) and was significantly restored by SKEO (500, 1000, 1500 ppm) and prednisolone treatment. The mean percentage of decreases of lipid peroxidation in SKEO (500, 1000, 1500 ppm)- and prednisolone-treated groups were 10.5, 28.5, 42.85, and 33.33 of control, respectively. The myeloperoxidase activity significantly increased in acetic acid-treated mice in comparison to the normal group (4.1 vs. 0.8 U/g) and significantly restored in SKEO (1000 and 1500 ppm)- and prednisolone-treated groups. The mean percentage of decreases of myeloperoxidase activity in SKEO (1000 and 1500 ppm)- and prednisolone-treated groups were 24.56, 50, and 52.63 of control, respectively. SKEO (1000 and 1500 ppm)- and prednisolone-treated groups showed significantly lower score values of macroscopic and microscopic characters when compared to the acetic acid-treated group. The beneficial effect of SKEO (1500 ppm) was comparable to that of prednisolone. Known antioxidant, antimicrobial, antiinflammatory, and antispasmodic potentials of Satureja Khuzestanica may be the mechanisms by which this plant protects animals against experimentally induced IBD. Proper clinical investigation should be carried out to confirm the activity in human disease.

Keywords
Inflammatory bowel disease, Satureja khuzestanica, Essential oil myeloperoxidase, Lipid peroxidation.

INTRODUCTION

Inflammatory bowel disease (IBD) comprises those conditions characterized by a tendency for chronic or relapsing immune activation and inflammation within the gastrointestinal tract. Crohn’s disease (CD) and ulcerative colitis (UC) are the two major forms of IBD. Despite extensive investigation, the pathophysiology of human IBD remains incompletely understood. Genetic factors, infective agents, immunological basis, smoking, medications, and pathological factors have been reported to be involved in IBD (Botoman et al. 1998; Gibson and Iser 2005; Hadjibabaie et al. 2005; Rezaie et al.
Commonly used medications include aminosalicylates, glucocorticoids, antibiotics, and immunomodulators. Glucocorticoids are given in acute attacks of the disease and offer immediate relief. Aminosalicylates like sulphasalazine and mesalamine are given chronically to maintain remission and prevent relapses.

Several studies have shown that oxidative stress is involved in the pathogenesis of IBD even as an etiologic factor (Grisham and Granger 1988; Grisham 1994; Jahanshahi et al. 2004). If there are too many free radicals or too few antioxidants for protection, a condition of oxidative stress develops, which may cause chronic and permanent damage to cells. One of the markers of oxidative stress is increased lipid peroxidation of the cells, which is determined by measuring thiobarbituric acid reactive substances (TBARS) (Abdollahi et al. 2004). The enzyme myeloperoxidase (MPO) is an enzyme found in neutrophils and in much lower concentrations in monocytes and macrophages. This enzyme catalyzes the oxidation of electron donors (e.g., halides) by hydrogen peroxides. In case of inflammatory conditions like IBD, the levels of neutrophils in inflamed tissues, and consequently MPO enzyme, increase. The assessment of MPO activity is well established for quantitation of intestinal inflammation (Krawisz et al. 1984), especially in the acetic acid mouse model of colitis. Acetic acid-induced colitis is an easily inducible model of IBD, and the similarity of the inflammatory mediators’ profile to IBD suggests that the inflammatory phase bears some resemblance to human intestinal inflammation (Elson et al. 1995).

*Satureja khuzistanica* Jamzad is an endemic plant of Iran that is widely distributed in the Southern part of Iran (Jamzad 1994). It is famous for its medical uses as analgesic and antiseptic in folk medicine (Zargari 1990). The genus *Satureja* belongs to the family Lamiaeae, subfamily Nepetoideae, and tribe Mentheae. One of the diagnostic characteristics of the subfamily Nepetoideae is that its representatives contain more than 0.5% of essential oil (El-Gazzar and Watson 1970). It has been reported that there are marked differences between and within the subspecies of *Satureja* essential oil composition (Slovkovska et al. 2001). During recent years, antiviral (Abad et al. 1999), antinociceptive and antiinflammatory (Hajhashemi et al. 2000; Amanlou et al. 2005), antibacterial and antifungal (Yamasaki et al. 1998; Azazet et al. 2002; Sokovic et al. 2002; Amanlou et al. 2004), antispasmodic and antidiarrheic (Hajhashemi et al. 2002), and vasodilatory (Sanchez de Rojas et al. 1999) effects have been reported for different species of *Satureja* growing in different parts of the world. Our recent study indicated that oral administration of *Satureja Khuzistanica* essential oil (SKEO) to rats induces a significant antioxidant, antidiabetic, antihyperlipidemic, and reproduction stimulatory effects without appearance of any toxicity or adverse effects.

