INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are chronic inflammatory conditions of the gastrointestinal system and are generally called inflammatory bowel diseases (IBDs). Although the etiology of the disease remains unknown, there is increasing evidence to link IBD to genetic and environmental factors as well as to the dysregulation of the mucosal immune system (1-8).

Intestinal epithelial cells have been shown to express multiple drug resistance (MDR) genes belonging to the ABC family of transporters. One of them, the ATP-dependent drug efflux protein P-glycoprotein (P-gp), encoded by the MDR gene MDR1, is known to be responsible for the active excretion of a variety of lipophilic cationic drugs as well as other harmful molecules from the intestines (1, 2, 7). Panwala et al. reported that mdr1a-/- knockout mice are susceptible to developing a severe, spontaneous intestinal inflammation that has a pathology similar to that of human IBD (1). If this is the case, either defective or stimulated functions of the efflux pump may alter the course of the disease models. In fact, Iizasa et al. demonstrated that following induction of colitis with dextran sodium sulfate (DSS) in mice, the expression of mdr1a and the pregnane X receptor (PXR) was reduced in the large intestine, and P-gp function was decreased; this was accompanied by severe inflammation (7). There are also human studies suggesting that people with genomic variants of MDR genes that are associated with lower intestinal P-gp expression are more susceptible to develop IBDs (4).

The PXR is a member of the nuclear receptor family of ligand-activated transcription factors, and their activation regulates the expression of the biotransformation (cytochrome P 450 (CYP)) enzymes and transport proteins involved in the metabolism and elimination of harmful chemicals and organisms from the body (3). The receptor is also reported to be a regulator of inflammation in the liver and intestines, which is critical for the maintenance of intestinal integrity (6, 8-11). Based on gene expression profiling in patients with CD and UC, Langmann et al. reported that dysregulation of xenobiotic metabolism and PXR activity in the gut are likely to contribute to the pathophysiology of UC (3).
by Shah et al. and Mencarelli et al. in mouse models of DSS-induced colitis and human cell lines, respectively (5, 6).

Nuclear factor kappa B (NF-kB), a key transcription factor of lymphocytes and macrophages, has important regulatory functions in the immune system and inflammatory processes (5, 12). Li et al. reported that it plays a pivotal role in the pathogenesis of UC, which might account for the up-regulation of the expression of tumour necrosis factor alpha (TNF-α) and intercellular adhesion molecule 1 (ICAM-1) (12). On the other hand, Zhou et al. observed that NF-kB target genes are up-regulated and small bowel inflammation is significantly increased in mice lacking the steroid X receptor (SXR) ortholog of PXR, thereby demonstrating a direct link between SXR and drug-mediated antagonism of NF-kB (13). They also demonstrated a reciprocal inhibition between PXR and NF-kB signalling pathways. This inhibition was shown to be PXR dependent and potentiated by PXR ligands, such as rifampicin (13). More recently, rifaximin, an analogue of rifampicin, was demonstrated to be beneficial in the treatment of IBD in humans as well as in experimental animals through human PXR-mediated inhibition of the NF-kB signalling cascade (14, 15).

St. John's wort (Hypericum perforatum) (SJW) is an herbal medicine that is frequently used for therapy of mild depression. It has been reported to have antioxidant, anti-inflammatory, and anti-bacterial properties, and it is known to be used for wound healing in Turkish folk medicine (16, 17). Recently, Dost et al. demonstrated that SJW can heal 2,4,6-trinitrobenzenesulfonic acid (TNBS) colitis of rats, and they attributed this effect to its antioxidant and anti-inflammatory properties (18).

It is well known that SJW has a strong enzyme-inducing property. It induces the nuclear receptor PXR and, consequently, CYP enzymes as well as P-gp and other multidrug resistance proteins (19-23). Gutmann et al. reported that MDR1 and CYP3A4 mRNA expression was induced by hyperforin and hypericin, chief active constituents of SJW (24).

