Efficacy of Setarud (IMOD®), a novel drug with potent anti-toxic stress potential in rat inflammatory bowel disease and comparison with dexamethasone and infliximab

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The inflammatory bowel disease (IBD) is an idiopathic, immune-mediated and chronic intestinal condition. In the present study, the effect of Setarud (IMOD®), a novel natural drug with known immunomodulatory, anti-inflammatory and antioxidant properties was investigated in experimental colitis in rats and compared with the dexamethasone and infliximab. Immunologic colitis was induced by intracolonic administration of a mixture of trinitrobenzene sulfonic acid (TNBS) and absolute ethanol in male Wistar rats. Animals were divided into 6 groups of sham (normal group), control (vehicle-treated), positive control (dexamethasone 1 mg/kg/day given orally and infliximab 5 mg/kg/day given subcutaneously) and 3 Setarud-treated groups (13.3, 20, 30 mg/kg/day given intraperitoneally). The treatment continued for 14 consecutive days and then animals were decapitated on the day 15 and distal colons were removed for macroscopic, microscopic, and biochemical assays. Biochemical markers, including TNF-α, IL-1β, ferric reducing/antioxidant power (FRAP), myeloperoxidase (MPO) activity and thiobarbituric acid-reactive substance (TBARS) were measured in the homogenate of colonic tissue. A remarkable reduction in macroscopic and histological damage scores was observed in the animals treated with Setarud. These findings were confirmed by decreased levels of TNF-α, interleukin-1β, MPO activity and TBARS, and raised levels of FRAP in the colon tissue. These observations confirmed the immunomodulatory, anti-inflammatory and antioxidant properties of Setarud in experimental colitis, which was comparable to those of dexamethasone and infliximab.

Keywords: Experimental colitis, IMOD®; Inflammatory bowel disease; colitis; Setarud; Oxidative stress; TNF-α antagonist, Toxicity

Inflammatory bowel disease (IBD) is a gastrointestinal (GI) disorder with complex pathogenesis. The disease is divided into two major idiopathic complications — Crohn's disease (CD) and ulcerative colitis (UC). In some cases, the exacerbation of disease is not limited to GI tract and may cause extra-intestinal manifestations, e.g. neurologic, skeletal, urinary and hepatobiliary system disorders. Some factors are considered having key role in the initiation and persistence of inflammation, e.g. impaired immunity, genetic, environmental and microbial factors. Toxic stress as the consequence of increased production of reactive oxygen species (ROS) and reactive nitric oxide species (RNS) plays a significant role in pathogenesis of IBD that harm cellular macromolecules, such as DNA, RNAs, proteins, cell-membrane polyunsaturated fatty acids, resulting in cell death.

Since the predisposing factors of IBD have not been clearly identified, there is no specific treatment for the IBD. Although salicylates are conventionally used in management of mild to moderate IBD, they are not without adverse effects. In addition, glucocorticoids and immunosuppressive drugs have been used for management of moderate to severe IBD. Recently, the benefit of synthetic or naturally-derived antioxidants like N-acetylcysteine (NAC), nicorandil, silymarin, Satureja, Zataria, Ziziphora, antimicrobials, and probiotics in IBD has been confirmed.

Setarud (IMOD®) is a patented drug (registered in USA and Europe) prepared from extracts from Rosa canina, Tanacetum vulgare and Urtica dioica, also comprising selenium and urea and treated with pulsed electromagnetic field of high frequency. It has been found usefulness in conditions associated with...
impaired immune system e.g. human immunodeficiency virus (HIV) infection and also for has shown potential in reduction of TNF-α and increasing IFN-γ and IL-2 levels\textsuperscript{19}. The safety of Setarud has been already approved\textsuperscript{20} and has been tested clinically\textsuperscript{21}. Recently, its benefit in experimental immune-based diabetes has been also reported\textsuperscript{22}.

In the present study, we have evaluated the effect of Serarud in experimental immune-based colitis by measuring TNF-α, IL-1β, ferric reducing/antioxidant power (FRAP), myeloperoxidase (MPO) activity, thiobarbituric acid-reactive substances (TBARS), as well as the macroscopic and histological damage scores in the colonic tissue.