Regarding the above-mentioned points, we expected that SKEO has a protective effect on IBD; therefore, the present study was designed to examine effects of this essential oil on an experimental model of IBD (acetic acid-induced colitis in mice) by measuring bowel TBARS level, MPO activity, and macroscopic and microscopic evaluations.

**METHODS**

**Chemicals**

2-Thiobarbituric acid (TBA), 1,1,3,3-tetraethoxy-propane, trichloroacetic acid (TCA), n-butanol, hexadecyl trimethyl ammonium bromide (HETAB), EDTA, O-dianisidine hydrochloride, hydrogen peroxide, acetic acid, prednisolone, and phosphate buffer from Merck Chemical Co. (Germany) were used in this study.

**Preparation of Essential Oil**

*Satureja Khuzistanica* Jamzad (Picture 1) is a subshrub, branched stem ± 30 cm high, densely leafy, and broadly ovaive-orbicular covered with white hairs. Base of the leaves is attenuate and petioliform. Each verticillaster has two to eight flowers, shortly pedunculate and remote. The plant was identified by the Department of Botany of the Research Institute of Forests and Ranglands (TARI), Tehran. A voucher specimen (No. 58416) has been deposited at the Herbarium of TARI. The aerial parts of plant were collected during the flowering stage of plant from the Khoaramabad of Lorestan province, air dried at ambient temperature in the shade, and hydrodistilled using a Clevenger-type apparatus for 5 h, giving yellow oil in 0.9% yield. The oils were dried over anhydrous sodium sulfate and stored at 4°C.

**Animals**

NMRI albino mice weighing between 20 and 30 g were used for the study. Animals were maintained under standard conditions of temperature (23 ± 1°C), relative humidity (55 ± 10%), and 12 h/12 h light/dark cycle, and fed with a standard pellet diet with water ad libitum. They were housed in standard polypropylene cages with wire mesh top. All studies were
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FIG. 1. Light micrograph of bowel tissue from normal or untreated animals, which did not receive any treatment. No major histological changes are apparent in micrograph (H & E, × 40 & 100).

FIG. 2. Light micrograph of bowel tissues from control mice, which received 0.1 mL of 6% acetic acid solution (once, intrarectally). Major histological changes are apparent in micrograph. Ed, edema; GT, granulated tissue; In, inflammation; NL, narrowed lumen; Ne, necrosis; DAC, disrupted architecture of the crypt; PMN, polymorphonuclears; MN, mononuclear; FUM, focal ulceration of mucosa (H & E, × 40 & 100).

FIG. 3. Light micrograph of bowel tissues from prednisolone-treated group, which received prednisolone (1.14 mg/kg, for 3 days) and acetic acid (0.1 mL, 6% solution, once, intrarectally). Minor histological changes are apparent (H & E, × 40 & 100).

FIG. 4. Light micrograph of bowel tissue from SKEO-treated animals, which received 7 days pretreatment with SKEO (500, 1000, 1500 ppm in drinking water) and 0.1 mL of 6% acetic acid solution; the prednisolone-treated group, received prednisolone (1.14 mg/kg, for 3 days) and acetic acid (0.1 mL, 6% solution, once, intrarectally). Acetic acid was administered intrarectally on eighth day. Drug treatment was continued until the tenth day. Prednisolone as standard drug was started on the day of acetic acid treatment and given orally as suspension containing 0.5% of sodium CMC.

For induction of colitis, overnight fasted animals were anesthetized using pentobarbital sodium (55 mg/kg, i.p.) and carried out using six mice in each group. All experiments on animals were considered carefully to be ethical. The experimental protocol was approved by the Ethic Committee of PSRC/TUMS.

