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Bile Salt Administration Ameliorated Polymicrobial Sepsis in Mice by Countering Oxidative Damage, Inflammation and Bacterial Load

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A B S T R A C T

Sepsis is a life threatening systemic inflammatory response to an infection or tissue damage. Bile salts are known to maintain intestinal barrier function. The current study investigated the protective role of bile salt against sepsis-induced lethality. Cecal slurry prepared from donor mice was administered intra-peritoneally to C57BL/6 male mice to induce sepsis. Animals subjected to cecal slurry were given either saline or bile salt (8 and 16 mg/ kg b.wt) intravenously 2 h before cecal slurry administration and observed for 10 days for survival, bacterial load, and hematological changes. Changes in oxidant-antioxidant level, inflammation, and histology were observed in lung, liver, and kidney. Acute toxicity of bile salt was conducted in accordance to OECD guidelines. Results showed that cecal slurry administration induced sepsis in mice as seen by increased bacterial load in peritoneal fluid and blood. 16 mg/kg b.wt of bile salt increased the survival rate against sepsis from 10% to 50% and diminished the bacterial load in peritoneal fluid and blood. Bile salt administration significantly ($P < 0.05$) ameliorated the cecal slurry induced increase in lipid peroxidation and myeloperoxidase activity; decrease in total thiol, catalase, and superoxide dismutase levels, spike in WBC count, and altered histological changes such as leukocyte infiltration and hemorrhage. Acute toxicity study of bile extract revealed no lethality along with any significant change in body weight. The findings of the current study indicate that bile salt administration increased survival rate possibly by effective bacterial clearance and protecting tissues from oxidative damage and inflammation.

Introduction

Overactive immune response is the hallmark of sepsis, which is a systemic inflammatory response to an infection. Despite advances in medical science, it remains one of the leading causes of death, accounting for approximately 53 million deaths annually across the globe (Fleischmann *et al.*, 2016). In developing nations like India, mortality ranges from 40-70% (Chatterjee *et al.*, 2017). It involves the secretion of pro-inflammatory cytokines, generation of free radicals (Riedemann *et al.*, 2003; Pinsky, 2004), release of bacterial toxins into the blood stream (endotoxemia) (Kritselis *et al.*, 2013)

and is often associated with multiple organ dysfunctions (Ziesmann and Marshall, 2018).

The pathophysiology of sepsis is a multifactorial process (Hattori *et al.,* 2017). A disturbance between the generation of reactive oxygen species (ROS) and its breakdown by cellular antioxidant enzymes is thought to have a crucial role in the development and progression of disease (Andrades *et al.,* 2009; Kurutas, 2015). An enhanced ROS generation by neutrophils at the site of inflammation cause endothelial dysfunction and tissue injury (Mittal *et al.,* 2014). Also, the progressive resistance of pathogenic microorganisms to multiple drugs in sepsis further aggravates the disease. Immunosuppression caused by impaired pathogen clearance after infection leads to a high state of morbidity and mortality (Sundar and Sires, 2013). Thus, novel therapies are required to improve the survival outcomes of patients with sepsis.

Bile salts are amphipathic cholesterol metabolites and steroidal anionic surfactants produced in the liver. They play a well-known role in the solubilization, absorption, and transport of lipids, as well as the enhancement of proteolytic cleavage of dietary proteins. Bile salts can affect mucosal immune cells (Keating and Keely, 2009), stimulate intestinal immunity (Soroka and Boyer, 2014), and maintain the integrity of the intestinal barrier to prevent bacterial translocation (Sun *et al.,* 2021). Even, Traditional Chinese Medicine reported use of bile from 44 different animals for treatment of a number of maladies (Camilleri and Gores, 2019). Ursodeoxycholic acid, one of the bile salts, has been shown to have liver protective properties by reducing oxidative stress, inhibiting apoptosis, stimulating bile flow, and increased detoxification of cholephilic compounds (Buryova *et al.,* 2013). It demonstrated antimicrobial activity, especially against gram-positive bacteria (Tyagi *et al.,* 2019). Patients with cirrhosis, who release much less bile salts than healthy individuals, have more intestinal bacteria and are more prone to systemic infections (Urdaneta and Casadesus, 2017). Also, exogenous administration of bile salt to primary cirrhosis patient decreased endotoxin accumulation in biliary epithelial cells (Sasatomi *et al.,* 1998).In view of all the mentioned characteristics of bile salt, it is evident that administration of bile salt may be useful in the treatment of sepsis, but its dose, means, and time of administration needs to be defined.

The current study focused to examine the potential protective effects of exogenous administration of bile salt against sepsis in mice. Sepsis is often accompanied by multiple organ dysfunctions (Lee *et al.,* 2016). Therefore, this study also examined the protective effect of bile salt on lung, liver, and kidney in terms of evaluating oxidative stress status, inflammation, and histopathology. Further, acute toxicity of bile salt was studied in mice as per OECD guidelines.

Materials and Methods

Animals

After receiving approval from the Institute's Animal Experimentation Ethics Committee, C57BL/6 male mice aged 8-10 weeks and weighing 24-30 g were collected and kept in the Institute's Animal House Facility. All animals were housed in a controlled environment of 12 h light: 12 h dark cycle and a temperature of 26 ± 2°C. Tap water and standard animal feed (Golden Feed, Delhi) were provided without restriction. All animal experiments were performed in accordance to the Committee for the Protection and Care of Small Experimental Animals (CPCSEA), Delhi, India, guidelines.