Materials and Methods

Chemicals

2,4,6-Trinitrobenzene sulfonic acid (TNBS), thiobarbituric acid (TBA), trichloroacetic acid (TCA), n-butanol, hexadeyl trimethyl ammonium bromide (HETAB), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), HCl, malondialdehyde (MDA), EDTA, dianisidine hydrochloride, hydrogen peroxide (H$_2$O$_2$), acetic acid, sodium acetate, folin-ciocalteu reagent, bovine serum albumin (BSA), ferric chloride (FeCl$_3$-6H$_2$O), sodium sulfate (Na$_2$SO$_4$), H$_2$SO$_4$, phosphoric acid (H$_3$PO$_4$), potassium dihydrogen phosphate (KH$_2$PO$_4$), potassium hydrogen diphosphate (K$_2$HPO$_4$), peroxide hydrogen (H$_2$O$_2$), sodium carbonate (Na$_2$CO$_3$), Na-K-tartarate, cupric sulfate (CuSO$_4$-5H$_2$O) from Merck (Tehran, Iran), infliximab (Remicade®) from Schering-Plough (UK), dexamethasone from Daru-Pakhsh (Tehran, Iran), rat specific TNF-α and IL-1β ELISA kits from Bender MedSystems Inc (Austria) were used.

Setarud (IMOD\textsuperscript{TM}) was obtained from Parsrus Research Group (Tehran, Iran), the owner of the patent. Setarud is a combination of three extracts (\textit{Rosa canina} fruit, \textit{Tanacetum vulgar} leaves and \textit{Urtica dioica} leaves ethanolic extracts), selenium and urea, having been exposed to a pulsed electromagnetic field as described in the patent\textsuperscript{19}.

Experimental animals and Experimental design

Male Wistar rats weighing between 220-230 g were housed single in standard polypropylene cages with wired-net top in a controlled room (temperature 23 ± 1, humidity 55 ± 10%, 12 h light-dark cycle) and fed a normal laboratory diet. Rats were deprived of food for 36 h prior to induction of colitis, but were allowed free access to tap water. Experiments followed a protocol approved by Ethics Committee of Pharmaceutical Sciences Research Center at Tehran University of Medical Sciences and all ethical criteria for working with animals were carefully met.

Forty-two rats were randomly assigned to 7 groups. Colitis was induced in all groups by administration of TNBS, except in the sham group animals which received saline instead of TNBS. Animals in the remaining 5 groups received dexamethasone (1 mg/kg/day, orally), infliximab (5 mg/kg/day, subcutaneously), Setarud (13.3, 20, and 30 mg/kg/day) and controls received the vehicle saline (10 ml/kg). Setarud was diluted 1:9 in saline and administered intraperitoneally. Doses of Setarud were selected on the basis of our previous experiment\textsuperscript{22}. The day of colitis induction was considered as 1st first day and all medications were administered for 14 days.

Induction of colitis

After 36 h of fasting state before colitis induction, animals underwent anesthesia by intraperitoneal administration of 45 mg/kg penobarbital sodium\textsuperscript{23}. For induction of colitis, rats were positioned on their right side and 0.3 ml of a mixture containing 6 vol. of 5% TNBS plus 4 vols of 99% ethanol was instilled intra-rectally using a rubber cannula (8 cm long)\textsuperscript{5}. After administration of TNBS, rats were maintained in a supine Trendelenburg position to prevent anal outflow of TNBS. Then administration of drugs was performed for 14 days. On the 15\textsuperscript{th} day, animals were anaesthetized as mentioned above, after which the abdomen was dissected open and 5 cm distal colon was removed.

All animals were sacrificed at the end of the procedure using an overdose of ether inhalation. The pieces of colons were cut open on ice, cleansed gently using saline and the macroscopic score of inflammation was determined with magnifier. Each sample was cut into two pieces, one piece for histopathology assessment (maintained in 5 ml formalin 10% as a fixative) and other for measuring biomarkers. The pieces for analysis of biomarkers were weighed and maintained in -20°C for 24 h. Then, the colonic samples were homogenized in 10 vols of ice cold 50 mM potassium phosphate buffer (pH 7.4), for which 100 µl of each sample was taken for antioxidant assay and maintained at -80°C until analysis. The remained homogenate for each sample
was then sonicated and centrifuged at 3500 g for 30 min. The plates were maintained separately and the supernatants were transferred into several microtubes for separate biochemical assays and maintained at -80°C until the analyses were performed\(^\text{10}\).

