The phytogenic feed additive Sangrovit modulates dextran sulfate sodium-induced colitis in rats

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ABSTRACT: The alkaloids of *Macleaya cordata* (Papaveracae) are active components of the phytogenic feed additive Sangrovit. The present study was conducted to evaluate the effects of Sangrovit on dextran sulfate sodium (DSS)-induced colitis using rats as an experimental model. Thirty-five male rats were randomly assigned to a control group (Group 1, *n* = 5), a Sangrovit group (Group 2, *n* = 20) and a DSS group (Group 3, *n* = 10). Group 1 received standard diet and tap water for 14 days. Group 2 received 500 ppm Sangrovit in their feed for 14 days and in the second week 5% DSS was added to the tap water. The animals in Group 3 were fed for seven days with standard diet and tap water and for the next seven days with standard diet and 5% DSS added to their tap water. The rats were sacrificed on day 14 and the following parameters were measured: disease activity (body and organ weight, colon length, presence of blood in stool), colon myeloperoxidase activity, expression of colon cyclooxygenase-2 (COX-2), hematological parameters, histological colitis score and selected parameters of oxidative stress. The animals treated with DSS for seven days (Groups 2 and 3) showed increases in liver and cecum weight, leukocyte count and colon shortening, decreases in hemoglobin and hematocrit associated with hematochezia. In comparison with Group 3 where DSS caused mucosal edema, cellular infiltration and epithelial disruption, the Sangrovit group showed less severe damage to the colon mucosa and decreased histological colitis scores. The Sangrovit group also showed diminished expression of DSS-induced COX-2, significantly mitigated myeloperoxidase activity in colon tissue and level of reduced glutathione in erythrocytes. In conclusion, some parameters of DSS-induced colitis were modulated by 500 ppm Sangrovit added to feed.

Keywords: *Macleaya cordata*; Papaveraceae; benzo[*c*]phenanthridines; protopines; feed additive; dextran sulfate sodium-induced colitis; cyclooxygenase-2; myeloperoxidase

List of abbreviations

AL = allocryptopine, **CH** = chelerythrine, **COX-2** = cyclooxygenase-2, **DHCH** = dihydrochelerythrine, **DHSG** = dihydrosanguinarine, **DSS** = dextran sulfate sodium, **GSH** = reduced glutathione, **GST** = glutathione transferase, **IBD** = inflammatory bowel disease, **MPO** = myeloperoxidase, **PR** = protopine, **QBA** = quaternary benzo[*c*]phenanthridine alkaloids, **SG** = sanguinarine, **TBARS** = thiobarbituric acid reactive substances, **TMB** = tetramethylbenzidine

　　In recent years, phytogenic feed additives for livestock have attracted increasing interest as an alternative feeding strategy for replacing antibiotic growth promoters, especially in the European Union where antibiotics have been banned as additives in livestock feed since 2006 (Council Regulation (EC) No 1831/2003 of the European Parliament and of the Council on additives for use in animal nutrition). Sangrovit, a natural appetizer feed additive is recommended for its mu-

colytic and anti-inflammatory effects on animal intestinal tracts (Mounsey, 2010). It contains as its active component a mixture of the powdered stems, leaves, capsules and seeds of *Macleaya cordata* (Willd.) R.Br. (plume poppy or *Bocconia cordata*, Papaveraceae) blended as a standard concentration of sanguinarine (SG). *M. cordata* contains the following major alkaloids: SG, its dihydroderivative (DHSG), chelerythrine (CH), protopine (PR), allocryptopine (AL) and phenolic acids (Kosina et al., 2010). In traditional Chinese medicine, the aerial part of *M. cordata* has long been used for its analgesic and anti-inflammatory properties (Chang and But, 1987). The anti-microbial, anti-inflammatory and immunomodulatory activities of *M. cordata* are attributed to the quaternary benzo[*c*]phenanthridine alkaloids (QBA) SG and CH (Simanek et al., 2003). These alkaloids exhibit local anti-inflammatory effects in the carrageenan-induced paw edema test in rats (Lenfeld et al., 1981). SG but not CH inhibits the signal transduction pathways critical to the inflammatory response leading to NF-κB activation (Chaturvedi et al., 1997). *M. cordata* is on the EFSA list of plants used as a component of feed additives in animal production (Franz et al., 2005).