Since the deficiency of the PXR gene (PXR/NR112) has been associated with an increased risk for IBD (3, 11) and since a reciprocal inhibition is known to exist between PXR- and NF-kB signalling pathways (13), it was proposed that PXR activation may inhibit the activity of NF-kB and the expression of its target genes, thus suppressing inflammation (5). Spironolactone (SPL) is a ligand for rodent PXR (25). In a previous study, we used SPL as a probe drug to investigate whether drugs that activate PXR can, in general, have therapeutic effects in the treatment of IBD (26); in fact, SPL ameliorated TNBS-induced colitis of the rats. However, we were unable to show the PXR-inducing effect of SPL due to some methodologic problems. This study was proposed to investigate if, like rifaximin, the PXR- and the P-gp-inducing effects of SJW and SPL contribute to their wound-healing properties in TNBS colitis of rats.

**MATERIALS AND METHODS**

**Animals**

Male Sprague-Dawley rats 3 months old (250 – 300 g) were housed in a room at a constant temperature of 22 ± 2°C with 12:12-h light/dark cycles and fed standard pellet chow and water ad libitum. The study was approved by the Marmara University School of Medicine, Animal Care and Use Committee (No: 17.06.2011- 34.2011.MAR).

**Experimental design**

Rats were assigned randomly to two different groups: the control group and the colitis group. Each group was then divided into three treatment subgroups with six rats in each.

- **Control (vehicle) group**: rectal alcohol (0.50 mL of 50% (v/v) ethanol) + p.o. 0.4% methylcellulose (MC)
- **TNBS group (colitis) group**: rectal TNBS + p.o. MC
- **SJW group**: rectal saline + p.o. SJW 300 mg/kg bid for 7 days
- **TNBS + SJW group (treatment group)**: rectal TNBS + p.o. SJW 300 mg/kg bid for 7 days
- **SPL**: rectal saline + p.o. SPL 80 mg/kg for 7 days
- **TNBS + SPL (treatment group)**: rectal TNBS + p.o. SPL 80 mg/kg for 7 days

**Induction of colitis**

Rats were fasted for 18 h before the experiments, with free access to water. Colitis was induced as previously described by Morris et al. (27). Rats were lightly anaesthetised by ether, and 30 mg of TNBS in a volume of 0.25 mL dissolved in 0.50 mL of 50% (v/v) ethanol was administered into the colon. For this purpose, a medical-grade polyurethane cannula with an external diameter of 2.0 mm was inserted intrarectally into the rat so that the tip was 8 cm proximal to the anus. After instilling TNBS, the cannula was left in place for a few seconds then gently removed. Rats in the control group received only 0.25 mL of saline intracolonic. The SJW (300 mg/kg bid) and SPL (80 mg/kg) were suspended in 0.4% MC and, beginning 24 hours after TNBS administration, were administered to rats in the treatment groups as oral gavage for 7 days; matched control groups received only 0.4% MC. The rats were checked daily for behaviour and stool consistency. At the end of the treatment period (on the eighth day) rats were sacrificed by cervical dislocation, and the trunk blood was collected. The abdomen was opened, and, after macroscopic evaluation of the intestines and the colon, tissue specimens were taken and frozen at –80°C for measurements of biochemical parameters or stored in formaldehyde for histological examination. Samples for histomorphological and histochemical analyses were immediately sent to the Department of Histology and Embryology.

**Assessment of colitis**

Macroscopic evaluation of the colon, ileum, and jejunum were performed as described by Wallace et al. (28). For each animal at postmortem laparotomy, 6 cm of the colon, extending proximally from 2 cm above the anal orifice, were removed along with ileum and jejunum tissues. Tissues first were split distally from 2 cm above the anal orifice, were removed along with ileum and jejunum tissues. Tissues first were split longitudinally, slightly cleaned in physiological saline to remove faecal residues, weighed, and pinned out onto a card. An independent observer (a pathologist) who was blinded to the treatment, scored the macroscopic appearance of the intestinal mucosa.