**Macroscopic and microscopic assessment of colonic damage**

The severity of colonic damage in macroscopic examination was evaluated using the following scoring system: 0 (normal appearance with no damage), 1 (localized hyperemia without ulceration), 2 (localized hyperemia with ulceration), 3 (linear ulceration with inflammation at one site), 4 (two or more sites of ulceration and extending more than 1 cm along the length of colon), and 5-8 (damage extending more than 2 cm along the length of colon and the score was enhanced by 1 for each increased cm of involvement). In addition, the fixed segments in formalin 10\(^\circ\) were embedded in paraffin and stained with hematoxyllin and eosin to examine the tissues microscopically. The scoring was performed by an observer blind to the treated groups. Microscopic scores were determined as follows: 0 (no damage), 1 (focal epithelial edema and necrosis), 2 (disperse swelling and necrosis of the villi), 3 (necrosis with neutrophil infiltration in submucosa); and 4 (wide spread necrosis with massive neutrophil infiltration and hemorrhage)\(^\text{12,13}\).

**TNF-α and IL-1β**

The colonic samples were homogenized in 10 vols of ice cold 50 mM potassium phosphate buffer (pH 7.4) and used for determination of TNF-α and IL-1β using an ELISA kit as instructed by the kit brochure. TNF-α and IL-1β levels were expressed as pg of cytokine per mg protein of tissue\(^\text{10}\).

**Ferric reducing antioxidant power (FRAP)**

Total anti-oxidant capacity of colon tissue was evaluated by measuring the ability to reduce Fe\(^{3+}\) to Fe\(^{2+}\). Interaction of TPTZ with Fe\(^{2+}\) results in formation of a blue color with a maximum absorbance at 593\(^\text{24}\). Data were expressed as μM Fe\(^{3+}\) ions reduced to Fe\(^{2+}\) per mg protein of tissue.

**Myeloperoxidase (MPO) activity**

The colonic samples were homogenized in 10 vols of ice cold potassium phosphate buffer (50 mM, pH 7.4), sonicated and centrifuged at 3500 g for 30 min. The supernatant was centrifuged at 12000 g for 20 min and then 0.1 ml of supernatant was added to 50 ml of phosphate buffer containing 0.167 mg/ml α-dianisidine and 0.0005% H\(_2\)O\(_2\) that resulted in an orange complex. The absorbance was measured for 3 min spectrophotometrically in 460 nm. One unit of MPO activity was described as the change in absorbance per min at room temperature in the final reaction and expressed as unit per mg protein of colon tissue. Details of the procedure have been described elsewhere\(^\text{12}\).

**Thiobarbituric acid-reactive substances (TBARS)**

Lipid peroxidation occurs because of toxic stress and can lead to serious cellular damages. MDA, the main byproduct of polyunsaturated fatty acids oxidation is known as a biomarker of lipid peroxidation and its concentration was assessed in colon tissue using thiobarbituric acid-reactive substances (TBARS) assay. The data were reported as μM per mg protein of colon tissue\(^\text{12}\).

**Total protein**

Protein concentration of colon homogenate was determined according to Lowry method using BSA as standard\(^\text{25}\).

**Statistical analysis**

Results were expressed as mean ± standard error of the mean (SEM). The data were analyzed by one-way ANOVA, followed by Turkey’s post hoc test for multiple comparisons to ensure the variances of data were distributed properly. A P-value less than 0.05 was considered significant.

**Results**

**Macroscopic and microscopic evaluation of the colonic damage**

Severe inflammation, ulceration, dilatation, adhesion and wall thickening was caused by treatment of animals with TNBS/ethanol in the controls, whereas colons of sham group were normal (P<0.05). Administration of both dexamethasone and infliximab improved macroscopic scores significantly in colitis rats (P<0.05). Administration of Setarud reduced colonic damage in an almost dose-dependent manner (P<0.05) comparable to that of positive control groups.

Microscopic evaluation of control group showed severe, intense transmural inflammation and/or diffused necrosis (P<0.05), whereas in sham group, features of colons were within normal limits. Histological examination of Setarud-30 group showed mild inflammation of the mucosa. In Setarud-20
group, moderate inflammation of the mucosa and superficial sub-mucosa and superficial ulceration were observed. In Setarud-13 group, severe mucosal and submucosal inflammation, crypt distortion and goblet cell depletion were observed. Rats treated with infliximab also exhibited mild mucosa inflammation and crypt damage. Minimal mucosal inflammatory cell infiltration was observed in the histological evaluation of dexamethasone-treated group (Table 1 and Fig. 1).