One crucial aspect of current animal food production is the use of protective feed ingredients as anti-inflammatory agents (Windisch et al., 2008). Anti-inflammatory feed components are a key to optimal growth and livestock health. Inflammatory bowel disease (IBD) refers to a chronic inflammatory disease of the gastrointestinal tract, including the stomach, small intestine and colon. In animal models, acute or chronic colitis can be induced by dextran sulfate sodium (DSS) in the drinking water (Gaudio et al., 1999). Histologically, DSS-induced colitis is characterized by mucosal congestion, severe submucosal edema, deep erosion and thickening of the colon wall, and polymorphonuclear leukocyte and lymphocyte infiltrations. Some natural compounds, such as alkaloids and polyphenolics can modulate intestinal inflammation. The alkaloids berberine (Shu et al., 2006), tetrandrine (a bisbenzylisoquinoline) (Zhang et al., 2009), 4-methoxy-5-hydroxycanthin-6-one (Liu et al., 2007) and oxymatrine (Zheng et al., 2005) ameliorate DSS-induced colitis through inhibition of the inflammatory response, improvement in colon structure and disease activity index. Caffeic acid, a phytochemical found widely in plant foods, decreases the expression of pro-inflammatory genes

Il-17, iNOS, and increases IL-4 gene expression in DSS-treated mice (Ye et al., 2009). Green tea polyphenol extract significantly decreases DSS-induced colitis in mice (Oz et al., 2005).

Recently we have studied the effects of long term administration of *M. cordata* herb and its purified alkaloid extract *sanguiritrin* (a mixture of SG and CH) on pig and rat metabolism and its possible genotoxicity (Kosina et al., 2004; Psotova et al., 2006b; Stiborova et al., 2008). We also showed that there were no toxic effects associated with the oral administration of 7000 ppm of Sangrovit to rats (Zdarilova et al., 2008). These experiments confirmed that neither herb nor extract used as feed preparations, had any adverse effects on selected clinical chemical parameters and were thus safe. *Sanguiritrin* is successfully used in veterinary medicine (Levchik et al., 1999; Fox, 2008). The aim of the present study was to investigate the effect of *M. cordata* constituents on a rodent model of DSS-induced colitis. For the purpose of the study, intact *M. cordata* was administered as the feed additive Sangrovit.

MATERIAL AND METHODS

Feed additive and chemicals. Sangrovit was obtained from Bioferm CZ, Brno, Czech Republic. For determination of alkaloid content, Sangrovit was extracted with acidified (1% HCl) methanol and analyzed by HPLC. The content of isoquinoline alkaloids was 19.33 ± 1.19 (g/kg) sanguinarine, 10.44 ± 0.43 chelerythrine, 5.98 ± 0.23 protopine, 3.50 ± 0.19 allocryptopine and 1.76 ± 0.26 dihydrosanguinarine. Dextran sulfate sodium (36 000 to 50 000 Da) was obtained from MP Biomedicals LLC (Ohio, USA). Western blotting luminol reagent for chemiluminescent horseradish peroxidase (HRP) detection, rabbit anti-cyclooxygenase 2 (H-62) and goat anti-actin (I-19) antibody, rabbit anti-goat and goat anti-rabbit HRP-conjugated secondary antibodies were purchased from Santa Cruz Biotechnology (USA). Other chemicals were purchased from Sigma-Aldrich Co (USA).

Diets. Standard diet. A powdered commercial diet for laboratory animals (KrmiMo, Tetcice, Czech Republic) was used.

Experimental diet. Sangrovit (500 mg) was blended with standard diet (1000 g) to make feed pellets. The diets were prepared weekly and analyzed by HPLC/MS periodically to confirm the al-

kaloid content. The pellets were stored in paper bags and kept dry.

Animals. The study was approved by the Ethics Committee, Ministry of Education, Czech Republic and conducted in compliance with the Experimental Animals Protection Act No. 167/1993 L.C. Male Wistar rats (200 ± 10 g bw) were purchased from Velaz s.r.o., Prague, Czech Republic. The rats were acclimatized one week before the experiment. They were kept in plastic cages containing dust-free sawdust, two animals in a cage. On the day of treatment, the animals (n = 35) were 12 weeks old with a mean body weight of 221 ± 11 g. The animals were randomly assigned to three groups: a control group (Group 1, n = 5, 220 ± 12 g), a Sangrovit + DSS group (Group 2, n = 20, 220 ± 10 g) and a DSS group (Group 3, n = 10, 224 ± 14 g).