Induction of Colitis and Treatments

The protocol of the study, including doses and duration of treatment, and groups were all designed according to previous studies (Krawisz et al. 1984; Abdollahi et al. 2003a; Jagtap et al. 2004). The study comprised six different groups as follows: normal or untreated animals, did not receive any treatment; control animals received 0.1 mL of 6% acetic acid solution (once, intrarectally); SKEO-treated animals received 7 days pretreatment with SKEO (500, 1000, 1500 ppm in drinking water) and 0.1 mL of 6% acetic acid solution; the prednisolone-treated group, received prednisolone (1.14 mg/kg, for 3 days) and acetic acid (0.1 mL, 6% solution, once, intrarectally). Acetic acid was administered intrarectally on eighth day. Drug treatment was continued until the tenth day. Prednisolone as standard drug was started on the day of acetic acid treatment and given orally as suspension containing 0.5% of sodium CMC.
then 0.1 mL of 6% acetic acid solution was instilled into the rectum of the mouse. Animals were allowed to hang in air by holding their tails for 1 to 2 min. This prevented spillage of the solution from the rectum. After 48 h animals were sacrificed by cervical dislocation and dissected open to remove colon. A 5-cm long piece of colon was flushed gently with saline, cut open, and scored for inflammation based on the macroscopic characteristics. Then tissues were fixed in 10% formalin saline and examined histopathologically. Biochemical evaluation of colon inflammation was done using assay of MPO activity and TBARS concentration.

Assay of Colon Macroscopic Characters

Mice colon (5 cm long) was scored for macroscopic features using following scoring pattern:

<table>
<thead>
<tr>
<th>Percentage area affected</th>
<th>Score</th>
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<tbody>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1—5</td>
<td>1</td>
</tr>
<tr>
<td>5—10</td>
<td>2</td>
</tr>
<tr>
<td>10—25</td>
<td>3</td>
</tr>
<tr>
<td>25—50</td>
<td>4</td>
</tr>
<tr>
<td>50—75</td>
<td>5</td>
</tr>
<tr>
<td>75—100</td>
<td>6</td>
</tr>
</tbody>
</table>

Assay of Colon Microscopic (Histologic) Characters

To process for microscopic studies, 5-µm thick paraffin sections were stained in hematoxyline and eosin. The stained sections were examined for eight inflammatory changes as follows: edema, granulated tissues, ulceration or thickening of mucosa, inflammation, existence of polymorphonuclears and mononuclears, necrosis, disrupted architecture of the crypt, and narrow lumen. The sections were all recorded by a histopathologist and a sign score between 0 and 3 for each sign and a total of 24 were used to determine the severity of colon inflammation.

Each of these signs was scored between 0 and 3 on the basis of pathologist observation.

Assay of Colon MPO Activity

Colonic samples were minced on ice and homogenized in 10 mL of ice-cold 50 mM potassium phosphate buffer (pH 6.0), containing 0.5% hexadecyltrimethyl ammonium bromide (HETAB) and 10 mM EDTA. The homogenates were then sonicated and centrifuged for 20 min at 12,000 g. MPO activity was measured spectrophotometrically as follows: 0.1 mL of supernatant was combined with 2.9 mL of 50 mM phosphate buffer containing 0.167 mg/mL O-dianisidine hydrochloride and 0.0005% H$_2$O$_2$. The change in absorbance was measured spectrophotometrically (Shimadzu 160A UV-VIS spectrophotometer), at 460 nm. One unit of MPO activity is defined as the change in absorbance per minute at room temperature, in the final reaction. MPO activity (U/g) = X/weight of the piece of tissue taken where X = 10 × change in absorbance per minute/volume of supernatant taken in the final reaction (Krawisz et al. 1984).

Assay of Colon TBARS Concentration

Malondialdehyde (MDA) is the main end-product of the oxidation of polyunsaturated fatty acids and its concentration in the medium is an established measure of lipid peroxidation extent. In this test, the reaction of thiobarbituric acid (TBA) with lipid peroxide products makes a complex, which is determined spectrophotometrically, and lipid peroxidation in samples are assessed in terms of thiobarbituric acid reactive substances (TBARS) produced. Briefly, the colonic samples were homogenized in buffered saline (1:5) and then 800 µL
of trichloroacetic acid (TCA, 10% w/v) was added to 400 μL of this mixture and centrifuged in 3000 g for 30 min. Then, 600 μL of the supernatant was added to 150 μL of TBA (1% w/v). Then the mixture was incubated for 15 min in a boiling water bath and then 4 mL n-butanol was added; the solution was centrifuged, and cooled; and absorption of the supernatant was recorded in 532 nm by UV-160-A Shimadzu double beam spectrophotometer (Japan). The calibration curve of a 1,1,3,3-tetraethoxypropane standard solution was used to determine the concentrations of TBA-MDA adducts in samples. The molar absorption coefficient of 1,1,3,3-tetraethoxypropane is 156,000 M⁻¹L⁻¹. TBARS level was presented as μmol/g of colon (Satho 1978).

**Statistical Analysis**

Values are reported as mean ± SEM. Statistical significance between groups was computed by analysis of variance (ANOVA) and Tukey multiple comparison posthoc tests. P values lower than 0.05 were considered significant.