Mucosal injury also was evaluated macroscopically according to methods described in Wallace et al. (28) using the following scale:

1. Focal hiperemia, no ulceration
2. No ulceration or hyperemia, thickening of the intestinal wall
3. Ulceration and inflammation at a single site
4. Ulceration and inflammation at two or more sites
5. Major damage > 1 cm
6-10 Major damage > 2 cm

**Biochemical analyses of tumor necrosis factor-α and nuclear factor-kB in plasma**

Plasma TNF-α and NF-kB were quantified according to the manufacturer's instructions and guidelines using enzyme-linked
immunosorbent assay (ELISA) kits specific for the mentioned rat cytokines. These particular assay kits were selected for their high degree of sensitivity, specificity, inter- and intra-assay precision, and small amount of plasma sample required to conduct the assay.

**Myeloperoxidase (MPO) assay**

The MPO activity was measured in tissue samples using a procedure similar to that documented by Hillegas et al. (29). Tissue samples (0.2 – 0.3 g) were homogenised in 50 mM potassium phosphate buffer (PB) (50 mmol/L K2HPO4, pH 6.0) and centrifuged at 41,400 g for 10 min at 4°C, and the supernatant was discarded. The pellets were rehomogenised with an equivalent volume of 50 mM/L PB containing 0.5% (w/v) hexadecyl trimethyl ammonium bromide (HETAB). Following three freeze/thaw cycles with sonication between each cycle, the samples were centrifuged at 41,400 g for 10 min. Aliquots (0.3 mL) were added to 2.3 mL of reaction mixture containing 50 mM PB, o-dianisidine 2HCl, and 20 mM H2O2 solution. The MPO activity was assessed by measuring the H2O2-dependent oxidation of o-dianisidine 2HCl. One unit of enzyme activity was defined as the amount of the MPO present per gram of tissue weight that caused a change in absorbance of 1.0/min at 460 nm and 37°C.

**Chemiluminescence (CL) assay**

Chemiluminescence assays were carried out with lucigenin (bis-N-methylacridiniumnitrate) and luminol (5-amino-2,3-dihydro-1,4-phthalazinedione). Measurements were taken at room temperature using a Mini Lumat LB 9509 luminometer (EG&G Berthold, Germany). Tissue samples were collected into two vials, and CL counts were obtained after the addition of lucigenin or luminol, 0.2 mM each. All counts were obtained at 15-s intervals for 5 min and corrected for milligram amount of the tissue sample. Data were expressed as the area under the curve of relative light units (30).

Superoxide radicals were determined using lucigenin, while hydroxyl and hydroperoxide radicals were determined using luminol.

**Histopathological evaluation**

For the light microscope investigations, jejunum, ileum, and colon samples were fixed in 10% formaldehyde, dehydrated in an alcohol series, cleared in toluene, and embedded in paraffin. Paraffin sections (5 µm) were stained with haematoxylin and eosin and examined under a photomicroscope (Olympus BH 2, Tokyo, Japan). All tissue sections were examined microscopically for the characterisation of histopathological changes by an experienced histologist who was unaware of the treatment conditions.

**Immunohistochemistry**

Immunohistochemical staining was performed using the streptavidin-biotin-peroxidase method. Briefly, sections of 4 µm were cut from the paraffin-embedded tissue and mounted on positively charged slides. The sections were dried overnight at 37°C, deparaffinised in xylene, and hydrated in graded ethanol solutions. Endogenous peroxidase activity was blocked by incubating the slides in 3% hydrogen peroxide diluted in methanol followed by washing under running tap water. To unmask the antigens, the slides were microwave-treated in 10 mM citrate buffer (pH 6.0) for 20 min. The tissue sections were rinsed in phosphate-buffered saline (PBS, pH 7.4), and, to minimise the background staining, the sections were incubated with serum-blocking solution for 10 min. Sections were then incubated with antibodies to P-gp at a dilution of 1:40 and PXR at a dilution of 1:200 for 60 min at room temperature. After washing with PBS, the bound primary antibodies were detected by incubation with the biotinylated secondary antibodies followed by peroxidase-conjugated streptavidine according to the manufacturer. Staining was visualised with 3,3’-diaminobenzidine tetrahydrochloride. The slides were counterstained with Mayer haematoxylin, dehydrated in ethanol, and coverslipped.

