Original article

# Effect of sodium butyrate on gastric ulcer aggravation and hepatic injury inflicted by bile duct ligation in rats

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## ARTICLE INFO

## ABSTRACT

*Background and Aim:* Cholestasis is positively associated with an increased risk of peptic ulceration. The present study investigated the aggravating effect of cholestasis on piroxicam-induced gastric ulceration. The study also evaluated the effect of sodium butyrate (SoB) on piroxicam-induced gastric ulceration in cholestatic animals and its effect on hepatic tissues and both effects were compared to ursodeoxycholic acid (UDCA) as a standard anticholestatic drug.
*Methods:* Bile duct ligation was adopted for induction of cholestasis in rats. The cholestatic animals received saline, SoB (P.O, 400 mg/kg, twice daily) or UDCA (P.O, 30 mg/kg/day) for 4 days starting from the first day of surgery. On the 4th day, blood samples were collected for determination of serum hepatic markers, then gastric ulcers were induced by piroxicam administration (P.O, 50 mg/kg) and 4 h later, the stomach was isolated and gastric mucosa was collected for biochemical determinations. The ulcer indices for the investigated drugs were compared to omeprazole as a standard acid suppressive drug.
*Results:* Piroxicam-induced ulceration was exacerbated in cholestatic rats. Gastric mucosa showed a significant elevation of MDA and TNF-α together with a significant decrease in GSH &VEGF levels. SoB treatment significantly attenuated ulcer development. The afforded protection was higher than that provided by UDCA and was not significantly different from that afforded by omeprazole. SoB significantly decreased gastric mucosal MDA and TNF-α level, whereas UDCA failed to alter these parameters. Both drugs significantly elevated GSH, VEGF and IL10 levels. Similar to UDCA, SoB showed a significant reduction in AST, ALT, GGT, ALP and bilirubin level. Histopathological examination confirmed the attenuating effect of SoB on gastric and hepatic injury.
*Conclusions:* Sodium butyrate effectively protected gastric and hepatic tissues against cholestasis-induced damage. Gastroprotection was mediated through antioxidant, anti-inflammatory and angiogenic activities.

## 1. Introduction

Cholestasis could be defined as an impairment of bile flow due to a decrease in bile secretion by hepatocytes or due to an obstruction of intra-or extrahepatic bile ducts. Accumulation of cholephilic components particularly bile acids and bilirubin in biliary tracts leads to bile ducts devastation and hepatocytes damage (Santiago et al., 2018). Cholestasis may also result in several extra-hepatic complications such as renal dysfunction (Holt et al., 1999), cardiomyopathy (Nam et al., 2014) and even endotoxemia (Papakostas et al., 2003). In addition, the incidence of peptic ulceration has been found to be significantly higher in patients suffering from cholestasis as compared to normal populations (Mansour-Ghanaei et al., 2008). Patients suffering from obstructive jaundice are also susceptible to the development of fatal post-operative upper gastrointestinal (GI)

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bleeding (Dixon et al., 1984). Experimentally, several reports have shown that cholestatic animals are more vulnerable to the development of gastric ulceration. Exposure of bile duct ligated rats to water-immersion stress results in exacerbated gastric mucosal damage as compared to sham-operated animals (Moezi et al., 2010). In addition, ethanol (Moezi et al., 2010) and non-steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin (Moezi et al., 2010) and aspirin (Dehpour et al.,1998), are reported to induce aggravated gastric lesions in cholestatic rats.

Several factors are thought to contribute to the etiology of ulcer aggravation in cholestatic patients or animals such as increased gastric acid secretion (Sasaki et al., 1986), overproduction of reactive oxygen species (ROS) and pro-inflammatory cytokines (Kiarostami et al., 2006; Polat and Emre 2006), decreased gastric mucosal blood flow (Beck et al., 1992) and diminished prostaglandin synthesis (Beck et al., 1993).

Butyrate, a short-chain fatty acid, is present in dairy products and it is synthesized by anerobic intestinal microflora through fermentation of undigested carbohydrate and fibers in the colon. It serves as the main source of energy for intestinal epithelial cells (Leonel and Alvarez-Leite, 2012). It also inhibits the growth of intestinal pernicious bacteria (Wen et al., 2012) and regulates gut hormones secretion (Clarke et al., 2014). In addition, it plays a role in the maintenance of the integrity of the gastrointestinal barrier (Ploger et al., 2012).

Butyrate has been recognized as a promising therapeutic agent for several disorders of the digestive system. Butyrate has been found to be effective against inflammatory bowel diseases such as Crohn's disease (Sabatino et al., 2005) and ulcerative colitis (Chen et al., 2018). In a case with familial intrahepatic cholestasis, butyrate has shown an improvement in bile secretion and a significant hepatoprotection (Gonzales et al., 2012). In addition, experimental data have shown the protective effect of butyrate against liver injury induced by endotoxin (Yang et al., 2014) or in ischemia-reperfusion model (Liu et al., 2014). Moreover, treatment of mice with sodium butyrate (SoB) has provided a significant protection against ethanol-induced gastric ulcers (Liu et al., 2016).

