Glycosides Based Standardized Fenugreek Seed Extract Ameliorates Bleomycin-induced Liver Fibrosis in Rats Via Modulation of Endogenous Enzymes

Amit D. Kandhare, Subhash Laxmanrao Bodhankar, Vishwaraman Mohan¹, Prasad A. Thakurdesai¹

Background: Liver fibrosis a complex process of excess collagen deposition resulted in disturbance of hepatic cell function. Glycosides based standardized fenugreek seed extract (SFSE-G) has potent anti-inflammatory, antioxidant, and anti-fibrotic properties. Objective: The aim of this study is to evaluate the hepatoprotective potential of SFSE-G against bleomycin (BLM)-induced liver fibrosis in laboratory animals. Materials and Methods: Sprague-Dawley rats (180–220 g) were assigned to various groups, namely, normal, sham, BLM control, SFSE-G (5, 10, 20, and 40 mg/kg, p.o.), methylprednisolone (10 mg/kg, p.o.), and sildenafil (25 mg/kg, p.o.). Liver fibrosis was induced in various groups (except normal and sham) by single intratracheal BLM (6 IU/kg) injection. Various biochemical, molecular (reverse transcription polymerase chain reaction) and histological parameters were evaluated. Results: Intratracheal BLM administration caused significant induction (P < 0.001) of hepatotoxicity and liver fibrosis reflected by elevated levels of serum aspartate transaminase (AST), alanine transaminase (ALT), total as well as direct bilirubin, and gamma-glutamyl transferase (GGT). Administration of SFSE-G (20 and 40 mg/kg, p.o.) significantly reduced (P < 0.001) levels of AST, ALT, and GGT and significantly increased (P < 0.001) the level of serum albumin. BLM-induced elevated liver oxidative stress and decreased total antioxidant capacity was significantly restored (P < 0.001) by SFSE-G (20 and 40 mg/kg) treatment. It also significantly inhibited BLM-induced alteration in liver Farnesoid X receptor (FXR) mRNA expression. SFSE-G treatment reduced histopathological alteration induced by BLM in liver. Conclusion: SFSE-G exerts its hepatoprotective potential via inhibition of oxido-nitrosative stress and modulation of FXR mRNA expression thus ameliorates BLM-induced liver fibrosis.

Keywords: Farnesoid X receptor, hepatotoxicity, nitric oxide, oxidative stress, total antioxidant capacity

INTRODUCTION
Liver fibrosis a complicated process of new fiber formation with excess collagen deposition resulted in disturbance of hepatic cell function.[1] An array of chronic hepatic diseases leads to liver fibrosis. It has been well documented that various stimuli such as (congenital, metabolic, inflammatory, parasitic, vascular, toxins, or drugs such as bleomycin (BLM) responsible for induction of highly orchestrated cellular and molecular events in hepatic cells caused hepatic fibrosis.[2-4]

It has been reported that activation of alteration pathways in peroxisomes and the endoplasmic reticulum in...
response to an inundated capacity of mitochondrial oxidation leads to excessive production of a metabolite. These metabolite serves as a major source of reactive oxygen species (ROS) that caused mitochondrial DNA damage, cellular degradation, elevated lipid peroxidation thus the release of pro-inflammatory cytokines (tumor necrosis factor-alpha [TNF-α], interleukin (IL)-1 β, and IL-6)]. These events cause increased transforming growth factor-β1 (TGF-β1) expression, as well as activation of hepatic stellate cells that eventually leads to the synthesis of scar-forming collagen, and therefore, liver fibrosis is developed. The researcher also proved that TGF-β mediated transduction and regulation of Smads proteins played a vital role in liver fibrosis.[10]

BLM is an antibiotic produced by the bacterium, *Streptomyces verticillus* which is used as chemotherapeutic agents in the treatment of lymphomas, testicular carcinomas, and squamous cell carcinomas.[11] Various clinical trials have reported the incidence of serum enzyme elevations in 10–40% of patients who are monitoring chemotherapy with BLM.[12-16] BLM has the ability to DNA damage via formation of Fe (II) BLM complex that resulted in the generation of ROS, including superoxide and hydroxyl radicals. This ROS along with lipid peroxidation caused inhibition of collagen degradation thus collagen production is stimulated leads to liver fibrosis.[17] Over several decades, animal models have been used to develop newer therapeutic moieties with anti-fibrotic potential.[18] Current treatments regimen for liver fibrosis included the use of corticosteroids, penicillamine, or colchicine which aimed at reversing the fibrosis, but they lack in efficacy.