**TNF-α and IL-1β levels**

TNF-α level was drastically elevated in control group as compared to sham ($P = 0.00$). Administration of Setarud at doses 13.3, 20 and 30 mg/kg remarkably decreased TNF-α level ($P = 0.004, 0.00$ and $0.00$, respectively). The TNF-α levels in Setarud-20 ($P = 0.396$) and -30 ($P = 0.895$) were similar to that in dexamethasone group, whereas in Setarud-13 group were significantly higher ($P = 0.003$) than that of dexamethasone group. All Setarud groups demonstrated significantly higher TNF-α levels, as compared to infliximab ($P = 0.00, 0.004$ and $0.03$, respectively). The TNF-α levels were not significantly different between Setarud-20 and -30 ($P = 0.963$) groups (Fig. 2A).

A significant increase in IL-1β was observed in control group, as compared to sham ($P = 0.00$). Administration of Setarud in doses of 13, 20, and 30 caused a marked reduction in IL-1β level ($P = 0.00$ for all). The IL-1β levels in Setarud-20 and -30 groups demonstrated no significant difference with that of dexamethasone group ($P = 0.913$ and $1.00$ for Setarud-20 and -30, respectively).

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**Table 1—Extent of colonic damage based on macroscopic and microscopic scores**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Macroscopic score (Mean ± SEM)</th>
<th>Microscopic score (Mean ± SEM)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Median (Min-Max)</td>
<td>Median (Min-Max)</td>
</tr>
<tr>
<td>Sham</td>
<td>(0.0 ± 0.0)</td>
<td>(0.0 ± 0.0)</td>
</tr>
<tr>
<td>Control</td>
<td>(5.75 ± 0.48)ª</td>
<td>(3.75 ± 0.25)ª</td>
</tr>
<tr>
<td></td>
<td>5.5 (5.0-7.0)</td>
<td>4 (3.0-4.0)</td>
</tr>
<tr>
<td>Dexa</td>
<td>(0.67 ± 0.33)b</td>
<td>(0.67 ± 0.33)b</td>
</tr>
<tr>
<td></td>
<td>0.5 (0.0-2.0)</td>
<td>0.5 (0.0-2.0)</td>
</tr>
<tr>
<td>Infliximab</td>
<td>(1.0 ± 0.25)b</td>
<td>(1.33 ± 0.21)b</td>
</tr>
<tr>
<td></td>
<td>1.0 (0.0-2.0)</td>
<td>1.0 (1.0-2.0)</td>
</tr>
<tr>
<td>Setarud-13</td>
<td>(2.17 ± 0.40)b</td>
<td>(2.83 ± 0.31)b</td>
</tr>
<tr>
<td></td>
<td>2.5 (1.0-3.0)</td>
<td>3.0 (2.0-4.0)</td>
</tr>
<tr>
<td>Setarud-20</td>
<td>(1.83 ± 0.40)b</td>
<td>(2.33 ± 0.42)b</td>
</tr>
<tr>
<td></td>
<td>1.5 (1.0-3.0)</td>
<td>2.0 (1.0-4.0)</td>
</tr>
<tr>
<td>Setarud-30</td>
<td>(1.5 ± 0.22)b</td>
<td>(1.67 ± 0.33)b</td>
</tr>
<tr>
<td></td>
<td>1.5 (1.0-2.0)</td>
<td>1.5 (1.0-3.0)</td>
</tr>
</tbody>
</table>

Dexa group: treated with 1 mg/kg/day orally dexamethasone; Infliximab group: treated with 5 mg/kg/day subcutaneously infliximab; Setarud-13, -20, and -30 groups: treated with 13, 20 and 30 mg/kg/day respectively intraperitoneally. Colons were within normal limits including crypts (a) and submucosal tissue (b) in Sham group, but extensive severe transmural inflammation (c), granuloma with necrosis and crypt destruction (d) were seen in control group. Minimal mucosal inflammation was observed in histological evaluation of Dexe group (e). Features of colons were within normal limits including mucosal and submucosal tissue (f) in infliximab treated group. Cytotoxic destruction (g), crypt abscess (h) with granuloma (i) without necrosis were seen in Setarud-13 groups. Mild crypt distortion and inflammatory cell infiltration (j) were seen in Setarud-20 group. Histological examination showed a mild inflammation of mucosa in Setarud-30 group (k). Magnification of all images was 100]
Setarud-20 and -30, respectively), while Setarud-13 was not as effective as dexamethasone ($P = 0.024$). Similar results were observed with Setarud groups, as compared to infliximab ($P = 0.02, 0.97$, and $1.00$, respectively). Although, no significant difference was observed in IL-1β levels between Setarud-13 and -20 ($P = 0.194$) or between Setarud-20 and -30 ($P = 0.981$) groups, but IL-1β level in Setarud-30 group was significantly lower than that of Setarud-13 ($P = 0.048$).