Chemicals

Bile salt was purchased from Millipore (Sigma-Aldrich, USA). Nitroblue tetrazolium, glutathione reduced, glutathione oxidized, 1-chloro-2,4-dinitrobenzene, NADPH, 1,2-dithio-bisnitrobenzoic acid, nicotinamide adenine dinucleotide phosphate, and Folin’s reagent were supplied by Sisco Research Laboratories (Mumbai, India). Sodium carbonate, trichloroacetic acid, and culture media were purchased from Himedia (Mumbai, India). P-

nitrophenylphosphate was purchased from Calbiochem (CA, USA). Bovine serum albumin, thiobarbituric acid, and aluminium chloride were bought from Sigma-Aldrich (MO, USA).

Efficacy study of bile salt

Experiment I: Sepsis was induced by administration of cecal slurry (CS) as per the method described elsewhere (Lee *et al.*, 2016) with some modifications. In order to prepare CS, donor mice were taken and sacrificed by CO_2 inhalation. Donor mice cecam was removed and the contents of their cecam were isolated aseptically. CS was made with sterile saline at 0.5 g/mL concentration and filtered through a 70µm filter. To induce sepsis, 0.5 mL of CS (0.5 g/mL) was administered intraperitoneally into the mice.

For efficacy studies, a dose-dependent effect of bile extract was evaluated in sepsis induced mice. The mice were separated into four groups (n = 10 mice/group): (a) Control- normal saline, (b) CS only, (c) CS + bile salt (8 mg/kg b.wt), and (d) CS + bile salt (16 mg/kg b.wt). Mice were intravenously (i.v.) administered with bile salt dissolved in normal saline, 2 h before CS administration. Survival, and body weights, wasmonitored for 10 days after CS administration.

Experiment II: Based on efficacy study, most efficacious dose was chosen for further studies. For tissue-specific studies, mice were divided into three groups (n = 6): (a) Control, (b) CS only, and (c) CS + bile salt.

Animals were sacrificed at 48 h after CS administration. Peritoneal fluid and peripheral blood were taken out to access the bacterial load. Lung, liver, and kidney were harvested and a 10% homogenate was prepared in phosphate buffer saline (PBS) for oxidative stress and inflammatory marker analysis. A part of tissues were also kept in 10% normal buffered formalin for histology. All the enzyme activity and colorimetric detection was done using ELISA reader (Biotek, VT, USA).

Peripheral blood analysis

Hematological analysis of peripheral blood was done using automated hematology analyzer (Celtac-α, Nihon Kohden, Japan). Haemoglobin (Hb), platelets, white blood cells (WBC), and red blood cells (RBC) were all measured.

Bacterial load

Peritoneal fluid and peripheral blood obtained from mice were subjected to 10-fold serial dilution with sterile normal saline and plated on blood agar plates. After 24 hours of incubation at 37°C, the appearance of bacterial colonies on agar plates were counted and calculated as colony forming units per mL (CFU/mL).

Evaluation of oxidative stress markers

For lipid peroxidation (LPx), generation of malondialdehyde (MDA) was measured according to the method of Ohkawa *et al.*, (1979). Further, total thiol (T-SH), catalase (CAT, EC 1.11.1.6), and superoxide dismutase (SOD, EC 1.15.1.1) activity was estimated as per the protocol of Sedlak and Lindsay (1968); Claiborne (1985) and Nishikimi *et al.*, (1972) respectively. Bradford method was used to quantify protein (Bradford, 1976).

Determination of inflammation

For inflammation, myeloperoxidase (MPO) activity in the homogenates of lung, liver, and kidney tissues were analyzed using a commercial enzyme-linked immunosorbant assay kit (Abcam, UK) as per the protocol mentioned by the manufacturer.

Histological analysis

Formalin-fixed lung, liver, and kidney tissues were processed in different gradient of alcohol, embedded in paraffin and cut into 5-µm sections. The sliced tissue sections were stained with hematoxylin and eosin (H & E stain), and then were analyzed using light microscopy (Axioscope D1, Carl Zeiss, Gottingen, Germany).

Toxicity study of bile salt

Male mice were used for the acute toxicity study as per OECD guidelines (OECD guidelines 420). 20 animals were taken and divided into four groups- i) control, ii) efficacious dose based on the survival experiment, iii) 5-times of efficacious dose, and iv) 10-times of efficacious dose per kg b.wt of bile salt. Mice were fasted overnight before treatment.

Different doses of bile salt dissolved in sterile normal saline was administered intravenously (i.v) and control animals were administered saline (i.v). Change in body weight and mortality were recorded daily since the initiation of dosing till 14th day.

At the end of study, mice were sacrificed and hematological and biochemical changes and organ weight/body weight ratios were determined. Blood was drawn directly from the abdominal vena cava and placed in tubes with or without EDTA before being centrifuged to collect the serum at 3000 g for 10 minutes at 4°C.

Using a fully automated biochemistry analyzer (Erba; Model No: EM-360), serum levels of total bilirubin, blood urea nitrogen (BUN), serum glutamic-oxaloacetic transaminase (SGOT), alkaline phosphatase (ALP), serum glutamic pyruvic transaminase (SGPT), creatinine, uric acid, triglycerides, and total cholesterol were determined.

Hematological parameters viz. WBC, RBC, Hb, mean corpuscular volume (MCV), and platelets were determined in blood samples using hematology analyzer (Celtac-α, Nihon Kohden, Japan).