**Chemicals and reagents**

Chemicals and reagents used for the first part of the study included TNBS (Fluka Chemie, Germany), SJW (standardised extracts by Solgar Vitamin and Herbs, Istanbul, Turkey), SPL (Aldactone tablets, Ali Raif Pharmaceuticals, Istanbul, Turkey), ELISA kits specific for rat cytokines NF-κB and TNF-α (Biosource International, Nivelles, Belgium), HETAB (Sigma-Aldrich, St. Louis, USA), o-dianisidine 2HCl (Sigma-Aldrich), lucigenin (bis-N-methylacridiniumnitrate), and luminol (5-amino-2,3-dihydro-1,4-phthalazinedione (Sigma-Aldrich). Chemicals and reagents used for immunohistochemistry were serum-blocking solution (Histostain Bulk Kit, Invitrogen LABSA Detection System, Paisley, UK) and antibodies to P-gp (JSB-1, Abcam, UK) and PXR (bs-2334R, Bioss, Salem MA, USA). All other chemicals used in this study were analytical grade.

**Statistical evaluation**

All values in the figures and text are expressed as the arithmetic mean ± standard error of the mean (mean ± S.E.M.). The data were evaluated using Graph Pad Prism Version 4.0 software (GraphPad Software, San Diego, USA). The statistical significance of any difference in each parameter among the groups was evaluated by one-way analysis of variance (ANOVA) followed by Tukey’s post-hoc tests. Histology or immunohistochemistry data were expressed as the median (range). Values of P <0.05 were considered statistically significant.

**Table 1.** Changes in the body weight, and the volume (weight /length) of the colonic tissue of the rats. (spironolactone (SPL), 80 mg/kg po, St John’s wort (SJW): 300mg/ kg bid, po). Data are expressed as mean ±S.E.M.

<table>
<thead>
<tr>
<th>Group n=6</th>
<th>Control</th>
<th>Colitis</th>
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<tbody>
<tr>
<td></td>
<td>Saline</td>
<td>SPL</td>
</tr>
<tr>
<td>Delta body weight (g), 7 days</td>
<td>15.8±2.6</td>
<td>17.7±2.1</td>
</tr>
<tr>
<td>Colon weight:length ratio (g/cm), 7 days</td>
<td>0.85±0.07</td>
<td>0.94±0.07</td>
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*** P <0.001, significantly different from the control; **P <0.01; **P <0.001 significantly different from the colitis group.
RESULTS

After intracolonic administration of TNBS, rats became anorexic, and colitis caused severe diarrhea in the majority of animals. Seven days after treatment with TNBS, there was a marked loss in body weight (P <0.001) (Table 1). There were inflammatory changes in the intestinal tract that were associated with a significant increase of weight/length of the rat colon (P <0.001) (Fig. 1). Macroscopic inspection of the colon showed a flaccid appearance, loss of normal morphology, and evidence of bowel wall thickening, inflammation, and ulcers. This was most significant in the colon. Lesions in the colon, ileum, and jejunum tissues were quantified using a macroscopic damage score described by Wallace et al. (28). Treatment with SJW or SPL for 7 days after induction of colitis prevented significant (P <0.01) loss in body weight and lessened the increase in tissue weight; however, values were still significantly higher compared to the control tissues (P < 0.01). Treatment with SPL or SJW also attenuated the extent and severity of the colonic injury, reducing the macroscopic damage score; however, the damage was not completely healed during this treatment period and was still significantly higher than the controls (P <0.001) (Table 2).