The current study was designed to evaluate the aggravating effect of cholestasis on piroxicam-induced gastric ulceration. The study also aimed to investigate the possible gastroprotective effect of SoB against piroxicam-induced gastric ulceration and its effect on hepatic tissues in cholestatic animals. Both gastric and hepatic effects of sodium butyrate were compared to ursodeoxycholic acid (UDCA) as a standard anticholestatic drug in a trial to find out an effective therapy for patients suffering from the comorbidity of peptic ulceration and cholestasis.

## 2. Materials and Methods

### 2.1. Drugs and chemicals

The drugs used in the current study included:- SoB (Sigma-Aldrich, St Louis, USA), UDCA (Ursofalk®, Minapharm Co., Egypt), omeprazole (Risek® - Julphar Co. Egypt), thiopental sodium (Anapental® Sigma-Tec Co, Egypt) and benzathine benzylpenicillin (Pencitard ®, NCPC North best Co., Ltd). All other chemicals and reagents were purchased at the highest grade of purity available from commercial sources. Sodium butyrate solution (10% w/v), ursodeoxycholic acid suspension (2% w/v) and omeprazole solution (0.4% w/v) were freshly prepared in saline.

### 2.2. Animals

Adult male albino Sprague Dawley rats, weighing 230–270 gm, were obtained from the animal house of Faculty of Pharmacy-

Alexandria University. The animals were kept in plastic cages at ambient temperature with free access to water and standard rat chow, containing 19% protein (AL- Fajr feed Co., Egypt). The animals were left to acclimatize to laboratory conditions for a period of one week before the commencement of the experiments. All experimental procedures were approved by the Animal Care Use Committee of the Faculty of Pharmacy, Alexandria University.

### 2.3. Induction of cholestasis

To induce cholestasis, the bile duct was isolated and double ligated using a modified method of Cameron and Oakley, (1932). Before the operation, each animal received an injection of benzathine benzyl penicillin (300,000 U/kg, I.M) to guard against bacterial infection. The animals were anaesthetized with an intraperitoneal injection of thiopental (50 mg/kg body weight). A middle abdominal incision was made and the hepatic ligament was exposed, then the common bile duct was double ligated with 4–0 surgical silk sutures below the hepatic biliary ducts junction without resection between the two ligatures. The same procedure was conducted in sham-operated (SO) rats except for the ligation of the common bile duct. Blood samples were collected from retro orbital sinus on the 4th day of surgery prior to ulcer induction and were used for determination of serum hepatic markers.

### 2.4. Induction of gastric ulceration

On the third day of surgery, SO or bile duct ligated (BDL) rats were fasted for 24 h with free access to water and the animals were housed individually in cages with a wire-mesh floor to prevent coprophagy. On the fourth day, gastric ulcers were induced by oral administration of piroxicam (50 mg/kg), then four hours later the rats were sacrificed with an overdose of thiopental. The stomach of each rat was isolated, opened along the greater curvature, washed in ice-cold saline and dried between filter papers. The stomach was examined by the naked eye for the presence of hemorrhagic spots and ulceration to calculate ulcer index according to the reported scale (Abouzeit-Har et al., 1982). Gastric mucosa was then scrapped and used for biochemical determinations.

### 2.5. Experimental protocol

Twenty-four rats were divided into four experimental groups, each containing six animals: SO group receiving piroxicam (Group 1), BDL group receiving piroxicam (Group 2), SoB-treated BDL group receiving piroxicam (Group 3), and UDCA-treated BDL group receiving piroxicam (Group 4). Group 1&2 received the vehicle (saline) whereas, Group 3& 4 were treated orally with SoB (400 mg/kg twice a day) (Liu et al., 2016) or UDCA (30 mg/kg once daily) (He et al., 2015), respectively. All treatments started from the 1st day of surgery for 3 consecutive days. On the fourth day, an additional dose was given 30 min before piroxicam administration.

A separate group of BDL-piroxicam treated rats (n = 6) was given the standard acid suppressing drug; omeprazole, as a positive control, to assess the potential gastroprotective activity of the investigated drugs on ulceration index. Omeprazole (10 mg/kg once daily) was orally administered for 3 consecutive days with an additional dose given on the fourth day one hour before piroxicam administration.

A preliminary experiment was carried out to investigate the potential ulcer protective activity of the investigated drugs in piroxicam-treated SO rats. Three groups were used, each of six rats and they were treated with SOB, UDCA or omeprazole (positive control) in the same treatment regimen described above. The ulcer

indices in these groups were compared to piroxicam-treated SO rats receiving saline (Group 1).

## 2.6. Determination of gastric mucosal parameters

Gastric mucosal homogenate (5%) was prepared in saline and was used for spectrophotometric determination of malondialdehyde (MDA) (Uchiyama and Mihara, 1978), reduced glutathione (GSH) (Richardson and Murphy, 1975) and nitric oxide (NO) levels (Sun et al., 2003). A more concentrated gastric mucosal homogenate (20%) was used for the determination of gastric mucosal content of tumor necrosis factor-alpha (TNF-$\alpha$), interleukin-10 (IL-10) and vascular endothelial growth factor (VEGF) using commercial rat ELISA Kits. ELISA Kit for TNF-$\alpha$ was purchased from eBioscience, San Diego, CA, USA, whereas ELISA Kit for IL-10 and VEGF were purchased from Bosterbio, Pleasanton, CA, USA.