Statistical analysis

The results were represented as mean ± standard deviation (SD) and analyzed with GraphPad prism 7 software (GraphPad Software Inc., USA). The differences between two groups were compared employing the Student t-test. The survival rate was determined using the Kaplan-Meier method. P values of less than 0.05 were considered significant.

Results and Discussion

Effect of bile salt on survival rate

Figure 1A, showed that CS administration significantly ($P < 0.05$) reduced the survival rate in mice. Only 10% of mice survived till day 10 in CS group. Pre-treatment with bile salt (8 and 16 mg/kg b.wt) significantly ($P < 0.05$) increased the survival rate by 40% and 50%, respectively. The body weight of mice with CS group dropped significantly ($P < 0.05$) (Figure 1B) in comparison to control. The maximum body weight loss observed in CS + bile salt group was 12.17% in comparison to 14.74% of CS group. Further, 16 mg/kg b.wt was selected to analyze tissue specific changes due to highest survival efficiency (50%).

Effect of bile salt on peripheral blood analysis

Injection of CS, resulted in significant ($P < 0.05$) drop of 31.7% in WBC count at 24 h post-CS administration while the count increased by 38.2% at 48 h in comparison with control (Figure 2A). Pre-treatment with bile salt significantly ($P < 0.05$) decreased the elevated WBC counts at 48 h by 13.3%. CS caused significant ($P < 0.05$) loss of platelets (a decrease of 30% and 47.6% at 24 h and 48 h respectively) in comparison to control. Bile salt administration didn't impose any increase to the low platelet count and the count remained to diminish by 47.5% and 40.4% at 24 h and 48 h respectively, in comparison to control (Figure 2D). The CS alone group depicted a significant ($P < 0.05$) drop in Hb levels at all time points (Figure 2 C). Bile salt administration enhanced the lowered Hb level by 1.5% and 12.5% at 24 and 48 h respectively in comparison to CS group, but the change was non-significant. Similarly, RBC count decreased significantly ($P < 0.05$) in the CS group versus the control group (Figure 1B). Contrary to this, bile extract administration replenished the RBC loss caused due to CS administration in a time-sensitive manner and the changes noticed were 15.7% and 33% at 24 h and 48 h respectively in comparison to CS group.

Effect of bile salt on bacterial load

CS caused an increase in the number of CFU at 24 h in blood as well as in peritoneal fluid, in comparison to control where CFU remain undetected (Figure 3A and 3B). In comparison to the CS group, pre-treatment with bile salt (16 mg/kg b.wt) resulted in a significant ($P < 0.05$) decrease in the number of bacteria in the blood (49.2% reduction in CFU) and peritoneal fluid (53.2% reduction in CFU).

Effect of bile salt on oxidative stress

Figure 4A demonstrates that the MDA levels in the kidney, liver, and lung tissues. The CS alone group resulted into significantly increased ($P < 0.05$) MDA level relative to the control group. In comparison to the CS group, the administration of bile salt (16 mg/kg b.wt) significantly ($P < 0.05$) reduced the elevated MDA levels in all tissues. Contrarily, 24 hours after CS administration, there was a significant (P 0.05) decrease in the activities of all the antioxidant enzymes TSH, SOD, and CAT in all tissues against the control (Figure 4B-D). Compared to the CS group, bile salt treatment increased TSH levels in the lung (1.8-fold), liver (1.58-fold), and kidney (1.52-fold) (Figure 4B). Bile salt administration also significantly ($P < 0.05$) replenished the SOD activity in lung (1.8-fold hike) and kidney (1.3-fold hike), in comparison to CS (Figure 4C). Pre-treatment with bile salt caused a 2.4, 2.3, and 1.6-folds enhancement in CAT activity in lung, liver, and kidney respectively, when compared to CS group.

Effect of bile salt on inflammation

Leukocyte infiltration as a signal for inflammation was assessed by determining MPO activity in lung, liver, and kidney tissues (Figure 4E). As opposed to the control group, the CS group had significantly ($P < 0.05$) higher MPO activity in all tissues (2.4, 5.5, and 1.6-fold increases in lung, liver, and kidney, respectively). Pre-treatment with 16 mg/kg b.wt of bile salt caused significant ($P < 0.05$) reduction of MPO activity in lung (2.1-fold reduction) and kidney (1.6-fold reduction) when compared to CS group. However, in liver, bile salt was unable to contradict the spike in MPO activity, in comparison to CS group.

Effect of bile salt on structural changes in lung, liver, and kidney

The lungs of control group showed normal histological features such as normal alveolar wall thickness and air space (Figure 5). The administration of CS to mice caused haemorrhage, thickening of alveolar wall, leukocyte infiltration into the interstitium and alveoli, and significant reductions in alveolar air space. Bile salt administration attenuated the structural changes in lung by reducing haemorrhage, leukocyte infiltration, reducing alveolar thickening, and increasing alveolar space. Histological examinations of the control group's liver revealed normal cell structures (Figure 5). In comparison to control group, marked histopathological changes were observed in the CS group including haemorrhage and inclusion of intranuclear body. Bile salt treatment attenuated the pathological changes in liver by reducing haemorrhage and intranuclear inclusion body and bought the structural integrity near to control group. CS caused subtle changes in kidney histology with marked hemorrhage and cellular infiltration (Figure 5). Pre-treatment with bile salt didn’t control the hemorrhage caused by CS but prevented cellular infiltration in kidney.