Design of the experiment. The animals consumed *ad libitum* either the standard diet (Groups 1 and 3) or the diet containing 500 ppm of Sangrovit (Group 2) for 14 days. For the first week all the animals drank tap water. In the second week Group 1 drank tap water, Groups 2 and 3 drank tap water with 5% DSS which was prepared daily.

During the acclimatization period and during the experiment, the conditions in the animal room were as follows: temperature (23 ± 2 °C; checked daily); relative humidity (30 to 70%); light/dark cycle 12 h/12 h. The animals had free access to their respective diets, water or water containing DSS. Feed consumption was checked three times a week. The health of the animals was checked daily. The disease activity was determined by changes in body weight twice a week and prior to sacrifice and stool hemoccult positivity on days 10 and 13.

Sample collection and preparation. The animals were deprived of feed 12 h before terminal *i.m.* anaesthesia by fentanyl (4 µg/100 g bw), medetomidin (20 µg/100 g bw) and diazepam (0.5 mg/100 g bw). After opening the abdominal cavity, the main organs of the gastrointestinal tract were examined macroscopically. Blood was collected from the aortic bifurcation into Na_2EDTA-tubes (Sarstedt, Germany) and the blood count was determined. The rest of the blood was centrifuged (450 × g, 10 min, 4 °C) to obtain the plasma. Plasma aliquots were stored at –80 °C for determination of the parameters of oxidative stress. Erythrocytes were washed with phosphate buffered saline and were stored at –80 °C for determination of the parameters of oxidative stress. Liver and cecum were removed, washed in cold phosphate buffered saline

and weighed. The colon was removed, washed in cold phosphate buffered saline, measured in length and stored at –80 °C for determination of oxidative stress parameters and for alkaloid analysis. Small intestine and colon sections were examined histologically (see below).

Histopathology. The colon was dissected into four sections (Section 1 – ascending colon, Section 2 – transverse colon, Section 3 – descending colon, Section 4 – rectum). Between each part there were 3–4 cm long interspaces. The small intestine and colon specimens were fixed in Baker's solution for up to 24 h, embedded in paraffin and 7 µm sections were cut on a rotary microtome. Sets of histological sections were stained with hematoxylin-eosin and PAS. The histological evaluation was performed on an Olympus BX 40 light microscope using the following histological score (0 = normal, 1 = mild/focal, 2 = moderate/zonal, 3 = severe/diffuse). Histological morphological changes were assessed using the following eight parameters of colitis: destruction of epithelium, dilatation of crypts, loss of goblet cells, inflammatory infiltrate, edema, congestion, crypt abscesses and atrophy according to (Gaudio et al., 1999).The samples were evaluated by independent histologists.

Hematology. Hemoglobin, hematocrit, erythrocytes, thrombocytes and leukocytes were analyzed in Na_2EDTA blood using ABX ABC Vet Hematology Analyzer (Horiba ABX, France).

Parameters of oxidative stress. Lipid peroxidation was assessed by measuring the presence of thiobarbituric acid reactive substances (TBARS) in the plasma, erythrocytes and colon homogenates (Buege and Aust, 1978). The level of reduced glutathione (GSH) in erythrocytes and colon homogenate was determined according to Sedlak and Lindsay using Ellman's reagent (Sedlak et al., 1968). The plasma level of total SH-groups was determined according to Hu (1994). Glutathione transferase (GST) activity in colon tissue and erythrocytes was assayed by a modified method of Warholm et al. (1981). The protein concentration was determined by the Bradford method (Bradford, 1976).

Colon myeloperoxidase (MPO) activity was determined photometrically using 3,3',5,5'-tetramethylbenzidine (TMB) as a substrate according to Granell et al. (2004) with some modifications: colon (50 mg) was homogenized in 50mM phosphate buffer, pH = 5.4 containing 5 g/l hexadecyltrimethylammonium bromide (1 ml), sonicated for 30 s and centrifuged (10 min, 9700 × g, 4 °C). The

reaction mixture contained supernatant (20 μl), TMB (1.6mM, 10 μl), H_2O_2 (3.0mM, 20 μl) and 50mM phosphate buffer, pH 5.4 (150 μl). The reaction was started by H_2O_2 and stopped 3 min later with H_2SO_4 (2M, 10 μl). The measurement was performed at 450 nm.