Effects of St. John's wort and spironolactone on plasma cytokine levels

Nuclear factor kappa B has been reported to play a pivotal role in the pathogenesis of UC, which might account for the up-regulation of the expression of TNF-α. In this study, plasma TNF-α and NF-κB levels increased significantly (P <0.01 and P <0.001, respectively) in the colitis group compared to the control group. Treatment with SJW or SPL for 7 days significantly decreased the plasma TNF-α and NF-κB levels (Fig. 1).

Table 2. Histomorphological evaluation of the colon tissue.

<table>
<thead>
<tr>
<th>Colitis (7 days n=6)</th>
<th>Macroscopic Scoring of the Tissues</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
</tr>
<tr>
<td>Colon</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Ileum</td>
<td>0 ± 0</td>
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<tr>
<td>Jejunum</td>
<td>0 ± 0</td>
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Spironolactone (SPL), 80 mg/kg po, St John’s wort (SJW): 300mg/kg bid, p.o. Normal and mild to medium damage were evaluated together for chi-square test. *** P <0.001, significantly different from the control; * P <0.05, ** P <0.01, *** P <0.001 significantly different from the colitis group.

Fig. 1. Plasma nuclear factor kappa B (NF-κB) and tumor necrosis factor alpha (TNF-α) levels. Induction of colitis increased the plasma NF-κB and TNF-α levels significantly but treatment with spironolactone (SPL) or St John’s wort (SJW) for 7 days decreased the levels significantly, demonstrating the decrease in tissue inflammation. *** P <0.001 vs. control group; * P <0.05, ** P <0.01 vs. colitis group.
respectively) after induction of colitis, demonstrating the presence of severe inflammation. Both SJW and SPL treatments suppressed cytokine levels significantly ($P <0.01$), showing evidence that tissue inflammation was reduced (Fig. 1).

Effects of St. John's wort and spironolactone on tissue myeloperoxidase levels and on oxidative damage

Myeloperoxidase is an indicator of infiltration of the tissue with neutrophils and also is a characteristic of the TNBS-induced colitis. After induction of colitis, there was a marked increase in MPO activity in all tissue segments ($P <0.001$) (Fig. 2a-2c). Treatment with SJW or SPL significantly decreased the polymorphonuclear neutrophil infiltration in all intestinal segments ($P <0.05$).

Luminol and lucigenin tests were performed to demonstrate the accumulation of free radicals in the intestinal tissues. Superoxide radicals were determined with lucigenin; hydroxyl and hydroperoxide radicals were determined using luminol. Results of these tests indicate that colitis causes severe oxidative damage in all tissues, increasing free radicals significantly ($P <0.001$). Treatment of colitis with SJW or SPL significantly decreased the amounts of free radicals in all tissues ($P <0.05$) (Figs. 3a - 3c and 4a - 4c).

All of these results demonstrate that TNBS-induced colitis causes significant oxidative damage in the intestinal tissues, and this effect can be reversed by treatment with SJW or SPL.

Changes observed in tissue histomorphology in colitis and after treatment with St. John's wort or spironolactone

Paraffin blocks stained with haematoxylin and eosine were evaluated morphologically. In the colon, jejunum, and ileum tissues, the control (Fig. 5a, 5e, and 5i, respectively), SJW, and

![Fig. 2. Neutrophil infiltration which is represented by myeloperoxidase activity (MPO) in the colon (a), jejunum (b) and ileum (c) tissues caused by TNBS colitis and the effect of treatment with saline (SP), spironolactone (SPL) or St John's wort (SJW) for 7 days. MPO levels were increased significantly in colitis, but this was prevented significantly by both drugs; spironolactone appeared to be less efficient. *** $P <0.001$ vs. control group; *$P <0.05$, **$P <0.01$ vs. colitis group.](image-url)
SPL (images are not provided since they were similar to the control group) groups demonstrated regular epithelial and glandular morphology with abundance of goblet cells.

