**ABSTRACT**

Drug-induced liver injury is a common cause of acute liver failure. Dapsone is increasingly used in combination with rifampicin for the treatment of leprosy and also for several dermatological disorders. Clinically, abnormal liver function and focal bile duct destruction were reported after dapsone therapy. *Lagerstroemia speciosa* Pers., commonly known as Banaba has been traditionally used to treat various ailments including diabetes and obesity due to its antioxidant and anti-inflammatory efficacies. This study investigated the hepatoprotective effect of ethanolic banaba leaves extract (EBLE) against dapsone-induced hepatotoxicity in rats. Dapsone (30 mg/kg, i.p.) was administered twice daily for 30 days. In separate groups, rats were post-treated orally with EBLE (250 and 500 mg/kg) and silymarin (100 mg/kg) once daily for 30 days after dapsone administration. The marker enzymes of hepatotoxicity, oxidative stress markers, inflammatory markers and histopathology of liver were done. HPTLC analysis confirmed the presence of 12.87 µg of corosolic acid per mg of EBLE. Dapsone administration induced significant (*p* < 0.001) elevation of marker enzymes of hepatotoxicity in serum. This treatment also increased lipid peroxidation (*p* < 0.001) and pro-inflammatory markers (tumor necrosis factor-alpha, transforming growth factor-beta, and nuclear factor kappa-B) expressions (*p* < 0.001) and decreased antioxidants (*p* < 0.001) such superoxide dismutase, catalase and glutathione in the liver tissue. All these abnormalities were significantly (*p* < 0.001) mitigated after EBLE (500 mg/kg) and silymarin post-treatments. The results of this study suggest that silymarin and EBLE can be used for dapsone-induced hepatotoxicity.

**1. Introduction**

Drug-induced liver injury (DILI) is a common cause of acute liver failure (ALF) (Licata 2016, Ezhilarasan 2021). DILI is one of the leading causes of drug terminations at the preclinical and clinical levels (Norman 2020). The hepatotoxicity is very common with drugs like acetaminophen, anti-tubercular, anti-epileptic, dapsone, hormones, ferrous sulfate overdose, anti-retroviral, chemotherapeutic agents (Devarbhavi et al. 2010, Ezhilarasan et al. 2017, Hoofnagle and Björnsson 2019, Ezhilarasan 2021). The prevalence of viral hepatitis escalating worldwide and DILI also emerged as a major cause of acute hepatitis (Asrani et al. 2019). Devarbhavi et al. (2019) has reported that DILI is one of the common and important cause for acute and chronic liver failure in Asian patients. Despite significant advancements in diagnosis, hazard identification and risk mitigation, DILI remains to be a difficult task for drug development and a major health concern worldwide (Mosedale and Watkins 2017). Clinical studies from India also reported that fourteen percent of drug-induced ALF was mainly associated with dapsone, anti-tubercular, anti-epileptic, and anti-retroviral drugs (Devarbhavi et al. 2017, Devarbhavi et al. 2018).

Dapsone (DDS, 4, 4′-diaminodiphenylsulfone) belong to sulfone class (Fromm and Wittmann 1908) and it is commonly used in combination with rifampicin for the treatment of leprosy and also for several dermatological disorders like acne, dermatitis herpetiformis, psoriasis, and *Toxoplasma gondii* infections and *Pneumocystis carinii* pneumonia in AIDS patients (Semira et al. 2014, Wozel and Blasum 2014, Ghaoui et al. 2020). Clinically, hemolysis and hypoxia due to methemoglobinemia (dapsone syndrome or sulfone syndrome) are very common in patients with DDS therapy (Prasad 2000, Das and Jawed 2014, Keerty et al. 2020, Lewis and Jacobs, 2020). However, clinically, an abnormal liver function with primary sclerosing cholangitis was also reported in patients with DDS therapy for dermatitis herpetiformis (Kirby et al. 1999). DDS also induced cholangitis due to focal destruction of bile ducts (Itha et al. 2003). Follow-up clinical studies are also confirmed the hepatotoxic potential of DDS in human subjects (Devarbhavi et al. 2010, Devarbhavi et al. 2017). Therefore,
the need of the hour is to identify a novel therapeutic option for DDS-induced hepatotoxicity for clinical subjects.