**FRAP**

Due to colonic damage, a dramatic decrease of FRAP values was observed in control group, when compared to sham ($P = 0.00$), while the values were significantly elevated by administration of all doses of Setarud ($P = 0.001, 0.00$ and $0.00$, respectively). In comparison with dexamethasone, the least dose (Setarud-13) showed significantly lower value ($P = 0.018$), whereas Setarud-20 demonstrated an elevated non-significant value ($P = 0.927$) and Setarud-30 a significantly higher value ($P = 0.00$). As compared to infliximab, Setarud-13 and -20 groups showed significantly lower FRAP values ($P = 0.00$ for both), whereas the value was significantly higher ($P = 0.00$) in the Setarud-30 group. Although no significant difference was observed between Setarud-13 and -20 FRAP values ($P = 0.108$), in Setarud-30 group, FRAP value had a significant difference when compared with both groups ($P = 0.00$ for both, Fig. 3A).
MPO activity

Noticeable increase in colonic MPO activity was observed in control group as compared to sham group ($P = 0.00$). Administration of Setarud in doses of 13, 20, and 30 mg/kg caused a significant reduction in MPO activity ($P = 0.027$ for Setarud-13, $P = 0.00$ for Setarud-20 and -30). The reduction of MPO activity by Setarud-13 and -20 was lower than that of dexamethasone ($P = 0.00$ for both), whereas in Setarud-30 group it was comparable with that of dexamethasone ($P = 0.073$). In comparison with infliximab, Setarud at doses of 13, 20, and 30 mg/kg showed no significant difference ($P = 0.42, 1.00$, and 0.47 respectively). No significant differences were observed between Setarud-13 and -20 ($P = 0.480$) or Setarud-20 and -30 ($P = 0.164$) groups (Fig. 3B).

TBARS levels

Lipid peroxidation as TBARS in colon in various groups is shown in Fig. 3C. Induction of colitis in control group resulted in remarkable increase of TBARS ($P = 0.00$) in comparison to sham. TBARS levels were significantly decreased in Setarud-20 and -30 groups ($P = 0.00$), but no significant reduction was observed in Setarud-13 ($P = 0.787$). Although both Setarud-13 and -20 groups showed significantly elevated values when compared to dexamethasone ($P = 0.00$), but positive effect of Setarud-30 was close to that of dexamethasone ($P = 0.545$). Similarly, as compared to infliximab, TBARS values in Setarud-13 and -20 groups were significantly higher ($P = 0.00$), but Setarud-30 showed a non-significant raised value ($P = 0.90$).

Discussion

In the present study, we have demonstrated for the first time that treatment of IBD in rats with Setarud effectively ameliorates the immune-based colonic damage induced by TNBS/ethanol. Administration of Setarud causes a significant reduction in colonic macroscopic and microscopic damages in almost a dose-dependent manner, as revealed by declined pro-inflammatory cytokines, lipid peroxidation, and neutrophil sequestration. Of the various constituents of drug, *R. canina* has shown antioxidant properties$^{26}$. The galactolipid (an anti-inflammatory agent) derived from the fruit of *R. canina* has shown inhibitory effect on chemotaxis of human peripheral blood neutrophils$^{27}$. The extract of *R. canina* also exhibits inhibitory effect on inflammatory cytokines, such as IL-1$\beta$ and TNF-$\alpha$$^{28}$. Furthermore, *R. canina* has also shown free radical scavenging$^{29}$, anti-ulcerogenic$^{30}$ and anti-nociceptive$^{31}$ effects. On the other hand, flavonoid extracted from leaf and flower of *T. vulgare* exhibits anti-inflammatory effect (via inhibition of arachidonate metabolism pathway) in leukocytes$^{32,33}$. The low molecular weight fraction of fractionated acidic polysaccharides of *T. vulgare* has anti-inflammatory effect, whereas its high molecular fraction activates macrophage/monocyte capacity$^{34}$.

The Nettle (*Urtica dioica*), another constituent of Setarud has been traditionally used for a wide spectrum of ailments. Leaves of this plant have shown anti-inflammatory$^{35}$ and immunomodulatory$^{36}$ activities. The leaf extract inhibits the release of pro-inflammatory cytokines, such as TNF-$\alpha$, IL-1$\beta$ and nuclear factor-kappa B (NF-$\kappa$B)$^{38}$ and prevents the maturation of human myeloid dendritic cells and reduces primary T-cell responses$^{39}$. Selenium, another constituent of Setarud is reported to play an important role in a number of antioxidant metallo-enzymes, e.g. glutathione peroxidase$^{40}$ and thioredoxin reductase$^{41}$. Previous studies have shown its remedial effect as an anti-inflammatory/anti-necrotic agent on the inflammatory bowel disease$^{42}$.