## 2.7. Histopathological examination

Histopathological examination of gastric and hepatic tissues was performed to inspect the pathological alterations in these tissues. Gastric and hepatic specimens were fixed in 10% formalin and embedded in paraffin. Then, 5 $\mu$m thick sections were prepared and stained with Hematoxylin and eosin (H&E).

## 2.8. Determination of serum hepatic markers

Collected serum samples were used for determination of hepatic markers; aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) using commercial kits (Biosystem S. A., Barcelona, Spain). Total and direct bilirubin (TBIL & DBIL), and total bile acids (TBAs) were determined using commercial kits purchased from (Diamond Diagnostics, Cairo, Egypt) and (Spectrum Diagnostics, Cairo, Egypt), respectively.

## 2.9. Statistics

Data, expressed as mean ± S.E.M., were analyzed using one-way analysis of variance (ANOVA) test and post hoc multiple comparisons were done with Student-Newman-Keuls test. P-values < 0.05 were considered statistically significant.

## 3. Results

### 3.1. Effect of bile duct ligation on serum hepatic markers

Starting from the second day of BDL surgery, animals started to show signs of cholestasis such as jaundice and dark urine. Blood samples collected three days after bile duct ligation showed a significant rise in serum hepatic enzymes; AST& ALT as compared to SO animals. The increase mounted up to 8.42- and 18.82- fold, respectively. An increase in serum level of the hepatic biliary enzyme; ALP was also noted. This increase mounted up to 9.74-fold as compared to SO animals. In addition, the biliary tract enzyme; GGT was detected in serum following BDL and its level reached up to 31.00 U/L. Furthermore, serum indices of TBAs, TBIL and DBIL were markedly elevated following BDL. The elevation reached up to 17.00-, 10.40- and 61.56-fold, respectively (Table 1). None of the cholestatic rats showed signs of ascites at the time of the experiment. The manifestation of cholestasis were completely absent in SO animals.

### 3.2. Effect of bile duct ligation on piroxicam-induced gastric ulceration

As shown in Fig. 1, piroxicam-induced gastric mucosal damage was significantly higher in cholestatic rats. The ulcer index in cholestatic animals was almost duplicated compared to SO rats. Cholestasis-induced aggravation of peptic ulceration was accompanied with a significant elevation of gastric mucosal content of the lipid peroxidation marker; MDA, whereas the mucosal glutathione level was significantly decreased (Table 2). A marked increase of mucosal level of TNF-$\alpha$ was also observed in piroxicam-treated BDL rats whereas gastric mucosal level of IL-10 was not changed. In addition, a significant decrease in gastric mucosal level of VEGF was noted in piroxicam-treated BDL animals (Table 2).

### 3.3. Effect of sodium butyrate and ursodeoxycholic acid on serum hepatic markers in cholestatic animals

Both SoB and UDCA treatment significantly lowered the elevated serum hepatic markers; AST, ALT, ALP and bilirubin in a similar manner. Serum GGT level was also significantly reduced by either treatment, however SoB-induced reduction was significantly lower than that exerted by UDCA. The level of TBAs was not significantly altered by either treatment (Table 1).

### 3.4. Effect of treatment with sodium butyrate, ursodeoxycholic acid or omeprazole on piroxicam-induced gastric ulceration in sham-operated and cholestatic animals

As revealed from the preliminary experiment, treatment with either SoB or omeprazole significantly attenuated piroxicam-induced gastric ulceration in SO animals, whereas UDCA failed to offer any degree of protection. The ulcer indices in SoB and omeprazole were not significantly different (Fig. 1).

**Table 1**
Effect of sodium butyrate and ursodeoxycholic acid on serum hepatic markers.

| Groups/Parameters | SO | BDL | BDL + SoB | BDL + UDCA |
|---|---|---|---|---|
| AST (U/L) | 137.00 ± 13.31 | 1153.83 ± 16.03[*] | 1007.83 ± 42.50[#] | 944.50 ± 80.13[#] |
| ALT (U/L) | 43.67 ± 2.51 | 821.67 ± 53.16[*] | 659.83 ± 81.98[#] | 625.00 ± 36.51[#] |
| ALP (U/L) | 58.67 ± 8.51 | 571.33 ± 48.15[*] | 425.50 ± 20.99[#] | 442.83 ± 20.79[#] |
| GGT (U/L) | 0.0 | 31.00 ± 2.10[*] | 21.50 ± 1.18[#] [+] | 14.33 ± 1.76[#] |
| TBAs ($\mu$mol/L) | 20.72 ± 3.30 | 352.17 ± 9.27[*] | 288.5 ± 47.81 | 267.00 ± 22.23 |
| TBIL (mg/dl) | 1.23 ± 0.15 | 12.80 ± 0.58[*] | 10.18 ± 0.59[#] | 9.07 ± 0.48[#] |
| DBIL (mg/dl) | 0.18 ± 0.02 | 11.08 ± 0.23[*] | 8.53 ± 0.41[#] | 8.20 ± 0.65[#] |