*Lagerstroemia speciosa* Pers., (Lythraceae) commonly known as Banaba (Pride of India) has been traditionally used to treat various ailments including diabetes and obesity (Stohs et al. 2012). The leaves, stems, flowers, fruits, bark and roots of this plant contain a variety of phytocompounds like ellagic acids, glycosides, flavones, triterpenes (corosolic acid), and tannins, etc. (Stohs et al. 2012, Tiwary et al. 2017). The presence of corrosolic acid in leaves of *L. speciosa* was attributed to beneficial effects (Jayakumar et al. 2014, Rohit Singh and Ezhilarasan 2020, Zhang et al. 2020). Previous in vivo studies have shown the anti-inflammatory, anti-diarrheal, and anti-gout effects of banaba (Stohs et al. 2012). In the context of the liver, banaba petal extract attenuated carbon tetrachloride-induced oxidative stress and hepatotoxicity in mice (Tiwary et al. 2017). Our recent in vitro studies have explored the pre-apoptotic and cell cycle arresting potential of ethanolic banaba leaf extract (EBLE) in liver cancer cells (Rohit Singh and Ezhilarasan 2020, Thakur and Devaraj 2020). Therefore, in this study, we investigated the antioxidant and hepatoprotective effect of EBLE against DDS-induced hepatotoxicity in rats.

### 2. Materials and methods

#### 2.1. Drugs and plant extract

Dapsone (IP/17/008) was obtained as gratis from Atul Pharmaceutical Ltd. (Ahmedabad, Gujara, India). The EBLE was purchased from M/S. Quimico, herbal extract manufacturer, Bengaluru, India (Batch no. KAN/BE/1801009). According to the manufacturer’s certificate of analysis, EBLE contains 20% corrosolic acid. All the other chemicals used in this study were purchased from Sigma-Aldrich Company.

#### 2.2. Experimental animals

The male Wistar albino rats (150 – 200 g) were used for the study. They were housed in clean polypropylene cages and maintained under standard laboratory conditions at a temperature 22 ± 2 °C and 12 h alternating light-dark cycle. They were allowed free access to a standard pellet diet and water ad libitum. All the experimental procedures were done after obtaining necessary permission from the Institutional Animal Ethics Committee of Malla Reddy Institute of Medical Sciences, Hyderabad, India (3/IAEC/2017).

#### 2.3. High-performance thin layer chromatography (HPTLC) analysis

The EBLE (5 mg) was dissolved in 1 mL of chromatography-grade methanol and used for sample application on pre-coated silica gel 60 GF254 (10 × 10 cm with 250 μM thickness) used as stationary phase. The high-performance liquid chromatography grade chloroform and methanol (9:5:0.5) were used as mobile phase for corrosolic acid. The standard corrosolic acid was dissolved in methanol (50 μg/mL).

Development was done in ascending mode using CAMAG Automatic TLC Sampler 4 (ATS4), which was programmed through winCATS software.

#### 2.4. Experimental design

Thirty-six animals were divided randomly into six groups. Group I rats were administered dimethyl sulfoxide (1%) through i.p. route for the first 30 days and propylene glycol orally for the next 30 days and served as control. Group II rats were received DDS (30 mg/kg b.w., i.p.) (Veggi et al. 2005) twice daily for 30 days and sacrificed on day 31. Group III rats were administered DDS, twice daily for the first 30 days and left without treatment for the next 30 days. Group IV and V rats were received DDS for 30 days twice daily and were post-treated once daily with EBLE doses of 250 and 500 mg/kg and b.w., p.o. respectively (Mousa et al. 2019) for the next 30 days. Group VI rats were received DDS for 30 days and were post-treated once daily with silymarin (SIL) (100 mg/kg b.w. p.o.) (Devaraj et al. 2020) for the next 30 days (Figure 1). Except Group II (sacrificed at the end of day 30), all the groups of animals were sacrificed at the end of day 60. DDS was dissolved in dimethyl sulfoxide, EBLE was dissolved in distilled water. SIL was placed in propylene glycol.