Fenugreek, *Trigonella foenum-graecum* Linn. (Fabaceae) is a widely growing herb in India, Egypt, and Middle Eastern countries. Fenugreek seeds contain an array of phytoconstituents such as carbohydrate (mucilaginous fiber, galactomannan), proteins, alkaloids (trigonelline and choline), flavonoid glycosides (vicenin 1), furostanol glycosides (trigoneoside Ib), free amino acids (4-hydroxisoleucine, arginine, lysine, histidine), saponins (diosgenin, and yuccagenin), vitamins and mucilage.[19] Its potential to treat various diseases such as diabetes, hyperlipidemia, inflammation, and ulcers has been well documented in Ayurveda.[20] Furostanol and flavonoid glycosides present in fenugreek seeds are known to be responsible for its anti-inflammatory, antioxidant, anabolic, androgenic, and metal chelating properties.[21-27] Previous acute oral toxicity (AOT) and subacute toxicity studies carried out in our laboratory have shown the toxicological profile of glycosides based standardized fenugreek seed extract (SFSE-G) in laboratory animals with a lethal dose 50 more than 4350 mg/kg and no-observed-adverse-effect levels of 250 mg/kg.[28] Furthermore, a recent study revealed the anti-fibrotic efficacy of SFSE-G via its anti-inflammatory and antioxidant potential against BLM-induced pulmonary fibrosis.[17] However, its efficacy against BLM-induced liver fibrosis has not been evaluated yet. Hence, the aim of the present investigation was to assess the efficacy of SFSE-G against BLM-induced liver fibrosis in the laboratory rats by determining various biochemical, molecular, and histological changes.

**Materials and Methods**

**Animals**

Adult male Sprague-Dawley rats (180–220 g) were obtained from the National Institute of Biosciences, Pune (India). They were housed at 24 ± 1°C, with a relative humidity of 45–55% and 12:12 h dark/light cycle. The animals had free access to standard pellet chow (Pranav Agro-industries Ltd., Sangli, India) and filtered water *ad libitum* throughout the experimental protocol. All experiments were carried out between 09:00 and 17:00 h. The experimental protocol Committee for the Purpose of Control and Supervision of Experimentation on Animals (CPCSEA/08/2014) was approved by the Institutional Animal Ethics Committee of Poona College of Pharmacy, Pune and performed in accordance with the guidelines of CPCSEA, Government of India.

**Preparation, isolation, and characterization of glycosides based standardized fenugreek seed extract**

SFSE-G was prepared and characterized from the hydroalcoholic extract of fenugreek seeds as previously reported.[28] Two peaks namely Peak A (trigoneoside Ib) (76%), and Peak B (vicenin-1) (15%) were observed in high-performance liquid chromatography at RT = 2.2 min and 3.2 min, respectively. The corresponding compounds were isolated and characterized using thin layer chromatography, 1H- and 13C-NMR analysis.

**Chemicals, and kits**

BLM was purchased from Biochem Pharmaceutical Industries Limited, India. All other chemicals were purchased from S.D. Fine Chemicals, Mumbai, India, and LobaChem Pvt. Ltd., Mumbai, India. Total RNA extraction kit and one-step reverse transcription polymerase chain reaction (RT-PCR) kit was purchased from MP Biomedicals India Private Limited, India.

**Induction of liver fibrosis and drug treatment**

After an overnight fasting, fibrosis was induced by intratracheal administration of BLM hydrochloride (6 IU/kg in 0.9% NaCl) under anesthesia with 60 mg/kg ketamine hydrochloride and 5 mg/kg xylazine.[29,30] The
rats in the normal and sham control group were subjected to the same procedure, but BLM was substituted by saline. The rats were randomly divided into following groups (n = 12) as follows:

- **Group I**: Normal group: Rats did not undergo any surgery and did not receive BLM. They were administered a single daily dose of distilled water (10 mg/kg), p.o. for 28 days.
- **Group II**: Sham control group: Rats were undergone surgery and received saline. They were administered a single daily dose of distilled water (10 mg/kg), p.o. for 28 days.
- **Group III**: BLM control group: Rats were undergone surgery and received the intratracheal administration of BLM. They were administered a single daily dose of distilled water (10 mg/kg), p.o. for 28 days.
- **Group IV**: Methylprednisolone (10 mg/kg) treated group: (MP [10]): Rats were undergone surgery and receive the intratracheal administration of BLM. They were administered a single daily dose of methylprednisolone (10 mg/kg), p.o. for 28 days.
- **Group V**: Sildenafil (25 mg/kg) treated group: (Sild [25]): Rats were undergone surgery and received the intratracheal administration of BLM. They were administered a single daily dose of Sildenafil (25 mg/kg), p.o. for 28 days.
- **Group VI**: SFSE-G (5 mg/kg) treated group: [SFSE-G (5)]: Rats were undergone surgery and receive the intratracheal administration of BLM. They were administered a single daily dose of SFSE-G (5 mg/kg), p.o. for 28 days.
- **Group VII**: SFSE-G (10 mg/kg) treated group: (SFSE-G [10]): Rats were undergone surgery and receive the intratracheal administration of BLM. They were administered a single daily dose of SFSE-G (10 mg/kg), p.o. for 28 days.
- **Group VIII**: SFSE-G (20 mg/kg) treated group: (SFSE-G [20]): Rats were undergone surgery and receive the intratracheal administration of BLM. They were administered a single daily dose of SFSE-G (20 mg/kg), p.o. for 28 days.
- **Group IX**: SFSE-G (40 mg/kg) treated group: (SFSE-G [40]): Rats were undergone surgery and receive the intratracheal administration of BLM. They were administered a single daily dose of SFSE-G (40 mg/kg), p.o. for 28 days.

The doses of SFSE-G were selected on the basis of reported sub-AOT study (repeated dose 28 days), whereas a dose of methylprednisolone (10 mg/kg), and sildenafil (25 mg/kg) were based on previously reported methods. Vehicle or SFSE-G or methylprednisolone or sildenafil were given after the administration of BLM and the treatment was continued for 28 consecutive days.

**Serum biochemistry**

On day 28, the blood was withdrawn by a retro-orbital puncture for determination of serum parameters. The serum was separated by centrifugation using an Eppendorf cryocentrifuge (model no. 5810, Eppendorf, Hamburg, Germany), maintained at 4°C and ran at a speed of 7000 rpm for 15 min. The levels of serum albumin, direct bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin, and gamma-glutamyl transferase (GGT) were measured by a spectrophotometer (UV – visible spectrophotometer, Jasco V-530, Tokyo, Japan) using commercially available reagent kits according to procedure provided by manufacturer (Accurex Biomedical Pvt. Ltd., Mumbai, India).

**Liver biochemical analysis**

On day 28 after blood collection, animal were sacrificed by cervical dislocation and liver was collected for determination of various biochemical measurements, namely, total protein, superoxide dismutase (SOD), glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO), and total antioxidant capacity (T-AOC) according to earlier reported methods (Ghule et al., 2015; Gosavi et al., 2012; Goswami et al., 2015; Kandhare et al., 2012a, 2013a, 2014a). Another part of tissue samples were stored at –70°C for RT-PCR analysis of various markers. One liver tissue from each group was processed for histopathological examination.