Amongst several animal models of IBD, in TNBS-ethanol-induced colitis, ethanol breaks the mucosal barrier, whereas TNBS hampenizes colonic autologous or microbiota proteins representing them immunologic to host immune system. This model is very useful in studying gut inflammation, cytokine secretion patterns, cell adhesion and immunotherapy and is similar to human IBD$^{43,44}$. Pro-inflammatory cytokines, especially TNF-$\alpha$ and IL-1$\beta$ are excessively produced during primary stages of colitis. This leads to disequilibrium between pro-inflammatory and anti-inflammatory cytokines and is one of the most important elements involved in pathogenesis of human IBD$^{45,46}$.

TNF-$\alpha$, a 51-kDa cytokine secreted by monocytes, macrophages, and T-lymphocytes plays a determinant role in pro-inflammatory signaling, neutrophil recruitment to the site of inflammation, activation of coagulation and fibrinolysis pathways, and initiation of granuloma formation$^{47,48}$. It has been found that anti-TNF-$\alpha$ drugs (infliximab, adalimumab and certolizumab pegol) are useful in treatment of IBD$^{49,50}$. IL-1$\beta$, a key pro-inflammatory cytokine is produced by monocytes and tissue macrophages and has a regulatory effect on expression of some inflammatory process involved genes$^{51}$. In vitro,
IL-1β attracts and activates immune cells, and promotes leukocyte infiltration to inflamed tissues by expression of endothelial cell adhesion molecules expression. IL-1β is also responsible for diarrhoea, the major complication of IBD. The present results reveal that Setarud decreases colonic TNF-α and IL-1β levels, probably through inhibition of production and activity of these cytokines.

One of the major tissue-destructive mechanisms in IBD pathogenesis is toxic stress through excessive release of ROS by phagocytes, and a lesser extent by eosinophils, lymphocytes, and fibroblasts. The damage is caused by the imbalance between the production of oxidants and counterbalancing role of antioxidants. In order to evaluate the antioxidant power in colonic tissue samples, the FRAP method has been used. As expected, Setarud shows a strong antioxidant potential because of its free radical scavenging activity. Free radicals resulted from oxidation of macromolecules in the inflammed tissues can attract immune cells, such as polymorphonuclear neutrophils. Migration of these immune cells to the site of inflammation leads to overproduction of inflammatory agents including proinflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-8), adhesion molecules, prostaglandins, leukotriens, and platelet-activating factor (PAF). In addition, consumption of copious amount of oxygen by activated neutrophils leads to formation of more ROS and overexpression of oxidative enzymes like MPO. The MPO is a heme enzyme released from storage granules, following neutrophils activation by inflammatory stimuli. It catalyzes the formation of a number of reactive species. The present data reveal that Setarud is able to inhibit exacerbation of inflammatory condition resulted from release of destructive agents (by MPO and other oxidizing enzymes) subsequent to presence of neutrophils in the inflammation site.

Lipid peroxidation is another well-established mechanism of cell injury and is widely used as an indicator of oxidative stress in cells and tissues. The most used index of lipid peroxidation is malonaldehyde (MDA) formation, often measured with TBARS assay. MDA is a product of both radical-initiated lipid peroxidation and the enzymatic activity of thromboxane synthase. It has been shown that increased levels of TBARS are evident in the inflamed intestinal mucosa of IBD patients. The result of our study show that Setarud is capable of reducing lipid peroxidation because of its antioxidant potential. This antioxidant property is not only due to free-radical scavenging potential, but also refers to its fortifying effect on antioxidant enzymes. This is supported by the present findings that Setarud increases colon total antioxidant power even higher than normal levels.

In conclusion, our results demonstrate that Setarud exerts protective effects in murine model of colitis by improvement of inflammatory mediators, neutrophil infiltration, toxic stress, and colonic damage. Previous acute and chronic toxicology studies have shown safety of Setarud in animals even at doses of 10-30 times higher than maximal human recommended therapeutic dose and also in humans. However, further investigations and particularly clinical trials are required to elucidate its safety and efficacy in human IBD.

Acknowledgment

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References