The blood was collected at the end of days 15, 30, 45 and 60 from overnight fasted rats to investigate the progression of hepatotoxicity by analyzing serum marker enzymes. The blood samples were centrifuged for 10 min at 1500 rpm and serum was separated and was stored at −20 °C until the analysis was carried out. At the end of the study, livers were excised, washed in ice-cold saline, blotted to dryness and weighed. Liver tissues were homogenized with cold saline. After centrifugation at 3500 rpm for 10 min at 4 °C, the supernatant was collected to measure superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT) activities and 2-Thiobarbituric Acid Reactive Substances (TBARS) content in the liver.

#### 2.5. Estimation of serum marker enzymes of hepatotoxicity

The serum samples were used for the analysis of aspartate and alanine transaminases (AST and ALT), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (GGT), and lactate dehydrogenase (LDH) activities and bilirubin content and were estimated according to the instructions of commercially available diagnostic kits (Enzo lab diagnostic kits).

#### 2.6. Tbars assay

The level of lipid peroxidation was determined by quantifying the concentration of TBARS with the Ohkawa et al. (1979) method for determining the malondialdehyde concentration. To 0.5 ml of tissue homogenate, 1.5 ml of 20% acetic acid, 0.2 ml of SDS and 1.5 ml of TBA were added in a test tube and it was made up to 4 ml with distilled water and then heated for 60 min at 95 °C. After cooling, 4 ml of butanol-
pyridine mixture was added and shaken well. After centrifugation at 4,000 rpm for 10 min, the organic layer was taken and its absorbance was read at 532 nm.

2.7. Assay of SOD

To the tubes containing 0.5 ml of carbonate buffer, 0.5 ml of EDTA solution, required amount of homogenate containing enzyme was added and the final volume was made up to 2.5 ml. The reaction was initiated by the addition of 0.4 ml of epinephrine and the increase in absorbance at 480 nm was measured in a Shimadzu UV spectrophotometer (Japan). The enzyme activity was expressed as units/mg protein (Misra and Fridovich 1972).

2.8. Assay of CAT

About 0.05 ml of tissue homogenate was added to 1.2 ml of the phosphate buffer. To this, 1 ml of hydrogen peroxide was added to start the enzyme reaction. The decreased absorbance was taken at 620 nm for every 30 s intervals for 3 min. The enzyme blank was used with 1 ml of distilled water. The catalase activity was measured in nM of H₂O₂ decomposed/min/mg protein (Takahara et al. 1960).

2.9. Estimation of GSH

Reduced glutathione was assayed by the method of Moron et al. (1979). To 0.5 ml of tissue homogenate, 125 µl of 25% of trichloroacetic acid (TCA) was added to precipitate proteins. The tubes were cooled in ice for 5 min and the mixture was further diluted with 0.6 ml of 5% TCA and centrifuged at 9000 xg for 10 min. 0.3 ml of the aliquot was made up to 1 ml with 0.2 M sodium phosphate buffer (pH 8.0) and freshly made 5,5-dithio-bis-(2-nitrobenzoic acid) reagent (2 ml). After 10 min of incubation, the intensity of the yellow color produced was measured using a spectrophotometer at 412 nm.

2.10. Gene expression analysis

TRIzol reagent was used to extract total RNA from liver tissue according to the manufacturer’s instructions (Invitrogen). The quality and quantity of total RNA were detected by a spectrophotometer (NANODROP200, Thermo Scientific). The primers for tumor necrosis factor-alpha (TNF-α), transforming growth factor-beta (TGF-β), nuclear factor-kappa B (NF-κB), IκB (inhibitor of NF-κB) were synthesized from Sigma Genosys (Bangalore, India) (Table 1). M-MuLV Reverse Transcriptase and RNase Inhibitor were purchased from Thermo Scientific. PCR amplification was carried out on an Eppendorf Mastercycler ep (Eppendorf AG, Germany). The PCR was done using initial denaturation at 94°C for 1 min, 33 cycles of 94°C for 40 sec, 65°C for 40 sec, 72°C for 60 sec and a final extension at 72°C for 2 min. The amplified products were separated on a 1.5% agarose gel in tris buffer at 75 V for 3 h. The gel was stained with ethidium bromide and the amplified product was visualized and photographed on Gel Doc system. The location of a predicted product was confirmed by using a 100-bpladder (Applied Biosystems, USA) as a standard size marker. The gel was then photographed under UV transillumination. The intensity of PCR products was measured using a video image analysis system (Kodak Digital Science). The signal for each transcript was standardized against that of the β-actin mRNA from each sample and the results were expressed as transcript/β-actin mRNA ratio.