Acute toxicity study of bile salt

Animals administered 16 (effective dose), 80 (5-times effective dose), and 160 (10-times effective dose) mg/kg b.wt of bile salt displayed no mortality and significant change in body weight, 14 days after administration. No discernible differences were seen within the treatment group and the control group in the organ to body weight ratio of brain, liver, kidney, spleen, lung, and kidney (Table 1). The levels of SGOT, SGPT, creatinine, uric acid, BUN, triglycerides, and total cholesterol in mice treated with any of the 3 doses of bile salt did not differ

significantly from those in the control group (Table 2). However, only the group receiving 160 mg/kg b.wt of bile salt showed a significant ($P < 0.05$) loss in total bilirubin and ALP levels. RBC, Hb, MCV, and platelet count were not significantly affected by bile salt (Table 3). However, higher doses of bile salt (80 and 160 mg/kg b.wt) significantly ($P < 0.05$) showed surge in WBC count in comparison to control. Apart from this, 80 mg/kg b.wt group showed discoloration of tail while 160 mg/kg b.wt. group showed discoloration of tail along with hardening from day 2 onwards. Mice belonging to 160 mg/kg b.wt further exhibited partial tail loss from day 5 onwards

In this study, administration of bile salt (16 mg/kg b.wt) protected mice from CS-induced death which may be due to efficient bacterial clearance by bile extract which is evident in our study. This is further supported by the fact that bile salt possesses antimicrobial property (Tyagi *et al.,* 2019) and provides protection to gut mucosa from bacteria (Hofmann and Eckmann, 2006).

Hematological analysis in sepsis model is a good indicator for determining the degree of infection and is the first symptom that appeared immediately during sepsis. In this study, CS-induced sepsis showed depletion in RBC, platelets, and Hb whereas increased the number of WBC which clearly indicated the deteriorated hematology change after sepsis.

RBC is highly susceptible to sepsis induced damage which could be due to activated neutrophils releasing superoxide (Weis, 1980) and increased RBC destruction due to hemolysis. The decrease in RBC is further manifested as low Hb level which is clearly indicated in our study. Jung *et al.*, found that low level of Hb is correlated with mortality during sepsis (Jung *et al.,* 2019). Bile salt significantly compensated the RBC loss and also increased the Hb levels which indicated restored hematological changes. A common manifestation of bacterial sepsis is thrombocytopenia (Arif *et al.,* 2012) which was indicated as low platelet count in the study. Low platelet counts results from reduced production and increased consumption or destruction of platelets. Bile salt administration did try to recoup the platelet loss.

Oxidative stress is caused by a disruption in the balance between ROS and endogenous antioxidant compounds (Gutteridge, 1995). Apart from sepsis, studies have shown the participation of oxidative stress in various other biological processes (Morales-Gonzalez *et al.,* 2016; Kumar *et al.,* 2016). Administration of CS to mice is a widely used model of peritonitis/sepsis and is characterized by production of inflammatory and pro-oxidant molecule (Seemann *et al.,* 2017). We found that giving CS increased lipid peroxidation in the lung, liver, and kidney while decreasing anti-oxidant enzymes. This showed that oxidative stress was involved in the CS model. Decrease in endogenous antioxidant defence is related to progression of septic syndrome and vice-versa (Macdonald *et al.*, 2003). Polyunsaturated fatty acids are changed into peroxides and lipid hydroperoxides when lipid peroxidation happens. These can break down into cytotoxic products like MDA. An increase in MDA production in all the tissues 24 h after CS administration indicated occurrence of oxidative damage. Similar observations were also reported by others (Macdonald *et al.,* 2003; Takeda *et al.,* 1986). In addition, we also found enhanced level of TSH, CAT, and SOD in the tissues after administration of bile salt which play a role of primary defence against oxidative damage. Clinical studies showed revival of clinical outcomes in patients with sepsis after supplementation of antioxidants (Berger and Chiolero, 2007). Our results also indicated that supplementation of bile salt provides protection against CS-induced oxidative damage by enhancing antioxidant enzymes.

MPO is a marker of neutrophil infiltration/ inflammation and is known to cause tissue damage via production of ROS. Research reported that increase in MPO level caused tissue dysfunction and increased the risk of mortality (Schrijver *et al.*, 2017).

Fig.1 Effect of bile salt administration on CS-induced, mortality- A and change in body weight- B, of mice. Control group showed 100% survival rate as compared to CS group which showed only 10% survival, whereas CS + Bile salt group showed 40-50% survival. Data presented as mean ± SD of 10 mice/group. $^{*}P$ <0.05 significant in comparison to control; $^{\#}P$ <0.05 significant in comparison to CS. CS- cecal slurry, BS8- bile salt 8 mg/kg b.wt, BS16- bile salt 16 mg/kg b.wt.