Values are mean ± S.E.M., (n = 6).
SO; sham-operated, BDL; bile duct ligated, SoB; sodium butyrate, UDCA; ursodeoxycholic acid, AST; aspartate aminotransferase, ALT; alanine aminotransferase, ALP; alkaline phosphatase, GGT; gamma-glutamyl transferase, TBAs; total bile acids, TBIL & DBIL; Total bilirubin & direct bilirubin.
[*] Significantly different from SO group at $P < 0.05$.
[#] Significantly different from BDL group at P < 0.05.
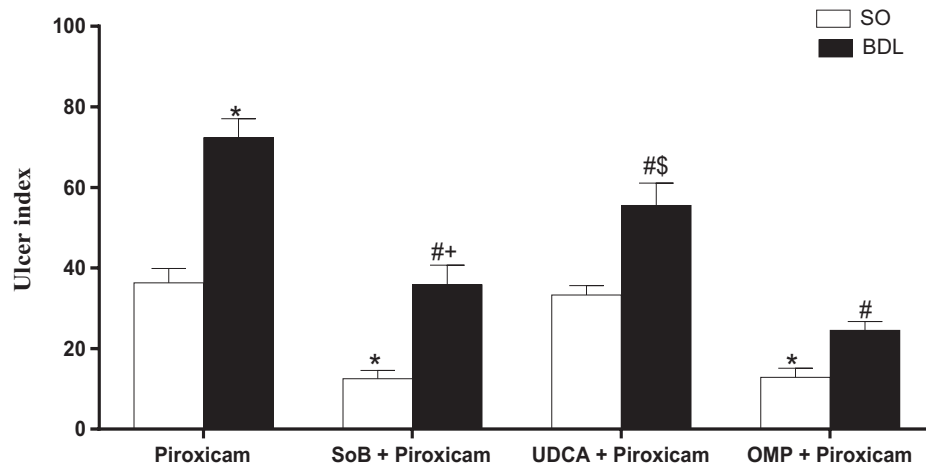[+] Significantly different from UDCA-treated group at P < 0.05. ANOVA followed by post hoc Newman-Keuls test.

**Fig. 1.** Effect of treatment with sodium butyrate (SoB; 400 mg/kg, twice a day), ursodeoxycholic acid (UDCA; 30 mg/kg, once daily) or omeprazole (OMP; 10 mg/kg, once daily) on piroxicam-induced gastric ulceration in sham-operated and cholestatic animals. Values are mean ± S.E.M., (n = 6). * significantly different from sham-operated (SO) group receiving piroxicam at $P < 0.05$ # significantly different from bile duct ligated (BDL) group receiving piroxicam at P < 0.05. $ significantly different from OMP-treated BDL group receiving piroxicam at P < 0.05. + significantly different from UDCA-treated BDL group receiving piroxicam at P < 0.05. ANOVA followed by post hoc Newman-Keuls test.

**Table 2**
Gastric mucosal content of MDA, GSH, TNF-$\alpha$, IL-10, NO and VEGF in piroxicam-treated groups (50 mg/kg).

| Groups/Parameters | SO + Piroxicam | BDL + Piroxicam | SoB + (BDL + Piroxicam) | UDCA+ (BDL + Piroxicam) |
|---|---|---|---|---|
| MDA (nmole/g tissue) | 116.83 ± 6.05 | 152.83 ± 7.73 * | 108.33 ± 15.06 # | 133.50 ± 6.87 |
| GSH ($\mu$g/g tissue) | 1103.83 ± 14.71 | 944.67 ± 39.04 * | 1556.30 ± 52.12 # + | 1199.00 ± 54.07 # |
| TNF- $\alpha$ (pg/mg protein) | 49.33 ± 6.21 | 90.50 ± 9.92 * | 59.23 ± 2.64 # | 86.00 ± 8.49 |
| IL- 10 (pg/mg protein) | 61.17 ± 6.68 | 61.83 ± 7.49 | 103.17 ± 4.44 # | 110.67 ± 4.99 # |
| NO ($\mu$g/g tissue) | 562.00 ± 10.37 | 529.50 ± 9.30 | 586.67 ± 47.22 | 646.50 ± 45.55 |
| VEGF(pg/mg protein) | 364.33 ± 30.14 | 253.67 ± 21.88 * | 397.31 ± 22.99 # | 416.67 ± 21.95 # |

Values are mean ± S.E.M., (n = 6).
SO; sham-operated, BDL; bile duct ligated, SoB; sodium butyrate, UDCA; ursodeoxycholic acid, MDA; malondialdehyde, GSH; glutathione, TNF-$\alpha$; tumor necrosis factor-alpha, IL-10; interleukin-10, NO; nitric oxide, VEGF; vascular endothelial growth factor
* Significantly different from SO group receiving piroxicam at $P < 0.05$.
# Significantly different from BDL group receiving piroxicam at P < 0.05.
+ Significantly different from UDCA-treated BDL group receiving piroxicam at P < 0.05. ANOVA followed by post hoc Newman-Keuls test.

On the other hand, treatment of cholestatic animals with SoB or UDCA significantly mitigated piroxicam-induced gastric mucosal lesions. The ulcer attenuating effect of SoB was significantly higher than that afforded by UDCA. Compared to omeprazole (a standard antiulcer drug; 10 mg/kg), the ulcer index in SoB-treated group was not significantly different whereas in UDCA-treated group, it was significantly higher (Fig. 1). The afforded protection ratios of omeprazole, SoB and UDCA were 66.13%, 50.46% and 33.87%, respectively.