Protein level of COX-2. The protein concentration of homogenate was determined by the Bradford assay and pooled samples were prepared. Proteins were separated by 15% SDS polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane. Detection of COX-2/actin was performed by incubation with a primary antibody (rabbit anti-COX-2 (H-62)/goat anti-actin (I-19) antibody) and then with a secondary HRP-conjugated antibody (goat anti-rabbit/rabbit anti-goat antibody). COX-2/actin expression was detected by chemiluminescence using Western blotting luminol reagent and autoradiography with XAR-5 film.

Determination of alkaloids. Colon tissue (0.5 g) was homogenized in methanol with 1% HCl (2 ml). The homogenates were vortexed for 1 min and centrifuged (3500 × g, 5 min, 4 °C). After supernatant evaporation (under N_2 at 40 °C), the solid residue was dissolved in aqueous methanol (70%, v/v; 250 μl) and centrifuged. Supernatant was analyzed by HPLC/MS as described below.

The HPLC chromatographic system Shimadzu (Shimadzu, Kyoto, Japan) equipped with a SCL-10Avp controller, a vacuum degasser, a binary pump (LC-10ADvp), an autoinjector (SIL-10ADvp), a column oven (CTO-10ACvp) and a UV-detector (SPD10Avp, 280 nm) was used. The system was coupled on-line to the ESI/ion-trap MS detector. The chromatographic column (150 mm × 2.1 mm, 5 μm) Eclipse XDB-CN (Agilent, USA) was used (t_R': SG 5.7, CH 6.7, dihydrochelerythrine (DHCH) 10.8, DHSG 11.8 min). The injection volume was 10 μl and the mobile phase consisted of methanol (solvent B)/2% acetic acid in 10% aqueous methanol, linear gradient elution: 0–9 min (10–55% B), 9–12 min (55–60% B), 12–12.1 min (60–10% B), 12.1–16 min (10% B). The mobile phase flow rate was 0.4 ml/min and the temperature of the column oven was set at 30 °C.

The quadrupole ion-trap MS detector LCQ Fleet (Thermo Scientific, Waltham, MA, USA) operating in a positive ESI mode was used for analysis. ESI-MS parameters were as follows: spray voltage (4.75 kV), transfer capillary temperature (375 °C), and capillary voltage 30 V. Nitrogen was used as sheath, auxiliary and sweep gas, and helium was used as the collision gas. The sheath, auxiliary and sweep gas flow rates were 50, 5, and 1 (as arbitrary units), respectively. Abundances of described MS^2 fragment ions (SG 332.17 → 304.17, CH 348.17 → 333.17, DHSG 334.17 → 319.17, and DHCH 350.25 → 335.17 m/z) were monitored for the compound analysis in real samples.

Statistical analyses. The data were analyzed using the nonparametric Wilcoxon two-tailed test (independent-samples) due to low number of subjects. The level of significance was 5%. Values are presented as means ± standard deviation.

RESULTS

Feed consumption

The diet consumption was 18 ± 1 g/day on average. No significant alterations in feed consumption were found for experimental groups compared to the control. The daily average consumption of Sangrovit in Group 2 was 9 mg/kg bw, i.e., 0.17 mg sanguinarine/kg bw.

Disease activity

The body weight gain curves followed a similar course in all experimental groups. On days 12 and 14 of the experiment a non-significant decreasing trend in body weight gain was observed in the DSS groups (Figure 1). A significant increase in liver and cecum weight and colon length shortening was found in Groups 2 and 3 (Table 1). The occult blood in feces in animals of Groups 2 and 3 was confirmed by a positive hemoccult assay. No deaths occurred during the experiment.
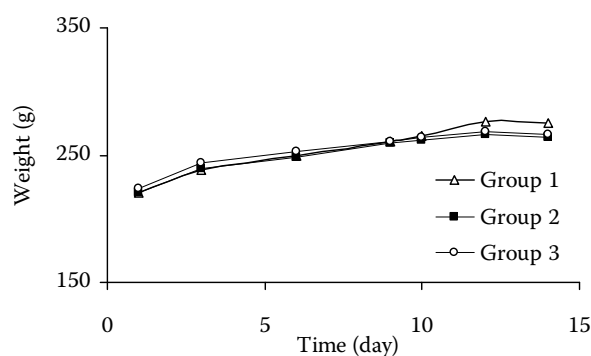