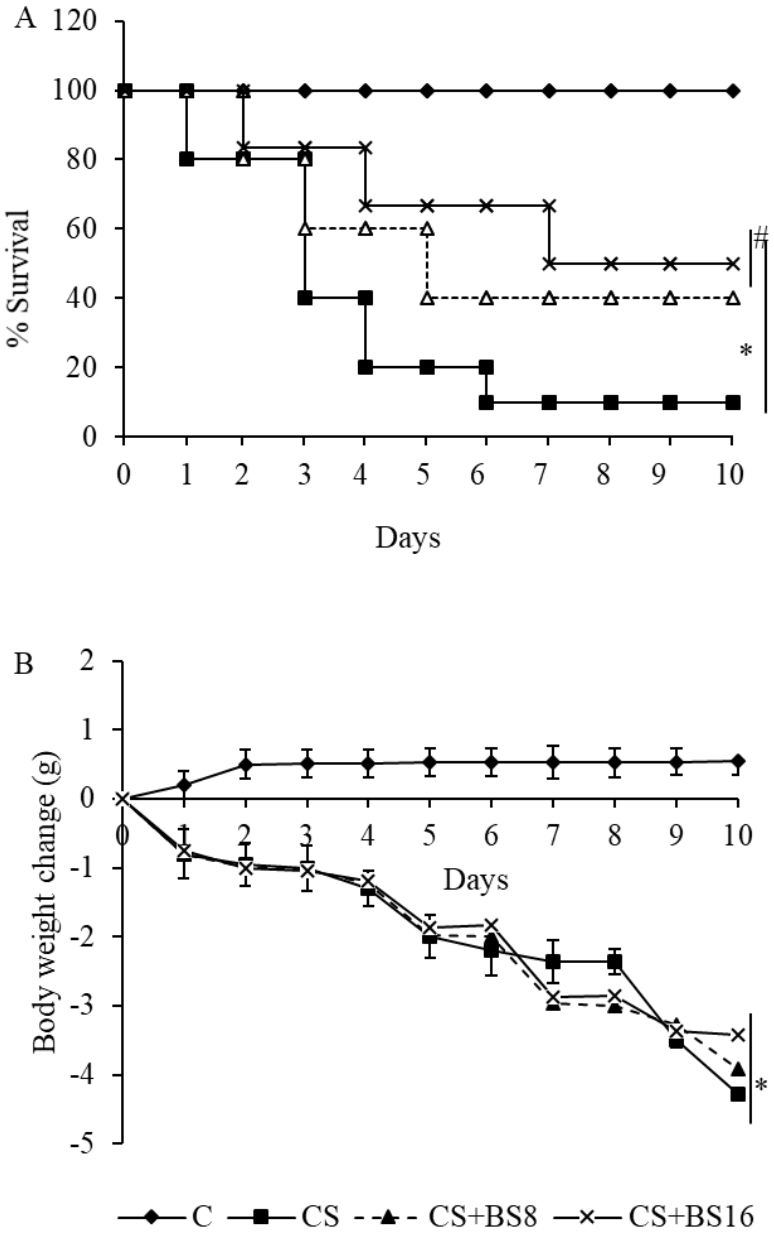

Fig.2 The effect of bile salt administration on hematological parameters in CS-induced peritonitis in mice. A- WBC, B- Platelets, C- Hb, and D- RBC. The results are presented as mean ± SD of 6 mice/group. *P≤0.05 significant when compared to control; #P <0.05 significant when compared to CS. CS- cecal slurry, BS- bile salt, WBC- white blood cells, RBC- red blood cells, Hb- haemoglobin.

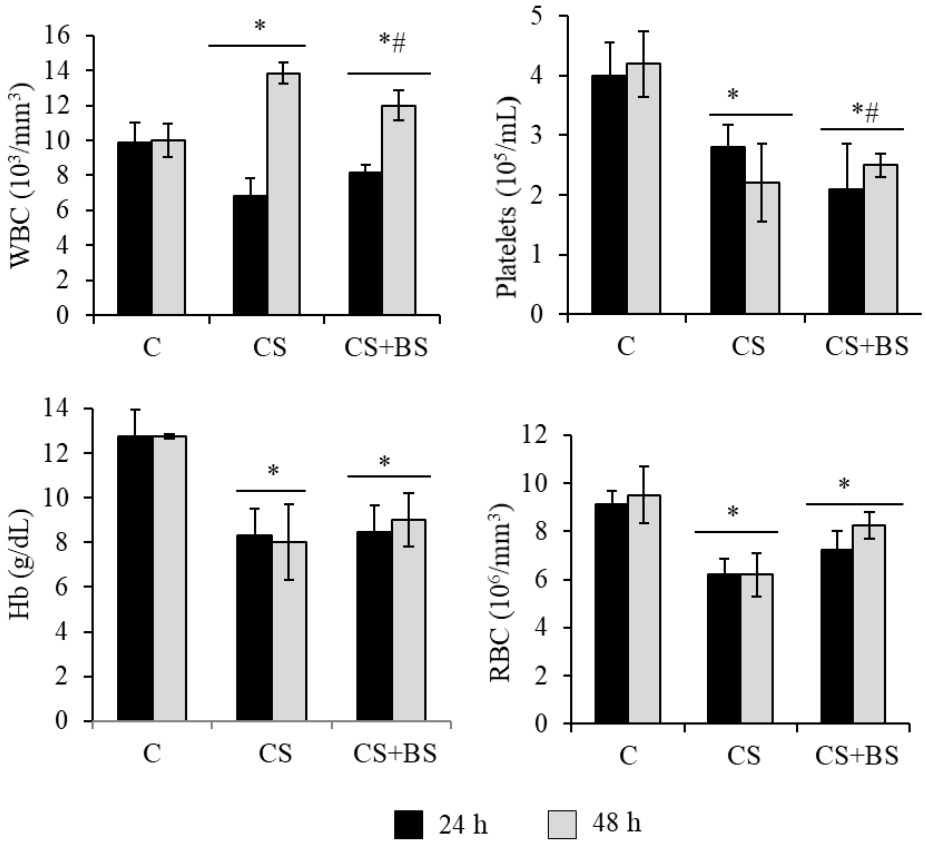

Fig.3 Effect of bile salt (16 mg/kg b.wt) administration on CS-inducedincrease in microbial load. A- Blood and B- Peritoneal fluid. Data shown as mean ± SD of 6 mice/group. *P <0.05 significant when compared to control; #P <0.05 significant when compared to CS. CFU- colony forming unit; C- control; CS- cecal slurry; BS- bile salt