The gastroprotective effect of SoB was accompanied with a significant reduction of gastric mucosal level of MDA and a significant elevation of GSH level. On the other hand, UDCA treatment significantly elevated GSH content, whereas it failed to lower MDA level. The increase in GSH level exerted by SoB was significantly higher than that afforded by UDCA treatment (Table 2).

In addition, SoB significantly lowered mucosal level of TNF-$\alpha$ and markedly increased the anti-inflammatory cytokine; IL-10. On the other hand, UDCA treatment significantly elevated the gastric mucosal IL-10 content but it failed to lower TNF-$\alpha$ level. Both SoB and UDCA significantly reversed the decrease in the gastric mucosal VEGF level (Table 2).

### 3.5. Histopathological examination

Histopathologic examination of hepatic specimens of BDL group showed hepatic and biliary tract damage with extensive neutrophilic infiltration. Rats treated with either SoB or UDCA showed a significant attenuation of hepatocytes degeneration and neutrophils infiltration (Fig. 2).

Examination of gastric tissues of piroxicam-treated groups also revealed extensive disruption of all gastric mucosal layers; however, higher mucosal damage was evident in BDL rats. Gastric specimens from BDL rats treated with omeprazole or SoB showed healthy looking mucosal lining. The cellular density in SoB-treated rats was even higher than that in omeprazole-treated rats. On the other hand, BDL rats treated with UDCA showed only mild regeneration of mucosal lining (Fig. 3).

### 4. Discussion and conclusions

The present study illustrated the aggravating effect of cholestasis on piroxicam- induced gastric ulceration in rats. A similar potentiation of induced gastric ulceration in cholestatic animals by other members of non-steroidal anti-inflammatory drugs (NSAIDs) was previously reported. Aspirin and indomethacin-induced gastric mucosal damage was found to be significantly aggravated in bile duct ligated animals (Dehpour et al., 1998; Moezi et al., 2010).

The main etiological factor contributing to the pathogenesis of piroxicam-induced peptic ulceration is the inhibition of prostaglandins synthesis ((Musumba et al., 2009). Piroxicam is known to be a relatively selective COX1 inhibitor (Cryer and Duboiso 1998). Inhibition of COX1 enzyme reduces the synthesis of PGI2 and PGE2, which are potent vasodilators and control almost all
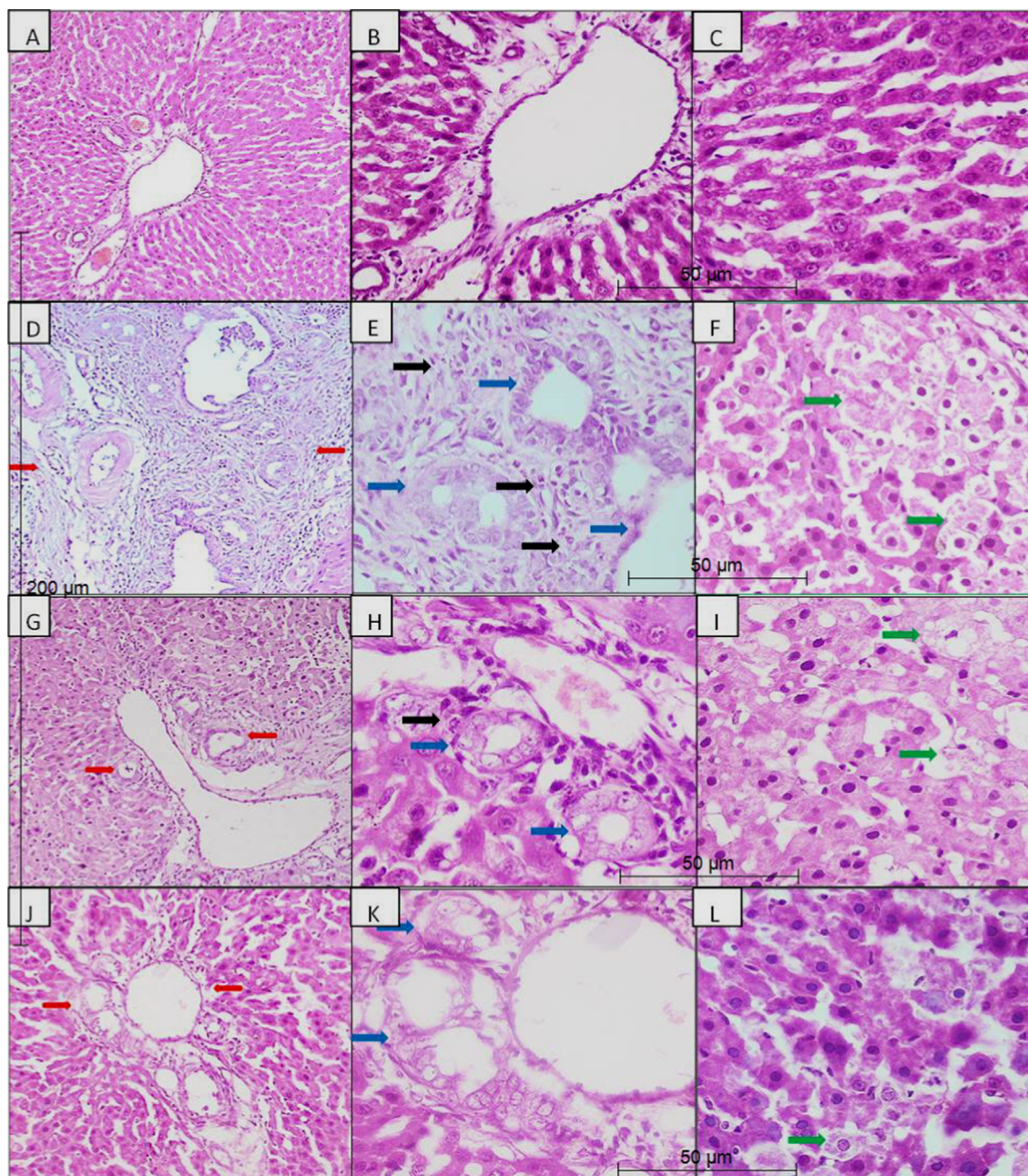
**Fig. 2.** Histopathologic examination of sections of the liver, stained by hematoxylin and eosin, from (SO group) (A to C) showing normal portal tract size and bile ducts (A, x100), with no inflammatory infiltrate (B, x400) and normal hepatocytes (C, x400). Sections from (BDL group) (D to F) show portal tract expansion by fibrous tissue (red arrows) (D, x100), the portal tracts are heavily infiltrated by neutrophilic infiltrate (black arrows) with bile duct proliferation (blue arrows) (E, x400), the hepatocytes show extensive ballooning degenerative changes (green arrows) (F, x400). Sections from (SoB-treated group) (G to I) show mild portal tract expansion (G, x100), the portal tract show minimal bile duct proliferation and neutrophilic infiltrate (H, x400), the hepatocytes show mild hepatocytes ballooning degeneration (I, x400). Sections from (UDCA-treated group) (J to L) show normal portal tract size (J, x100) with minimal bile duct proliferation and no neutrophilic infiltrate (K, x400) and rare hepatocytes ballooning degenerative changes (L, x400), (x100, scale bar 200 μm), (x400, scale bar 50 μm). SO; sham-operated, BDL; bile duct ligated, SoB; sodium butyrate, UDCA; ursodeoxycholic acid.