2.11. Histopathology of liver tissue

The liver tissue was fixed in 10% formalin, dehydrated in gradual ethanol (50–100%), cleared in xylene, and embedded in paraffin wax. The wax sections with 5–6 micron thickness were made using a rotary microtome. These tissue sections were then stained with hematoxylin and eosin, Masson’s trichrome and Sudan black B stains (Bancroft and Cook 1984). A scoring system was used to establish the severity of hepatic injury according to extent of (1) dilatation of sinusoids; (2) infiltration of inflammatory cell; (3) sinusoidal congestion; and (4) hydropic degeneration such as cytoplasmic...
vacuolization/swelling of hepatocyte), with features scored as 0 (normal), 1 (mild), 2 (moderate), or 3 (severe). The maximum score of 12 indicated the most severe hepatic injury (Akbulut et al. 2014).

2.12. Statistical analysis

Data obtained from the experiments are expressed as mean ± SD for six animals. The data were subjected to one-way analysis of variance (ANOVA) and the post hoc multiple comparison tests to assess the degree of significant difference between means of various treatment groups were performed by employing Tukay’s test, using SPSS software (version 22.0). The $p’$ value < 0.05 was considered significant.

3. Results

3.1. Phytochemical analysis of EBLE

The presence of corosolic acid in EBLE extract was analyzed by the HPTLC method. The chromatogram shows the presence of corosolic acid in EBLE with the Rf value of 0.77 vs. 0.79 of its corresponding standard Rf (Figure 2(A,B)). The quantitative analysis showed the presence of 12.87 µg of corosolic acid per mg of EBLE.

3.2. Effect of EBLE on DDS-induced changes in liver marker enzymes and bilirubin

In group II rats, 30 days of DDS administration caused significant ($p < 0.001$) elevation of serum AST, ALT, ALP, LDH, GGT and bilirubin level as compared to the control rats. DDS administration caused a nearly three to fourfold increase in liver marker enzymes that was measured at the end of days 15 and 30. The hepatotoxic potential of group II rats (sacrificed at the end of day 30 after DDS administration) was similar to that of group III (left without treatment after 30 days of DDS administration) and therefore DDS + EBLE and DDS + SIL post-treated groups were compared with group III. The EBLE treatments for 30 days at the doses of 250 and 500 mg/kg after DDS administration caused a significant ($p < 0.001$) fall in the elevation of AST, ALT, ALP, GGT and bilirubin in serum (Group IV and V). The EBLE post-treatments did not reverse the DDS induce LDH elevation. The SIL post-treatments for 30 days after DDS administration produced a significant fall ($p < 0.001$) in the activities of all the marker enzymes including LDH and decreased the bilirubin level in the serum of rats (Group VI) (Figure 3(A–F)).
Figure 3. Effect of ethanolic banaba leaves extract on dapsone (DDS) induced changes in the marker enzymes of hepatotoxicity and bilirubin in serum of rats. AST and ALT-aspartate and alanine transaminases, ALP-alkaline phosphatase, LDH-lactate dehydrogenase, GGT-gamma glutamyl transpeptidase. \( ^a p < 0.001 \) vs control; \( ^b p < 0.001 \) vs DDS + Recovery.