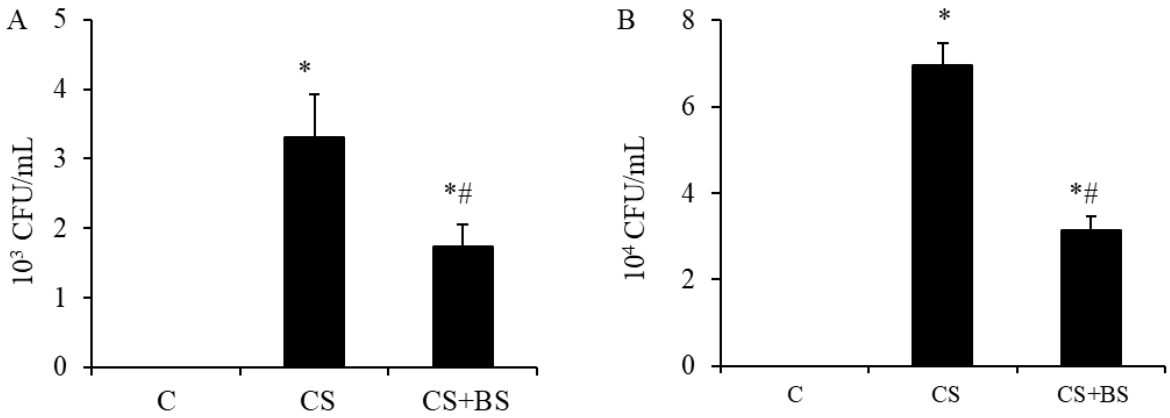

Fig.4 Effect of bile salt administration (16 mg/kg b.wt) in lung, liver, and kidney of mice subjected to CS. A- Lipid Peroxidation, B- Total thiols, C- SOD activity, D- Catalase activity, and E- MPO activity. Data shown as mean ± SD of 6 mice/group. *P <0.05 significant when compared to control; #P <0.05 significant when compared to CS. C- control, CS- cecal slurry, BS- bile salt, MDA- malondialdehyde, TSH- total thiols, NBT- nitrobluetetrazolium, SOD- superoxide dismutase, MPO- myeloperoxidase.