**RESULTS**

Colon macroscopic and microscopic changes in control and SKEO-treated groups are shown in Tables 1 and 2, respectively. Intrarectal instillation of acetic acid caused significant inflammatory reactions as indicated by macroscopic and microscopic changes (Tables 1 and 2). SKEO (1000 and 1500 ppm) and prednisolone-treated groups showed significantly (P < 0.01) lower score values of macroscopic and microscopic characters compared to the control group. Beneficial effects of SKEO (1500 ppm) in acetic acid-induced colon macroscopic and microscopic alterations was comparable to that of prednisolone.

The changes in the extent of lipid peroxidation in bowel homogenate of treated animals is shown in Figure 7. The TBARS level significantly increased in the control group in comparison to the normal group (4.88 vs. 3.02 μmol/g, P < 0.01). The mean percentages of decreases of MPO activity in SKEO (500, 1000, 1500 ppm)- and prednisolone-treated groups were 10.5, 28.5, 42.85, and 33.33 of control, respectively.

The changes in the activity of MPO in bowel homogenates of treated animals are shown in Figure 8. The MPO activity of the control group showed significant (P < 0.01) increase in comparison to the normal group (4.1 vs. 0.8 U/g). The MPO activity decreased (P < 0.01) in SKEO (1000 and 1500 ppm)- and prednisolone-treated groups compared to the control group.

The mean percentages of decreases of MPO activity in SKEO (1000 and 1500 ppm)- and prednisolone-treated groups were 24.56, 50, and 52.63 of control, respectively.

**DISCUSSION**

The overall results of the present study showed that SKEO has the potential to diminish colitis as evidenced by colon macroscopic, microscopic, and biochemical evaluations. Biochemical assays indicated that treatment with SKEO reduces MPO activity and TBARS concentrations. TBARS and MPO activity were employed in this study because these assays have been found to be reliable biomarkers of oxidative stress (Abdollahi et al. 2004; Arnhold 2004; Jahanshahi et al. 2004). In previous reports with rodent models of colitis, the utility of these two assays has been established for detection of oxidative stress in vivo. Moreover, MPO activity has been found to be correlated with intestinal, providing further basis for selection of these two biochemical assays (Rumi et al. 2004; Ogawa et al. 2002; Krawisz et al. 1984).

Interestingly, SKEO, especially at a dose of 1500 ppm, was very comparable to prednisolone, which showed significant protection against acetic acid-induced colitis as evidenced by biochemical, macroscopic, and microscopic examinations. The acetic acid-induced colitis model in mice is very similar to

### TABLE 1

**Evaluation based on macroscopic features in acetic acid-induced colitis**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean of macroscopic scores ± SEM</th>
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<tbody>
<tr>
<td>Control</td>
<td>3.39 ± 0.44</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>1.12 ± 0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SKEO 500</td>
<td>4.11 ± 0.51</td>
</tr>
<tr>
<td>SKEO 1000</td>
<td>2.17 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SKEO 1500</td>
<td>1.26 ± 0.21&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Observations were recorded by histopathologist and scored as described in methods section. Each value represents mean of macroscopic scores ± SEM of six animals in each group.

<sup>a</sup>Significant (P < 0.01) decrease in macroscopic score values compared to control.

<sup>b</sup>The difference in macroscopic score values of SKEO-treated and prednisolone-treated groups is not significant.

### TABLE 2

**Evaluation based on microscopic features in acetic acid-induced colitis**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean of microscopic scores ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.17 ± 0.61</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>5.5 ± 0.43&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SKEO 500</td>
<td>22.83 ± 0.48&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SKEO 1000</td>
<td>13.5 ± 0.76&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SKEO 1500</td>
<td>5.5 ± 0.43&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All histological findings were recorded by histopathologist and scored as described in methods section. Data are mean ± SEM of six observations.

<sup>a</sup>Significant (P < 0.01) decrease in macroscopic score values compared to control.