Figure 1. Effect of Sangrovit on body weight

Table 1. Effect of Sangrovit and DSS on body and organ weights on day 14

Weight (g)	Group 1	Group 2	Group 3
Body	274.6 ± 18.3	264.1 ± 17.4	266.3 ± 22.2
Weight increase (%)	28.4 ± 4.4	19.9 ± 5.5	19.0 ± 4.3
Liver	7.2 ± 0.6	8.1 ± 0.8*	7.9 ± 1.1
Cecum	1.3 ± 0.1	1.5 ± 0.3*	1.6 ± 0.3*
Feed consumption	18.5 ± 1.1	18.2 ± 2.7	17.4 ± 3.4
Organ weight/body weight (%)			
Liver	2.6 ± 0.1	3.1 ± 0.3*	3.0 ± 0.2*
Cecum	0.46 ± 0.05	0.58 ± 0.11*	0.58 ± 0.10*
Colon length (cm)	19.2 ± 0.6	14.7 ± 2.1*	15.8 ± 2.0*

*the value significantly different from control group ($P < 0.05$)

Histological damage score

Normal colonic mucosa was observed in control animals (Group 1). DSS (Group 3) caused severe ulcerative colitis throughout the colon, but was more pronounced in the rectum and the histological score was 1.8. There were diffuse mucosal lesions (epithelial damage, ulceration, inflammatory infiltrate, loss of goblet cells, dilatation of crypts, edema). In contrast, rats treated with Sangrovit (Group 2) had only focal epithelial lesions and a lower histological score (0.8) (Figures 2 and 3). In the small intestine, normal mucosa was observed in all groups (data not shown).

Hematological parameters

DSS (Group 2 and 3) modulated all monitored hematological parameters except for thrombocyte count. The inflammatory response in DSS-induced colitis animals was detected as a moderate increase in leukocytes in Group 3. In DSS treated groups a reduction in erythrocytes was associated as a decrease in hemoglobin and hematocrit. No improvement in selected DSS-modulated parameters in the Sangrovit group was found (Table 2). The decrease in erythrocytes (hemoglobin, hematocrit) was due to blood loss in stool.

Inflammatory and oxidative stress parameters

MPO activity was used as a quantitative index of polymorphonuclear neutrophil infiltration and inflammation. DSS-treated animals (Group 3) had significantly greater MPO activity in the colon than those in Group 1. Sangrovit significantly mitigated DSS-induced MPO activity. We found
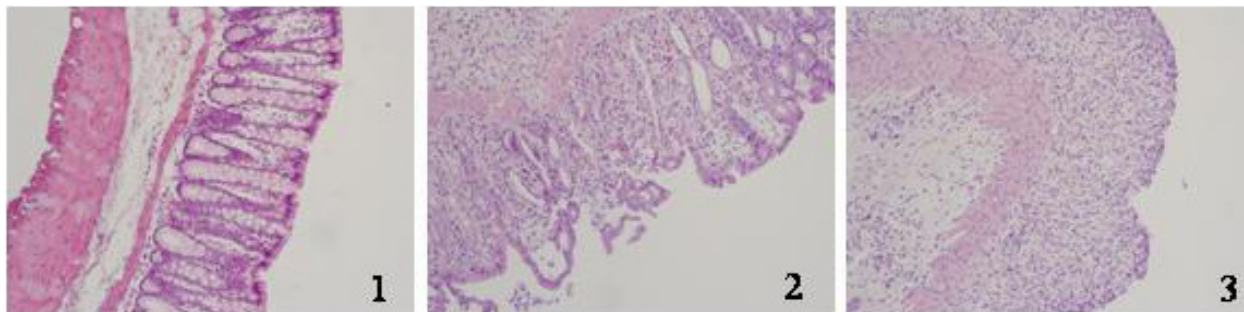