In the colon tissue, induction of colitis (Fig. 5b) degenerated the epithelial and glandular morphology with a high degree of accumulation of leukocytes. The SJW treatment (Fig. 5c) appeared to have reduced the degeneration more than the SPL treatment (Fig. 5d).

In the jejunum, induction of colitis caused severe desquamation in the epithelium, and this was accompanied by hypertrophy of the goblet cells (Fig. 5f).

In the ileum, there was degeneration with severe desquamation in the epithelium, and accumulation of leukocytes in the lamina propria was observed (Fig. 5j). In both ileum and jejunum tissues, SJW and SPL treatments led to a moderate regeneration in the epithelium, and results were quite similar for both drugs (Fig. 5g, 5h, 5k, and 5l).

**Pregnane X receptor staining**

For the colon tissue, the control group (Fig. 6a) showed prominent nuclear staining, whereas, in the colitis group (Fig. 6b), there was no staining. In both SJW and SPL treatment groups (Fig. 6c and 6d), moderate staining was observed (arrows), confirming that PXR expression was induced by each drug during treatment of colitis.

In the jejunum and ileum tissues, control groups (Fig. 6e and 6i, respectively) showed moderate staining, whereas there was minimal staining in the colitis groups (Fig. 6f and 6j, respectively). In both SJW and SPL treatment groups (Figs. 6g,
6h, 6k and 6l, respectively, for jejunum and ileum), moderate staining was observed, indicating activation of PXR expression (Fig. 6g and 6h).

P-glycoprotein staining

In the colon tissue, the control group (Fig. 7a) showed prominent epithelial membrane staining, demonstrating the presence of P-gp. However, in the colitis group (Fig. 7b), in accordance with the high damage score observed in this tissue, there was minimal staining, showing a severe decrease in P-gp expression. In both SJW and SPL treatment groups (Fig. 7c and 7d), moderate staining was observed (arrows), indicating the induction of P-gp expression during treatment of colitis with these drugs.

In the jejunum and ileum tissues, control groups (Fig. 7e and 7f) showed mild to moderate membrane staining, but there was almost no staining in the colitis groups (Fig. 7f and 7j). In both SJW and SPL (Fig. 7g, 7h, 7k and 7l, respectively) treatment groups, moderate staining of membranes was observed, demonstrating an increase in the expression of P-gp.

It is well known that P-gp expression increases from the jejunum to the colon, and our observation is in agreement with this; after treatment with SJW or SPL, induction of P-gp expression was more prominent in the colon tissue compared to the jejunum or ileum tissues.

In TNBS colitis, after treatment with SJW or SPL, P-gp expression increased, apparently due to induction by these drugs.

**DISCUSSION**

In this study, intestinal tissues obtained from rats with TNBS colitis exhibited loss of normal morphology and showed

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*Fig. 4. Oxidative damage, represented by lucigenin levels in the colon (a), jejunum (b) and ileum (c) tissues caused by TNBS colitis and the effect of treatment with saline (SP), spironolactone (SPL) or St John's wort (SJW) for 7 days. Luminol levels were increased significantly in colitis, but were decreased significantly after treatment for 7 days by either drug.

* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ vs. control group; + $P \leq 0.05$, +++ $P \leq 0.001$ vs. colitis group.
evidence of bowel wall thickening, inflammation, and ulcers. Treatment with SJW or SPL for 7 days prevented loss in body weight and attenuated the extent and severity of the colonic injury, reducing the macroscopic damage score and inflammatory changes in the intestinal tract. The treatments also decreased oxidative damage and tissue MPO activity. It has been reported previously that NF-\(\kappa\)B plays a pivotal role in the pathogenesis of UC (5, 12). Strong correlations between the nuclear level of NF-\(\kappa\)B and the tissue-positive expression of TNF-\(\alpha\) and ICAM-1, MPO activity, as well as the macroscopic and histological indices of TNBS-induced colitis also have been reported (5-12). Findings of the present study are in accordance with these previous observations. After drug treatment, parallel with the decrease in NF-\(\kappa\)B and TNF-\(\alpha\) levels, other indices of inflammation also decreased.