<sup>b</sup>The difference in macroscopic score values of SKEO-treated and prednisolone-treated groups is not significant.
human ulcerative colitis in terms of histologic features. It affects the distal colon portion with a nontransmural inflammation, massive necrosis of mucosal and submucosal layers, mucosal edema, neutrophil infiltration of the mucosa, and submucosal ulceration. The mechanisms by which acetic acid produces inflammation appear to involve the entry of the protonated form of the acid into epithelium, where it dissociates to liberate protons within intracellular acidification that most likely accounts for the epithelial injury observed. The inflammatory response initiated by acetic acid includes activation of cyclooxygenase and lipooxygenase pathways (MacPherson and Pfeiffer 1976; Sharon and Stenson 1985; Elson et al. 1998). Glucocorticoids act as anti-inflammatories by decreasing the recruitment of macrophages in the affected area. They suppress the synthesis of many inflammatory mediators (e.g., production of IL-1 from monocytes, production of IL-2 and tumor necrosis factor from lymphocytes). They also inhibit the enzyme phospholipase A2 and thus decrease availability of prostaglandins and leukotrienes (Katzung 2000). Thus, the first idea that may come to mind is that SKEO may have an effect on synthesis or release of these inflammatory mediators. Supporting this idea, Satureja Khuzestanica hydroalcoholic extract has been shown to have analgesic and anti-inflammatory properties when tested in rat formalin and carrageenan tests (Amanlou et al. 2005). In addition, Satureja macrostema Benth has been shown to contain one or several bioactive compounds with inhibitory effect on rabbit jejunum muscular contractility, confirming its antispasmodic effect. The mechanism of the inhibitory action on muscular contractility was not for effect on cholinergic, adrenergic, or 5-hydroxytryptamine receptors. The velocity
of the intestinal transit motility was significantly reduced by oral administration of the crude *Satureja* macrostema extract (Cortés et al. 2004). Thus, one mechanism for the beneficial effects of SKEO in colitis could be its possible interaction with cyclooxygenase or lipoxygenase pathways, which remains to be elucidated in further studies.

On the other hand, free radicals have been reported to play important roles in the pathogenesis of IBD (Grisham and Granger 1988; Grisham 1994; Jahanshahi et al. 2004). Supporting this idea, the activity of MPO was increased in the present colitis model. Together with the membranous NADPH oxidase, MPO is involved in the formation of reactive oxygen species and oxidation of biological material. It produces not only oxidative equivalents, but contributes also to the regulation in general response to invading microorganisms (Arnhold 2004). Increased TBARS as a marker of lipid peroxidation observed in association with higher MPO activity in the present experimental colitis model again confirms the role of free radicals in IBD. The chemical composition of the essential oil of *Satureja Khuzestanica Jamzad* from Iran has been examined by GC and GC-MS. The components were flavonoids, mainly carvacrol, eugenol, p-cymene, and thymol (Sefidkon and Ahmadi 2000; Farsam et al. 2004). Flavonoids are a class of plant phenolics with significant antioxidant and chelating properties. Their positive effects stem from their ability to inhibit lipid peroxidation, chelate redox-active metals, and attenuate other processes involving reactive oxygen species. In addition, carvacrol as the main constituent of SKEO has been found to have marked antioxidant properties (Lambert et al. 2001). In addition, SKEO has been found to increase blood total antioxidant power and decrease TBARS concentration (Abdollahi et al. 2003a). In supporting this idea, there is evidence that flavonoids have antiphosphodiesterase activity and thus could elevate intracellular levels of cyclic nucleotides (Abdollahi et al. 2003b). Recent studies well indicate that both cAMP and cGMP can diminish oxidative stress in many biological systems and diseases (Abdollahi et al. 2003c, 2003d; Saadat et al. 2004; Aghababaeian et al. 2005; Milani et al. 2005; Radfar et al. 2005; Zamani et al. 2005). Therefore, as a second mechanism of action, the beneficial effects of SKEO in IBD can be due to its strong antioxidant potential.

It should also be noted that many infective agents like special strains of *Escherichia coli*, Aerobacter aerogens, Proteus, and Staphylococcus sp. have been reported to have a role in the pathogenesis of IBD either directly or indirectly (Botoman et al. 1998; Gibson and Iser, 2005). Supporting this idea, the methanolic extract of cultivated and native *Satureja Khuzestanica* has been shown to have good inhibitory activity against G(+) and G(−) bacteria, especially *Staphylococcus aureus* and *Candida albicans* (Amanlou et al. 2004). Thus, antimicrobial activity of SKEO can be the third mechanism of action for its beneficial effects in IBD.

Taken collectively, it is concluded that SKEO is effective in suppressing acetic acid-induced colitis in mice. Known antioxidant, antimicrobial, antiinflammatory, and antispasmodic potentials of SKEO may be the mechanisms by which this plant protects animals against experimentally induced disease. Regarding the resemblance of acetic acid-induced colitis to human UC (Jurjus et al. 2004), it is suggested that SKEO is useful in treating UC in humans. Future studies should be focused on clinical studies on human IBD after establishing minimum toxicity parameters like determination of maximum tolerated dose and no adverse effect levels in animals.

**REFERENCES**