Figure 2. Effect of Sangrovit on colon tissue. Selected microphotographs of the colon of Group 1 (1) = no epithelial damage, Group 2 (2) = fed with Sangrovit and 5% DSS in tap water – focal mucosal damage, and Group 3 (3) = treated with 5% DSS in tap water – diffuse mucosal damage. Stained with hematoxylin-eosin; original magnification 10× for Group 1, 40× for Groups 2 and 3
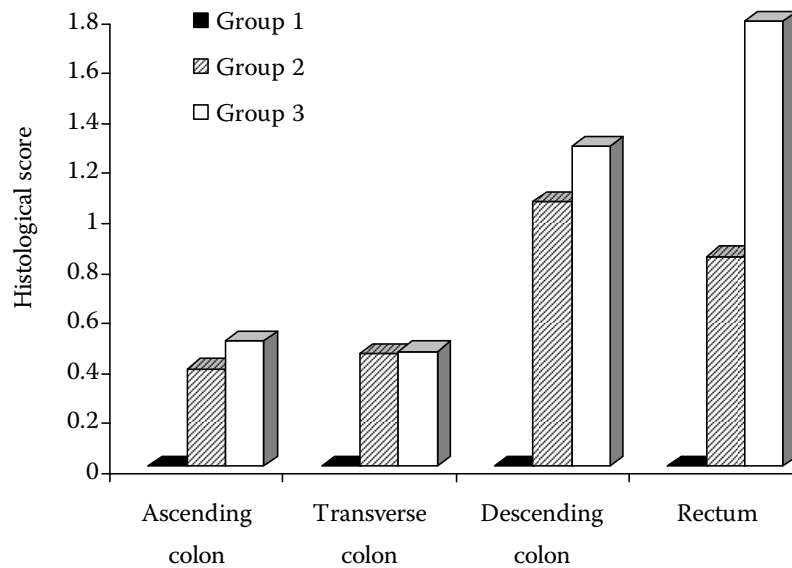
Figure 3. Effect of Sangrovit on colon histological score

increases in erythrocyte GSH in the DSS groups (Table 3). As shown in Figure 4 and Table 3, the COX-2 protein level was low in the colon tissue of control rats (Group 1), markedly increased in rats with DSS-induced colitis, but lower in rats treated with Sangrovit.

Table 2. Effect of Sangrovit and DSS on hematological parameters

Parameter	Group 1	Group 2	Group 3
Leukocytes (10^3/mm^3)	2.94 ± 0.41	5.48 ± 3.35*	5.33 ± 2.18*
Erythrocytes (10^6/mm^3)	8.74 ± 0.47	6.62 ± 2.19*	7.32 ± 2.16
Thrombocytes (10^3/mm^3)	719.6 ± 70.9	780.4 ± 89.7	799.9 ± 91.8
Hemoglobin (g/dl)	16.40 ±.0.87	12.16 ± 3.63*	13.32 ± 3.62*
Hematocrit (%)	49.2 ± 2.1	37.0 ± 11.7*	40.7 ± 11.7*

*the value significantly different from control group ($P < 0.05$)

Table 3. Effect of Sangrovit and DSS on oxidative stress parameters in plasma, erythrocytes and colon

Parameter	Group 1	Group 2	Group 3
GST[a,e] (nkat/g)	2.8 ± 1.2	2.6 ± 1.2	2.9 ± 1.1
GSH[a,e] (mmol/g)	160.7 ± 54.3	218.0 ± 85.3	305.7 ± 206.2*
TBARS[a,e] (nmol/g)	725.5 ± 161.3	569.9 ± 482.3	749.9 ± 655.5
TBARS[b,d] (nmol/g)	33.1 ± 10.0	31.0 ± 20.6	37.7 ± 23.1
SH-groups[b,d] (μmol/l)	7.9 ± 0.5	15.6 ± 34.5	8.6 ± 1.9
MPO[c,d] (nkat/g)	0.33 ± 0.07	0.77 ± 0.40*#	0.86 ± 0.31*
COX-2[c,d] (%)	100.0	110.7	169.9*
TBARS[c,d] (nmol/g)	210.8 ± 102.1	349.2 ± 478.6	559.9 ± 822.9
GSH[c,d] (mmol/g)	292.9 ± 134.2	325.6 ± 27.3	430.0 ± 110.8
GST[c,d] (nkat/g)	2.25 ± 0.92	3.05 ± 2.47	3.01 ± 1.53