St. John's wort has been demonstrated to have wound-healing properties in various experimental models, including peptic ulcer. Suntar et al. reported the wound-healing properties of ethanol extracts and ointments of \textit{H. perforatum} and claimed that the healing properties were due to their anti-inflammatory effects (16, 17). It has been recorded that the olive oil macerate of the flowering herb is used as a home remedy for the treatment of cuts and burns. Hyperforin was postulated as the active ingredient of the macerate (16, 17). St. John's wort is also known to have strong enzyme-inducing properties; it induces PXR as well as the expression of CYP enzymes and drug transport proteins of the ABC cassette family, including P-gps (20-24).

In this study, there may be several mechanisms contributing to the beneficial effects of SJW in the treatment of TNBS colitis of rats. It appeared to have significant antioxidant and anti-inflammatory effects, which is in accordance with the findings of Dost et al. (18). However, the main purpose of the present study was to investigate whether the PXR- and/or P-gp-inducing properties had any contribution to SJW's healing effects on intestinal damage. Our results confirmed that there is a positive correlation between the healing effects of SJW and an increase in the expression of intestinal PXR as well as P-gps in rats.

Spironolactone, a diuretic, also has strong enzyme-inducing activity. It is believed that induction of various CYP enzymes and transport proteins by SPL is regulated via activation of PXR, farnesoid X receptor, and the constitutive androstane receptor in humans and rodents (25, 31). It recently has been demonstrated that SPL induces the intestinal protein Mrp2 transcriptionally, with PXR being a potential mediator. These findings suggest that SPL could be of potential therapeutic application, particularly in situations of down-regulation of intestinal Mrp2 (32). Our previous study confirmed this observation, demonstrating that SPL- or similar PXR-activating agents can be of therapeutic

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**Fig. 5.** Histomorphologic observations of the control, TNBS colitis, St John's wort (SJW) treated colitis and spironolactone (SPL) treated colitis groups of the intestinal tissues (HE staining).

In the colon tissue - control group (a): intact colonic epithelium (arrows) and glands (*); colitis group (b): severe epithelial desquamation (arrows) and high density of leukocytes (arrowheads); St John's wort (SJW) treatment (c) and spironolactone (SPL) treatment (d) groups had reduced.

In the jejunum group - control group (e): regular epithelium (arrows); colitis group (f): severe desquamation (arrow) in epithelium besides hypertrophic goblet cells (*); colitis and St John's wort (SJW) treatment group (g): with mild regeneration and spironolactone (SPL) treatment group (h): with moderate regeneration of epithelium (arrows) and hypertrophy of goblet cells.

In the ileum tissue - control group (i): regular epithelium (arrows); colitis group (j): severe desquamation (arrow) in epithelium besides the high density of leukocytes in the lamina propria (*) and abundant distribution of goblet cells; colitis and St John's wort (SJW) treatment group (k) and spironolactone (SPL) treatment (l) group: regeneration of epithelium (arrows) and hypertrophy of goblet cells (arrowhead) degeneration with regenerated epithelium (arrows) and glands (*).
value in the treatment of IBDS that are linked to the down-regulation of PXR and/or Mrp2 (26). However, in that study, western blot analyses of PXR expression were inconclusive (26). In the present study, immunohistochemical staining studies demonstrated that there is an induction of PXR and P-gp by SPL, confirming this probability. Results of the studies for SJW were very similar to those for SPL; there was almost no staining for PXR in the inflammed tissues from rats with colitis, but they were restored to control levels after drug treatment.

Regarding the previously demonstrated reciprocal inhibition between PXR and NF-κB signalling pathways (13), our observation that NF-κB and TNF-α levels were significantly lower in the treated animals also suggests that SJW and SPL may have been effective through the activation of PXR.