Figure 4. Effect of ethanolic banaba leaves extract on dapsone (DDS) induced changes in the lipid peroxidation and enzymic and non-enzymic antioxidants level in the liver tissue of rats. Treatments were done as described in the material and method section. SOD-superoxide dismutase, CAT- catalase, GSH-reduced glutathione. DDS þ Recovery – 30 days DDS treatment followed by 30 days recovery. SIL-silymarin. ns-non-significant; \( ^a p < 0.001 \) vs control; \( ^b p < 0.001 \) vs DDS + Recovery.
3.3. Effect of EBLE on DDS-induced changes in lipid peroxidation and antioxidants

In the present investigation, in group II rats, DDS administrations for 30 days caused a significant ($p < 0.001$) increase in the TBARS levels in the liver tissue of rats. The TBARS level was also significantly ($p < 0.001$) increased in the group III rats compared to the control rats. Concomitantly, this treatment also caused a significant ($p < 0.001$) decrease in the activities of SOD, CAT and GSH content in the liver tissue of DDS administered rats (Group II and III). The EBLE and SIL post-treatments for 30 days after DDS administration significantly mitigated the TBARS levels in the liver tissue compared to the DDS group. The EBLE (at 500 mg/kg) and SIL post-treatments significantly mitigated the TBARS levels in the liver tissue compared to the DDS group. The EBLE at 500 mg/kg and SIL post-treatments significantly ($p < 0.001$) replenished the SOD and CAT activities near normalcy. It has to emphasize that at a low dose, EBLE treatment did not reverse SOD and CAT activities toward normalcy. The EBLE and SIL treatments significantly ($p < 0.001$) restored reduced GSH content in the liver tissue of rats (Figure 4(A,B)).

3.4. Effect of EBLE on DDS-induced changes in the pro-inflammatory marker genes

DDS administration for 30 days caused significant ($p < 0.001$) upregulation of TNF-α, TGF-β, and NF-κB and downregulation of IκB gene expressions in liver tissue of rats. EBLE treatments at 250 mg/kg did not downregulate the DDS-induced TNF-α and TGF-β gene expressions. However, high dose of EBLE and SIL post-treatments significantly downregulated TNF-α ($p < 0.001$) and TGF-β ($p < 0.05$) expressions. The NF-κB expression was significantly ($p < 0.001$) downregulated with concomitant upregulation of IκB expression ($p < 0.001$) after EBLE and SIL post-treatments (Figure 5(A,B)).

3.5. Effect of EBLE on histopathological changes induced by DDS in liver

The liver architectural distortion was investigated by H and E staining. The control liver tissue shows the normal architecture of the liver with intact central vein and radiating sinusoidal space. The liver tissue of DDS administered rats (group II) shows moderate periportal fibrosis (PPF) and peribiliary fibrosis (PBF) with the proliferation of connective tissues. The liver tissue of group III rats showed centrilobular necrosis. The liver tissue of rats post-treated with EBLE at 250 mg/kg after DDS administration showed mild PBF. While DDS + EBLE 500 mg/kg and DDS + SIL treatments showed nearly normal architecture of the liver. Masson’s trichrome staining was performed to access the fibrotic changes in the liver tissue. Control rats liver tissue shows normal liver architecture with

Figure 5. Effect of ethanolic banaba leaves extract (EBLE) on dapsone (DDS) induced changes in the expression of tumor necrosis factor alpha (TNF-α), transforming growth factor beta (TGF-β), nuclear factor kappa B (NF-κB), IκB (inhibitor of NF-κB) in liver tissue of rats. A. Qualitative expression of the above genes. 1 - Marker, 2 - Control, 3 - DDS, 4 - DDS + REC, 5 - DDS + EBLE 250 mg/kg, 6 - DDS + EBLE 500 mg/kg, 7 - DDS + SIL mg/kg. B. Quantitative analysis of gene expression. 
$a$ $p < 0.001$ vs control; $b$ $p < 0.001$ vs DDS + Recovery. $c$ $p < 0.05$ vs DDS + Recovery group.
no extracellular matrix accumulation, groups II and III rats liver tissue showed mild PPF and PBF. While EBLE 250 mg/kg post-treated rat livers showed moderate PBF and PPF with infiltration of inflammatory cells, DDS + EBLE 500 mg/kg and DDS + SIL treatments showed no apparent fibrotic changes in the liver tissue. In Sudan Black staining, control liver tissue shows no appearance of black color fat droplets. Rat liver tissues in groups II and III showed black fat droplets. In DDS + EBLE and DDS + SIL treated liver tissue architecture not contains a black stain of lipid droplets (Figure 6(A)). The liver injury score was significantly (p < 0.001) increased in DDS alone administered rats (group II and III). EBLE (p < 0.01 vs 250 mg/kg and p < 0.001 vs 500 mg/kg) and SIL post-treatments (p < 0.001) significantly reduced liver injury score (Figure 6(B)).