[a]erythrocytes, [b]plasma, [c]colon, [d]the value was expressed per 1 g of protein, [e]the value was expressed per 1 g of hemo-
globin
*the value significantly different from control group ($P < 0.05$)
#the value significantly different from DSS-treated group ($P < 0.05$)
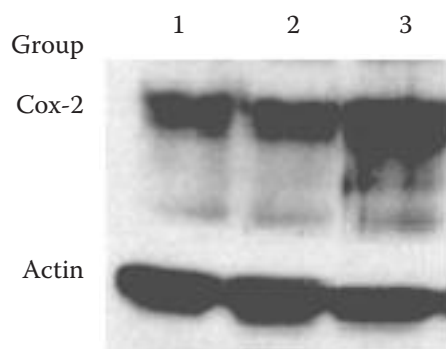
Figure 4. Effect of Sangrovit on colon COX-2 protein levels. Colon samples were homogenized in phosphate buffer and pooled, i.e., 5 or 20 or 10 samples in Groups 1, 2, 3, respectively and analyzed by immunoblotting

Colon tissue alkaloid content

The alkaloid content was determined only in the colon tissue of animals fed Sangrovit (Group 2) as 6.81 ng SG, 0.98 ng CHE, 0.60 ng DHSG and 0.36 ng DHCHE in 1 g of tissue.

DISCUSSION

Phytogenic feed additives are commonly defined as plant derived compounds incorporated into feed to improve the productivity of livestock (Windisch et al., 2008). A number of plant appetizers used in livestock are also used in traditional medicine. One of these, *Macleaya cordata*, is used in Sangrovit. The effects of Sangrovit are primarily ascribed to the isoquinoline alkaloids in *M. cordata*. The recommended dose for farm animals is 20–200 ppm Sangrovit in feed (Anonymous, 2010). The accepted experimental model of nonspecific IBD is rodent DSS-induced colitis. Treatment with DSS caused no injury to the small intestine but damaged the whole length of the colon, mostly in the rectums of rats in Groups 2 and 3. In the latter, tissue injury to the colon and rectum was characterized by multifocal areas of mucosal erosion, colonic epithelial cell injury and significant mucosal infiltration of neutrophils. Colon damage was also confirmed by the presence of blood in the stool of animals in both groups. This finding corresponded with the decreased hematocrit and hemoglobin levels that were observed. The animals in groups treated with DSS showed increases in liver and cecum weight, colon shortening and changes in leukocyte count. The histological colitis score was higher in DSS-treated rats (Group 3) than in Sangrovit-treated rats (Group 2, Figure 3). Thus, oral administration of Sangrovit at a dose of 500 ppm in feed (9 mg/kg bw per day) attenuated DSS-induced colitis, as shown by microscopic histological examination (Figure 2). One of the main pathological features of DSS-induced colitis is infiltration of polymorphonuclear neutrophils and mononuclear cells into colon tissues (Grisham et al., 1988). The activities of colon mucosal myeloperoxidase (MPO) and cyclooxygenase-2 (COX-2), which have been shown to correlate well with the severity of lesions in acute DSS-induced colitis, are established markers of neutrophil infiltration (Vicario et al., 2005). In our experiment, rats with colitis treated with Sangrovit (Group 2) showed significantly lower MPO activity than rats in Group 3 (Table 3). The positive effect of Sangrovit on DSS-induced colitis manifested as an inhibition of COX-2 expression (Table 3, Figure 4). One possible explanation of Sangrovit's protective effects may be linked to the anti-inflammatory activity of sanguinarine and its congeners. In the Sangrovit group we found these alkaloids retained in colon tissue. Their protective effect on colon/rectum tissue is therefore believed to be caused by direct contact with colon mucosa as the bulk of these alkaloids are excreted in the feces. Only a very small amount is absorbed and/or transformed to dihydroderivatives by nonspecific reductases (Psotova et al., 2006a). We can conclude that Sangrovit ameliorates experimental colitis measured as reduced gross mucosal injury in the rectum and decreased MPO activity and COX-2 protein levels. In summary, the experiment presented in this report showed that DSS-induced colitis in rats was modulated by the addition of 500 ppm Sangrovit to feed.

Acknowledgement

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