aspects of gastric mucosal defense (Musumba et al., 2009). Most NSAIDs including piroxicam also exert direct cytotoxic effect through interaction with membrane phospholipids (Lichtenberger et al., 2006).

The exact mechanisms underlying the aggravation of NSAIDs-induced gastric ulceration in cholestatic animals have not yet been fully elucidated. Oxidative stress has been implicated in the pathogenesis of cholestasis-induced aggravation of peptic ulceration (Kiarostami et al., 2006; Polat and Emre, 2006). Coinciding with this, our results revealed a marked elevation of gastric mucosal

MDA content and a decrease in mucosal GSH level in piroxicam-treated cholestatic animals. Decreased glutathione level could be explained in view of the reported reduction of glutathione reductase activity in cholestatic animals (Montilla et al., 2001). Normally, this enzyme is responsible for replenishing reduced glutathione stores (Mannervik, 1987). Both the reduction in glutathione content and the increase of MDA level reflect an increase in the load of ROS in gastric tissues which could be secondary to the elevation of serum TBAs concentration in cholestatic rats. Bile acids, besides their direct cytotoxic effect, exert a pro-oxidant
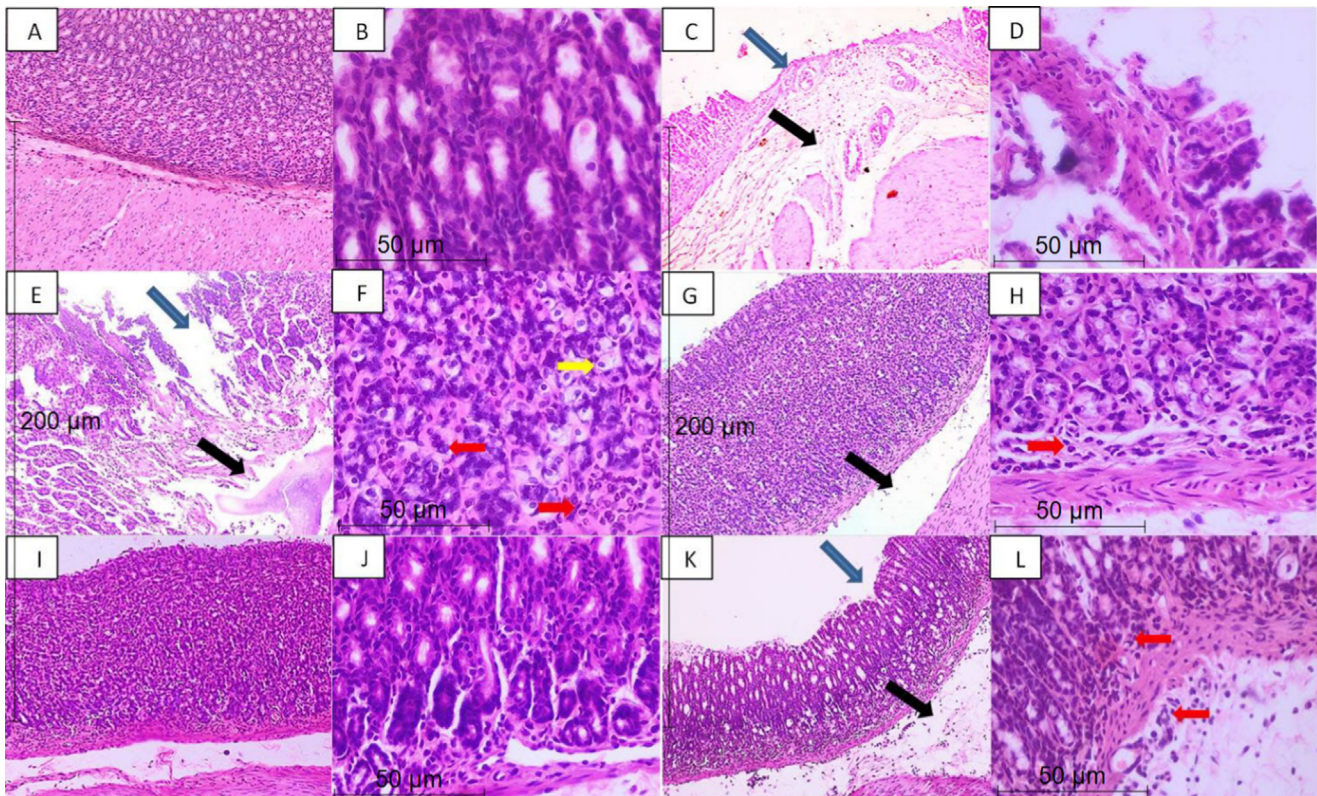
**Fig. 3.** Gastric tissue sections stained by hematoxylin and eosin showing control rats with normal gastric mucosa, submucosa and muscular layer (A, x100), the mucosal glands show normal architectural and cytological features (B, x400). Piroxicam-treated SO rats show mucosal ulceration (blue arrow) and submucosal edema (black arrow) (C, x100), the mucosal ulceration and the adjacent tissues show mild inflammatory infiltrate (D, x400). Piroxicam-treated BDL rats show mucosal ulceration (blue arrow) with extensive inflammatory infiltrate and submucosal edema (black arrow) (E, x100), the mucosal epithelial cells show cytoplasmic vacuolization (yellow arrow) and severe inflammatory infiltrate (red arrows) (F, x400). OMP–treated BDL group receiving piroxicam shows a moderate regeneration of the mucosal epithelium and a decrease of the submucosal edema (black arrow) (G, x100), and a decrease in the inflammatory infiltrate (red arrows) (H, x400). SoB-treated BDL group receiving piroxicam shows a remarkable restoration of the mucosal epithelium, a