4. Discussion

Dapsone is one of the FDA-approved drugs, frequently used for various microbial infections (Ezhilarasan 2021). In clinical subjects, DDS hypersensitivity syndrome was commonly associated with increased liver hepatotoxic marker enzymes such as AST, ALT, ALP and GGT in serum (Itha et al. 2003, Vinod et al. 2013, Quaresma et al. 2015). Unfortunately, the hepatotoxic potential of DDS is not well-studied (Ezhilarasan 2021). Therefore, the need of the hour is to understand the toxic potential of DDS and also to identify an effective alternative remedy for DDS-induced liver injury. The liver marker enzymes are confined in hepatocytes and in response to liver injury or hepatocellular membrane degeneration cause the release of liver marker enzymes in serum (Devaraj et al. 2020,
Ezhilarasan et al. 2012, Ezhilarasan and Karthikeyan 2016). Therefore, the elevation of hepatotoxic marker enzymes found in serum after DDS administration could be taken as an indication of the onset of hepatotoxicity. Furthermore, previous experimental studies have shown the toxic potential of DDS on biliary system, for instance, DDS impairs bile flow and induces cholestasis (Veggi et al. 2002, Veggi et al. 2005). Clinically, jaundice and hyperbilirubinemia are commonly reported adverse effects after DDS treatment (Itha et al. 2003, East and Blanton 2012, Devarbhavi et al. 2017). Therefore, the onset of jaundice could be the possible reason for the elevation of bilirubin observed after DDS administration. In agreement with the present study, our previous studies have reported the presence of gallic acid and berberin in EBLE (Rohit Singh and Ezhilarasan 2020, Thakur and Devaraj, 2020) and these phytoconstituents have hepatocellular membrane stabilizing properties (Ezhilarasan et al. 2014, Sharmin et al. 2018). The ethanolic banaba extract treatment was shown to reduce serum marker enzymes of liver toxicity in CCl₄-induced mice (Tiwary et al. 2017). Therefore, the hepatoprotective effects were observed in this study can be attributed to the hepatocellular membrane-stabilizing property of the phytoconstituents present in EBLE.

In this study, DDS administration induced lipid peroxidation and also concomitantly decreased the first-line antioxidants such as SOD, CAT and GSH in liver tissue of rats indicating the onset of oxidative stress. DDS undergoes bio-transformation in the liver mainly by N-acetylation and N-hydroxylation. The N-hydroxylation pathway is catalyzed by cytochrome P₄₅₀, flavin monooxygenase or prostaglandin H-synthetase enzymatic system (Coleman 1993, Zhu and Stiller 2001). In the N-hydroxylation pathway, DDS converted into DDS-hydroxylamine (DDS-NOH), which subsequently converted into nitrosodapsone. The DDS-NOH is a highly reactive intermediate, which induces oxidative stress in the liver (Irshaid et al. 1994). The N-hydroxylation of DDS also liberates superoxide radicals and induces lipid peroxidation thereby causing oxidative stress (Veggi et al. 2008). Therefore, bio-transformation of DDS in the liver and subsequent liberation of free radicals could be the possible cause for lipid peroxidation and decreased antioxidants levels. The hepatotoxic DDS-NOH metabolite of DDS was implicated for severe side effects, including hemolytic anemia, agranulocytosis, and methemoglobinemia including liver injury (Kirby et al. 1999, Coleman 2001, Chougule et al. 2008, Ezhilarasan 2021). Hence, it is reasonable to assume that DDS-NOH may be responsible for the hepatocellular regeneration and subsequent elevation of liver marker enzymes in the serum. Besides, in vitro and in vivo antioxidant and free radical scavenging potentials for banaba extract have been reported.