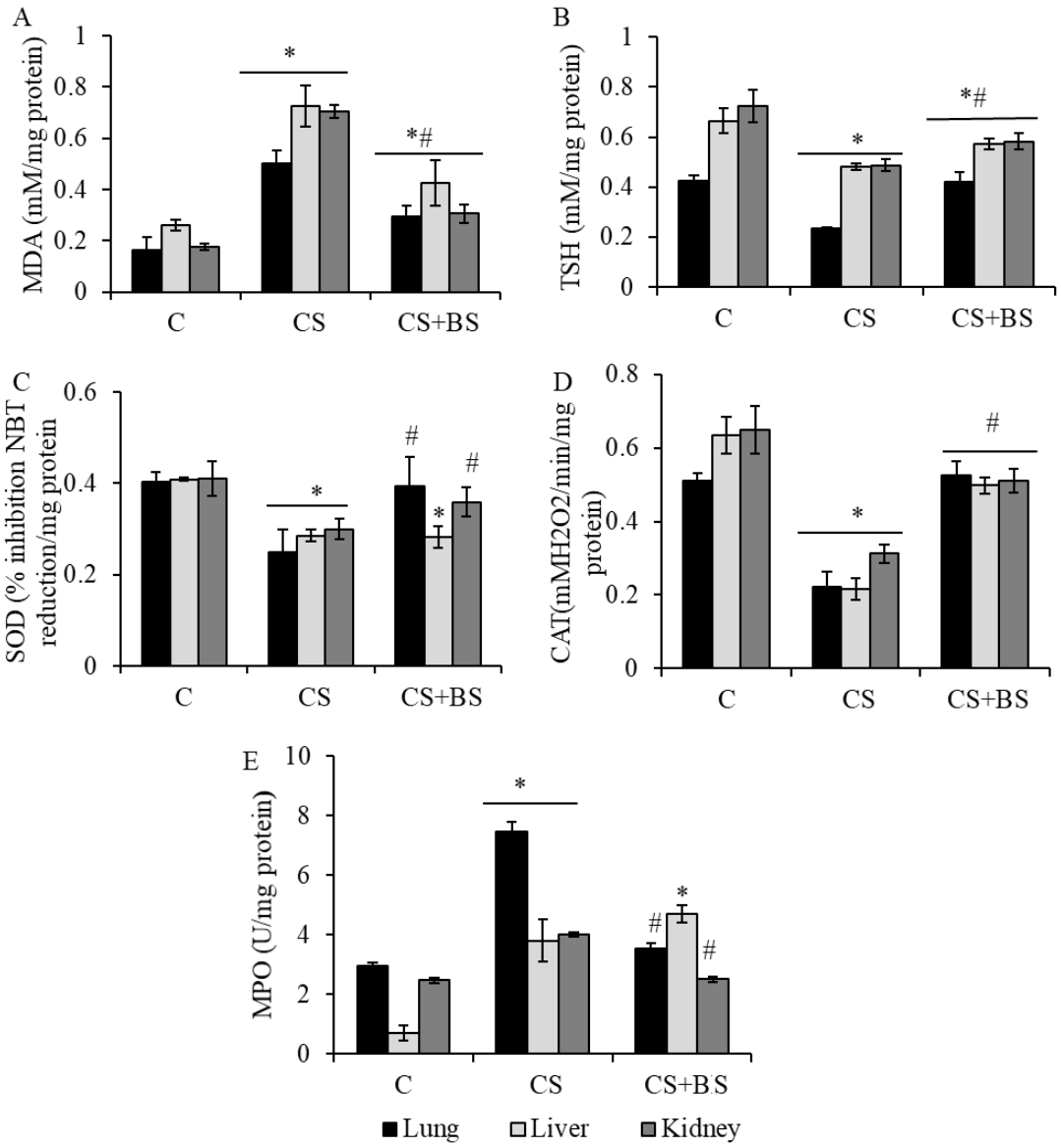

Fig.5 Effect of bile salt(16 mg/kg b.wt) administration on CS-induced structural changes in organs.The results show H&E staining of lung, liver, and kidney tissue sections from the indicated group (x400). The damaged sites are depicted by black circle- hemorrhage, black square- alveolar congestion, black arrow- alveolar wall thickening, black dotted arrow- *intranuclear inclusion body*,and red dotted arrow- mononuclear cell infiltration.C- control, CS- cecal slurry, BS- bile salt.

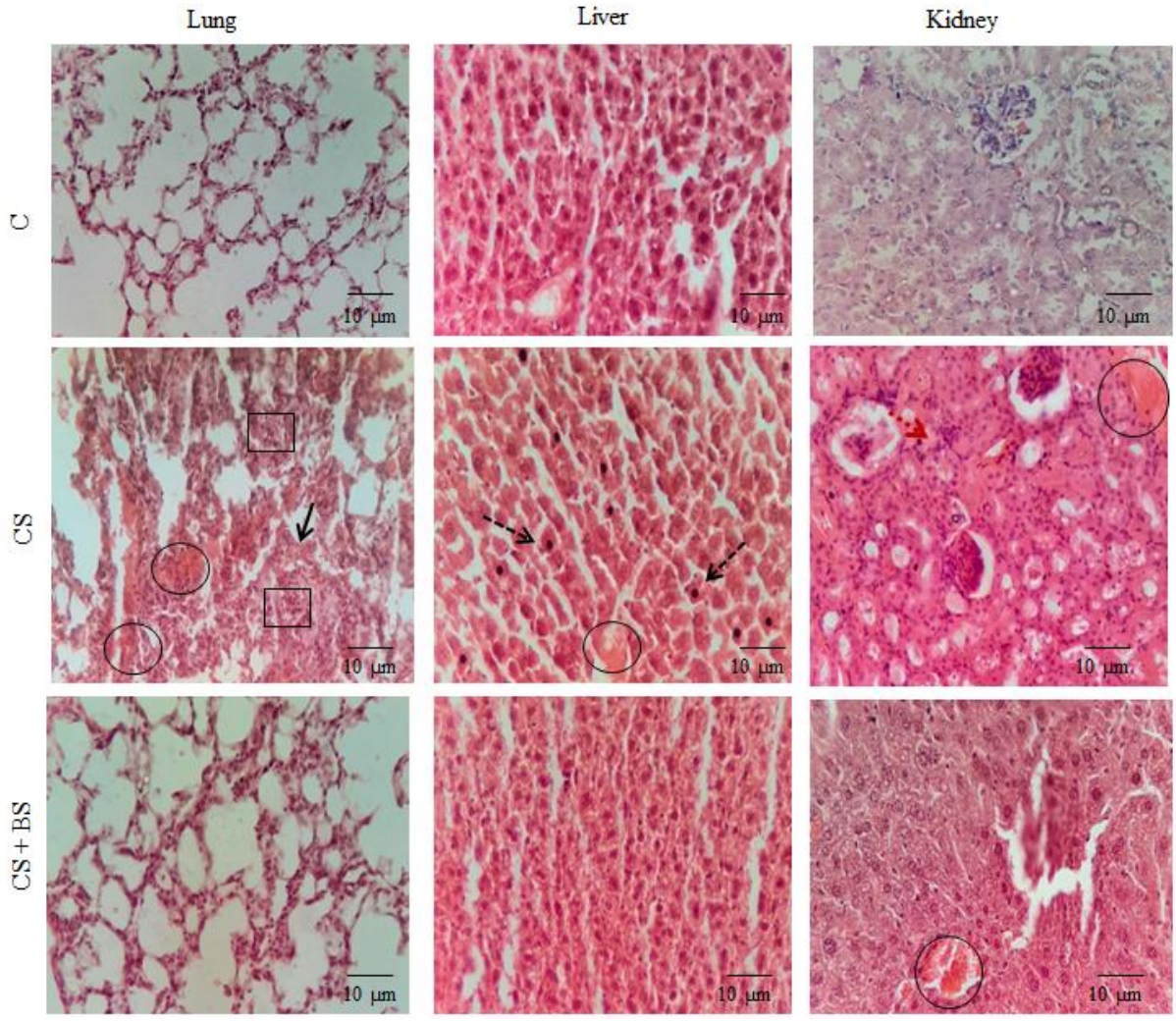

Table.1 Effect of bile salt on organ to body weight ratio of mice

Organ	Control	Bile salt (mg/kg b.wt)		
		16	80	160
Lung	0.78±0.62	0.75±0.82	0.7±0.35	0.71±0.26
Liver	4.91±0.2	4.72±0.07	4.81±0	4.89±0.60
Kidney	1.30±0.31	1.31±0.02	1.28±0.27	1.25±0.14
Spleen	0.37±0.1	0.42±0.55	0.51±0.42	0.56±1.06
Brain	2.05±0.04	2.1±0.05	2.26±0.27	2.18±0.09