Recent studies have demonstrated that activation of the SXR by drugs, such as rifampicin, in humans inhibits the activity of NF-κB (13) and that there is a reciprocal inhibition between PXR and NF-κB signalling pathways (13). Rifaximin, an analogue of rifampicin, was demonstrated to be beneficial in the treatment of IBD in humans as well as in the PXR-humanised mouse (14, 15). Cheng et al. reported that the therapeutic role of rifaximin in IBD is through human PRX-mediated inhibition of the NF-κB signalling cascade, suggesting that human PXR may be an effective target for the treatment of IBD (15). In fact, in the present study, plasma NF-κB levels that increased in colitis decreased after treatment with either SJW or SPL, probably in response to an increase in PXR expression in the intestinal tissues.

Wahl reported that in IBD the complex interplay of sensing and signalling pathways in maintaining healthy homeostasis of the intestines is disturbed; explaining that, as well as PXR another nuclear receptor, peroxisome proliferator-activated receptor (PPARγ) also produces beneficial effects in experimental IBD by repression of NF-κB (33). According to Wahl PPARγ when activated by the antidiabetic drugs thiazolidinediones, reduced mucosal damage and inflammation in animal models of intestinal inflammation; this was reflected in inhibition of NF-κB and stress kinase pathways mediating inflammation, as well as other indices of IBD. In fact, in a recent study Celinski et al. demonstrated the anti-inflammatory and therapeutic actions of PPARγ agonists rosiglitazone and troglitazone in DSS colitis of rats (34). Both drugs significantly reduced the levels of pro-inflammatory cytokines and increased the levels of inflammation-limiting cytokines. Troglitazone had a significantly stronger enhancement of anti-inflammatory cytokine expression than rosiglitazone (34).

These reports support our observations that activation of some nuclear receptors may suppress inflammation in experimental IBD.

In this study, for both SJW and SPL, induction of P-gp expression along the intestines appeared to be more significant compared to the activation of PXR expression. Induction of P-gps was reported to be independent of PXR induction in the gut wall (35), which might explain why P-gp expression was not correlated to the expression of PXR. Accordingly, both drugs may have exhibited their beneficial effects by an additional mechanism that involves a direct induction of P-gps in the epithelial cells, thus increasing the extrusion of noxious substances, such as bacterial products from the mucosa. It is
known that the P-gp expression increases longitudinally along the intestine, increasing from the duodenum to the distal regions of the small intestine, with the highest levels existing in the colon (36). In fact, in the present study, staining in the jejunum appeared to be weaker than the other tissues; interestingly, however, after treatment with SJW and SPL, staining appeared to be almost as strong as in the colon. This finding is in accordance with the observation of Buyse et al. (36), who reported an increase in P-gp activity in the non-inflamed ileum in DSS-induced rat colitis, suggesting that this may represent an adaptive mechanism to compensate for the impaired activity of P-gp in the colon.

An overall consideration of the observations of this study suggest that the expression of either PXR or P-gp increased moderately and that treatment with either SJW or SPL did not result in complete recovery. Treatment with a higher dose or for a longer period might have resulted in stronger induction of expression of PXR and/or P-gp and, consequently, an increased efficacy for both drugs. It is known that under experimental conditions, there is a spontaneous recovery of colitis. Thus, rather than extending the treatment period, it would be advisable to use higher doses of SJW or SPL in order to achieve more prominent effects. Experimenting with several different doses of SJW in different animal groups might have made it possible to compare the efficacy of higher doses in TNBS colitis of rats.

In conclusion, based on our findings, it may be suggested that in addition to their antioxidant effects, SJW and SPL were beneficial in ameliorating TNBS colitis of rats by increasing the expression of PXR and, thus, by suppressing the inflammation. The induction of P-gps in epithelial cells may have resulted in an increase in the extrusion of noxious substances from the mucosa, which also may have contributed to this healing effect. As a final comment, we do suggest that since IBDs are generally aggravated under stress or disturbed psychologic conditions,
anti-depressant and anti-inflammatory properties of SJW may have additive effects in the treatment of IBDs in humans.

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