**Figure 7.** Mechanism of dapsone (DDS)-induced hepatotoxicity and hepatoprotective effects of ethanolic banaba leaves extract (EBLE) and silymarin (SIL). AST and ALT-aspartate and alanine transaminases, ALP-alkaline phosphatase, LDH-lactate dehydrogenase, GGT-gamma glutamyl transpeptidase, LPO-lipid peroxidation, SOD-superoxide dismutase, CAT-catalase, GSH-reduced glutathione, TNF-α-tumor necrosis factor-alpha, TGF-B-transforming growth factor-beta, NF-κB-nuclear factor kappa B, IκB-inhibitor of NF-κB.
against a variety of pathological conditions including hepatotoxicity (Unno et al. 1997, Saumya and Basha 2011, Tiwary et al. 2017, Sharmin et al. 2018, Mousa et al. 2019). In a previous study, EBLE alleviated oxidative stress and restored antioxidants such as CAT and GSH (Mousa et al. 2019). The presence of corosolic acid was attributed to the antioxidant activity of EBLE since this phytocompound has been reported to have free radical quenching properties (Ikeda et al. 1999).

The pleiotropic cytokine TNF-α is secreted by liver macrophages and it acts as a mediator of hepatic inflammation (Schwabe and Brenner 2006). TNF-α is also considered one of the potent activators of NF-κB (Luedde and Schwabe 2011). NF-κB transcription factors play a major role in DILI. Under normal physiological conditions, NF-κB dimers are inactive in the cytoplasm by their interaction with IkB proteins (Liu et al. 2017). During liver injury or stress condition, NF-κB is activated after IkB phosphorylation and subsequently degraded by the proteasome, enabling the liberated NF-κB dimers to enter the nucleus and initiate transcription of genes with κB binding sites (Luedde and Schwabe 2011). TGF-β signaling plays an important role in the initiation and progression of liver diseases through inflammation, fibrosis, cirrhosis and cancer (Fabregat et al. 2016). The role of TGF-β in the progression of hepatic fibrosis was well-established (Ezhilarasan 2018, Ezhilarasan et al. 2018). The enhanced TNF-α, NF-κB, and TGF-β expressions were often reported as a primary cause for various hepatotoxic drugs induced liver injury (Lee et al. 2019, Abdelkader et al. 2020, Hassan et al. 2020). In this study, DDS induced the expression of these pro-inflammatory marker genes that are responsible for liver inflammation, and fibrosis and this could be the possible cause for the onset of liver inflammation and subsequent hepatotoxicity. In a previous study, 250 mg/kg of EBLE treatment significantly decreased the TNF-α expression during oxidative stress in the lung (Mousa et al. 2019). However, in this study, 250 mg/kg EBLE treatment did not reduce DDS-induced TNF-α and TGF-β expressions. At 500 mg/kg, EBLE treatment decreased TNF-α and TGF-β expressions and this could be due to its inherent anti-inflammatory potential as suggested by previous reports (Priya et al. 2008, Gupta et al. 2017, Sharmin et al. 2018, Mousa et al. 2019). The possible hepatotoxic effect of DDS and hepatoprotective effects of EBLE and SIL is presented in Figure 7.

The histopathology of the liver also confirms that DDS can induce architectural distortion, fibrotic changes and fat accumulation in the liver tissue. In general, EBLE post-treatment especially at 500 mg/kg and SIL treatment significantly reduced DDS-induced degenerative changes in the liver. Our biochemical and molecular reports are well supported by histopathological studies.

In conclusion, the results of the present study suggest that twice daily, 30 days administration of DDS can induce significant liver inflammation and hepatotoxicity in rats. The liver was not recovered from DDS intoxication even after 30 days left without any treatment (recovery group) and the toxicity was similar to the DDS alone group, which was sacrificed at the end of day 30. EBLE post-treatment especially at a high dose significantly reduced DDS-induced hepatotoxicity and therefore, EBLE could be further studied to develop as a potential drug against DDS-induced liver toxicity. The standard hepatoprotective agent SIL post-treatment significantly improved DDS-induced liver abnormalities and hence, it can be used for DDS intoxication.

Disclosure statement
The authors declare that they have no conflict of interest.

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