decrease of the submucosal edema (black arrow) (I, x100) and a marked decrease of the inflammatory infiltrate (J, x400). UDCA-treated BDL group receiving piroxicam shows a mild regeneration of the mucosal epithelium with focal surface erosions (blue arrow), a moderate submucosal edema (black arrow) (K, x100) and a moderate decrease of the inflammatory infiltrate (red arrows) (L, x400), (x100, scale bar 200 μm), (x400, scale bar 50 μm). SO; sham-operated, BDL; bile duct ligated, OMP; omeprazole, SoB; sodium butyrate, UDCA; ursodeoxycholic acid.

activity with a consequent increase in the generation of ROS (Perez and Briz, 2009). Elevated ROS damage cell membranes through the reaction with polyunsaturated fatty acids (Kunwar and Priyadarsini, 2011). They also oxidize cellular proteins such as the cytoskeleton protein with ultimate disruption of the mucosal barrier (Bandyopadhyay et al., 1999). In addition, ROS damage DNA molecules through the formation of DNA-radicals adduct with consequent cell death (Birben et al., 2012).

In the present study, elevation of the pro-inflammatory cytokine; TNF-α, was also noted in gastric mucosa of cholestatic rats. Histopathological examination revealed the presence of extensive inflammatory infiltrate in gastric tissues. Elevated TNF-α could be secondary to increased ROS production, as ROS are capable of activating the transcription of several proinflammatory genes (Gloire et al., 2006). In accordance with our finding, Ferraz et al., (1997) reported that gastric TNF-α expression was elevated in BDL animals and the elevation was linked to diminished synthesis of its negative modulator; prostaglandin E2 (Beck et al., 1993). TNF-α amplifies inflammatory cascade through further recruitment of macrophages and neutrophils (Vieira et al., 2009). TNF-α also stimulates caspase 3 in epithelial and endothelial cells thus contributing to apoptosis and gastric damage (Kim et al., 2000).

In addition, diminished gastric mucosal blood flow could contribute to the aggravation of piroxicam-induced ulceration in cholestatic animals. A significant decrease in gastric mucosal level of VEGF was observed in cholestatic animals. VEGF is an angiogenic

factor that is released from endothelial cells and it is involved in maintaining adequate mucosal blood flow (Ferrara et al., 1992).