The results are presented as mean ± SD of 5 mice/group.
$^{*}P < 0.05$ significant when compared to control.

Table.2 Biochemical parameters in blood serum of single-dose acute toxicity of bile salt

Parameters	Control	Bile salt (mg/kg b.wt)		
		16	80	160
Bilirubin total (mg/dL)	0.71±0.06	0.65±0.09	0.51±0.09	0.22±0.01*
SGOT (IU/L)	114±19.79	103±6.08	126.2±4.22	119±12.02
SGPT (IU/L)	36.5±8.48	31.5±2.26	30.3±1.97	34.85±17.32
ALP (U/L)	71.8±8.08	73.5±16.26	69±10.01	45±32.52*
Serum creatinine (mg/dL)	1.05±0.05	1.73±0.01	1.07±0.01	0.97±0.07
Serum uric acid (mg/dL)	0.51±0.07	0.61±2.05	0.69±0.84	0.83±0.70
BUN (mg/dL)	19.37±3.07	24.87±0.98	22.66±0.33	20.82±0.29
Triglycerides (mg/dL)	16.36±1.97	14.02±23.47	22.05±8.55	14.08±24.05
Total cholesterol (mg/dL)	88.32 ±16.61	86.85±12.37	98.6±3.39	77.6±2.82

The results are presented as mean ± SD of 5 mice/group. *P < 0.05 significantly different compared to control. SGOT- serum glutamic-oxaloacetic transaminase, SGPT- serum glutamate-pyruvate transaminase, ALP- alkaline phosphatase, BUN- blood urea nitrogen.

Table.3 Hematological parameters of single-dose acute toxicity of bile salt

Parameters	Control	Bile salt (mg/kg b.wt)		
		16	80	160
WBC (10^3/mm^3)	11.25±3.25	11.85±2.46	20.81±4.66*	22.46±7.14*
RBC (10^6/mm^3)	6.46±0.95	5.28±2.8	5.77±1.49	5.56±1.46
Hb (g/dL)	13.85±1.34	10.6±5.25	10.8±2.54	10.85±2.33
MCV (µm^3)	43.45±1.34	42.85±0.07	44.3±1.83	45.8±4.66
PLT (10^5/mL)	839±226.27	801±379.0	832±214.25	856±214.25

The results are presented as mean ± SD of 5 mice/group. *P < 0.05 significantly different compared to control. WBC- white blood cells, RBC- red blood cells, MCV- mean corpuscular volume, PLT- platelets.

Similarly, role of inflammation in various other disease have also been reported (Furman *et al.*, 2019; Kumar *et al.*, 2022). In the current study, CS administration led to sepsis that was induced by an inflammatory response as evidenced by rising MPO levels in all tissues. However, pre-administration of bile extract to mice subjected to sepsis resulted in decreased MPO activity, indicating that bile salt reduced the animal's neutrophil-induced inflammatory response. Our findings showed that CS administration exacerbated the histological character in the liver, lung, and kidney. It could be due to oxidative stress generated in tissues as evident in the study. Alteration of histological features could lead to organ injury and accounts for high mortality (Caraballo and Jaimes, 2019).

Shrum *et al.*, (2014) also reported similar organ disorganization by utilizing the same model for sepsis induction (Shrum *et al.*, 2014). Current study revealed the protective effect of bile salt in a sepsis model by attenuating edema, alveolar space degeneration, inflammatory cell infiltration, and hemorrhage in lung; hemorrhage in liver, cellular infiltration in kidney. The resultant change could be because of enhanced antioxidant mechanism.

Acute toxicity study of bile salt at an effective dose of 16 mg/kg b.wt reported no physiological change as observed through SGPT, SGOT, and other kidney function markers. An increase in WBC count was found during a haematological evaluation, which is indicative of potential immunomodulating properties of bile salt.

WBC is well known for its protective function through the production, movement, and antibodies distribution in response to infectious agent intrusion

(Nicholson, 2016). Administration of bile salt (80 and 160 mg/kg. b.wt) though caused local irritation at the site of administration but no mortality was observed.

It further leads to darkening and partial loss of tail which could be due to thrombosis as previously described by researcher (Choi *et al.,* 2021). However, single administration of bile salt didn't encounter any change either in body weight or organ weight.

The current study demonstrated the role of bile salt in prevention of CS induced mortality in mice by countering oxidative stress, inflammation, bacterial count, and restoration of hematological and histological architecture of lung, liver, and kidney. The acute toxicity study revealed no systemic toxicity at the effective dose.

Thus, our results suggested that bile salt has the potential to treat systemic inflammation, as it inhibits the activation of the oxidative stress cascade at its earliest stage.

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