In the present work, the gastroprotective activity of SoB against piroxicam-induced peptic ulceration was evaluated for the first time. Treatment of SO rats with SoB significantly attenuated piroxicam-induced ulceration, whereas, UDCA failed to provide any protection. The gastroprotective activity of SoB was comparable to that afforded by omeprazole. In accordance with our finding, Liu et al., (2016) reported that SoB effectively protected against the development of ethanol-induced gastric ulceration.

On the other hand, oral treatment of BDL animals with SoB or UDCA resulted in a significant reduction of piroxicam-induced gastric mucosal lesions. Notably, the gastroprotective effect afforded by SoB was not significantly different from that provided by the conventional antiulcer drug; omeprazole. Interestingly, histopathological examination revealed that the cellular density in SoB-treated group was even higher than that in omeprazole-treated rats. Both drugs showed a significantly higher protection ratio than that afforded by UDCA treatment.

The gastroprotective effect of SoB could be attributed to its ability to mitigate oxidative stress and lipid peroxidation as evidenced by lowered mucosal MDA level and elevated GSH content. Similarly, Liu et al., (2016) reported that the gastroprotection exerted by SoB against ethanol-induced gastric damage was accompanied with a marked reduction of gastric mucosal content of MDA. The anti-oxidant activity of SoB could be mediated through its ability

to induce translocation of the nuclear factor erythroid 2-related factor 2 (Nrf2) with a consequent increase in the expression of several downstream antioxidant enzymes such as superoxide dismutase and catalase (Xing et al., 2016). Enhanced activity of the antioxidant enzymes would lower the level of ROS and spare the mucosal content of GSH.

In the present study, SoB also showed a significant anti-inflammatory activity as evidenced by lowered TNF-α level. Similarly, the protective effect of SoB against ethanol-induced ulceration was reported to be accompanied with a significant decrease in serum level of pro-inflammatory cytokines; IL-1β, IL-6 and TNF-α (Liu et al., 2016). Decreased TNF-α transcription was previously linked to the ability of short chain fatty acids, including butyrate, to inhibit both the mitogen-activated protein kinase (MAPK) signaling pathways and the nuclear factor-kappa B (NF-κB) via activation of free fatty acid receptors or inhibition of histone deactylase activity (Li et al., 2018).

Elevation of the anti-inflammatory cytokine; IL-10, was also noted in the present study. A similar stimulatory effect of SoB on IL-10 production was documented in human monocytes (Saemann et al., 2000). Short chain fatty acids are capable of stimulating the differentiation of T-cells into IL-10 producing cells either directly or through inhibition of histone deacetylase enzymes (Sun et al., 2017).

The gastroprotective effect of SoB could also be mediated through its observed angiogenic activity. Sodium butyrate significantly elevated gastric mucosal VEGF content. A comparable stimulatory effect of SoB on VEGF expression was noted in other tissues. Kim and Chuang, (2014) documented the up-regulation of VEGF expression in brain tissues following SoB treatment. It was also reported that treatment of mice with SoB increased VEGF expression in adipose tissues. Increased VEGF transcription was previously linked to the ability of SoB to up-regulate PPAR-γ expression (Aguilar et al., 2018). VEGF is known to stimulate endothelial cells from preserved microvessels to migrate, proliferate and ultimately establish a microvascular network at the site of injury. VEGF would thus accelerate gastric ulcer healing though improvement of mucosal blood supply. VEGF is also involved in the induction of cellular proliferation (Jones et al., 1999). It was reported that administration of exogenous VEGF significantly enhanced the healing of acute gastric mucosal injury induced by ethanol in rats (Szabo et al., 1998).

The present study has demonstrated for the first time that in cholestatic animals, oral administration of SoB not only provided a potent gastroprotective effect but also it exerted a significant hepatoprotective effect. Oral treatment of BDL animals with either SoB or UDCA markedly reduced all elevated serum hepatic markers except for the level of TBAs. Histopathological examination showed that both drugs effectively attenuated hepatocytes degeneration, bile duct proliferation and neutrophils infiltration in cholestatic animals. The effectiveness of SoB as a hepatoprotective drug in different experimental models was previously reported. Sodium butyrate significantly protected against non-alcoholic fatty liver diseases induced by fructose (Jin et al., 2016) or a high fat diet (Sun et al., 2018). Sodium butyrate also exerted a hepatoprotective effect in a toxin-induced acute liver failure model (Yang et al., 2014).

The hepatoprotective effect of SoB could be attributed to its anti-inflammatory activity. Histopathological examination revealed a significant reduction in the neutrophilic infiltrate in SoB-treated group. Inflammatory cytokines are believed to be involved in the pathogenesis of cholestasis-induced hepatic damage through inhibition of bile acids export pumps which augments the hepatocellular accumulation of toxic bile acids (Kosters and Karpen, 2010). The hepatoprotective effect of SoB could also be an indirect effect through improvement of the gastrointestinal barrier (Ploger et al., 2012) which limits the transfer of gut endotoxins

to the systemic and portal circulation. Endotoxemia of gut origin is recognized as a key event in the pathophysiology of cholestasis-induced complications (Assimakopoulos et al., 2008).

In conclusion, the present study highlights the potential therapeutic usefulness of sodium butyrate in cholestasis. As the present study was conducted in animals, further studies on human subjects are needed to confirm the beneficial effect that can be exerted by sodium butyrate, in the management of cholestasis-induced complications.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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