**Reverse transcriptase polymerase chain reaction**

The levels of mRNA were analyzed in liver tissue using a RT-PCR approach as described previously (Kandhare et al., 2014, 2015a; Visnagiri et al., 2015). Briefly, single-stranded cDNA was synthesized from 5 μg of total cellular RNA using reverse transcriptase (MP Biomedicals India Private Limited, India) as described previously. The primer sequence for Farnesoid X receptor (FXR) (Forward: 5’-GACCCGAAAGACCCATTGCT-3’, Reverse: 5’-TCTCCACTGCCTCTTATCT-3’) and β-actin (Forward: 5’-GTCACCCACAGTCGCCCCATC-3’, Reverse: 5’-ACAGAGTACTTGCGCTCAGGAG-3’) was provided by 3B BlackBio Biotech Private Limited, Spain. Amplification of β-actin served as a control for sample loading and integrity. PCR products were detected by electrophoresis on a 1.5% agarose gel containing ethidium bromide. The size of amplicons was confirmed using a 100-bp ladder as a standard size marker. The amplicons were visualized, and images were captured using a gel documentation system (Alpha Innotech Inc., San Leandro, CA, USA). Gene expression was assessed by generating densitometry data for band intensities in different sets of experiments, by analyzing...
Histological examination
Liver tissues were stored in 10% formalin for 24 h. The specimen was dehydrated and placed in xylene for 1 h (3 times) and later in ethyl alcohol (70, 90, and 100%) for 2 h. The infiltration and impregnation were carried out by treating with paraffin wax twice, each time for 1 h. Tissue specimens were cut into sections of 3–5 mm thickness and were stained with hematoxylin and eosin. The specimen was mounted on the slide by use of distrene phthalate xylene as a mounting medium. Sections were examined under a light microscope for the inspection of the histopathology features of specimen and infiltration of cells. The various changes in histological features were graded as Grade 0 (not present or very slight), Grade 1 (mild), Grade 2 (moderate), and Grade 3 (severe) as described earlier.[35]

Statistical analysis
Data are expressed as mean ± standard error mean. Data analysis was performed using GraphPad Prism 5.0 software (GraphPad, San Diego, CA, USA). Data were analyzed using one-way repeated analysis of variance (ANOVA), and Dunnett’s test was applied for post hoc analysis. A value of $P < 0.05$ was considered to be statistically significant.

**Results**

**Effect of glycosides based standardized fenugreek seed extract on bleomycin-induced alteration in body weight and liver weight to body weight ratio (liver index) of rats**

There was significant decreased ($P < 0.001$) in the body weight and significant increased ($P < 0.001$) in relative liver weight of BLM control group as compared to normal and sham control group. Treatment with SFSE-G (10, 20 and 40 mg/kg) significantly inhibited ($P < 0.001$) BLM-induced decrease in body weight as compared to BLM control group. When compared with BLM control group, methylprednisolone (10 mg/kg) as well as sildenafil (25 mg/kg) showed significant increased ($P < 0.001$) in the body weight [Table 1].

When compared with BLM control group, relative liver weight was significantly and dose-dependently decreased ($P < 0.01$ and $P < 0.001$) in the SFSE-G (20 and 40 mg/kg) treated group. Methylprednisolone (10 mg/kg) as well as sildenafil (25 mg/kg) treatment also showed the gel images in the ImageJ program (Version 1.33, Wayne Rasband, National Institutes of Health, Bethesda, MD, USA) semi-quantitatively. The band intensities were compared with constitutively expressed β-actin. The intensity of mRNAs was standardized against that of the β-actin mRNA from each sample, and the results were expressed as PCR-product/β-actin mRNA ratio.

### Table 1: Effect of glycosides based standardized fenugreek seed extract on bleomycin-induced alteration in body weight, relative liver weight, serum aspartate transaminase, serum alanine transaminase, serum bilirubin, serum gamma-glutamyl transferase and serum albumin of rats ($n=4$)

| Parameters               | Normal          | Sham            | BLM control     | SFSE-G (10) | SFSE-G (20) | SFSE-G (40) |
|--------------------------|-----------------|-----------------|-----------------|-------------|-------------|-------------|
| Body weight (g)          | 26.8±0.5-6.0    | 26.1±0.4-6.0    | 26.3±0.2-6.0    | 26.4±0.5-6.0| 26.5±0.5-6.0| 26.6±0.5-6.0|
| Relative liver weight    | 0.22±0.01-0.04 | 0.20±0.02-0.01 | 0.23±0.02-0.01 | 0.24±0.02-0.01| 0.25±0.02-0.01| 0.26±0.02-0.01|
| AST (IU/I)               | 139±1.17        | 140±1.16        | 159±2.15        | 173±2.13    | 175±2.13    | 175±2.13    |
| ALT (IU/I)               | 32±6.58         | 32÷6.69         | 32±6.58         | 32±6.58    | 32±6.58    | 32±6.58    |
| Direct bilirubin (mg%)   | 0.50±0.01       | 0.49±0.01       | 0.51±0.01       | 0.51±0.01  | 0.51±0.01  | 0.51±0.01  |
| Total bilirubin (mg%)    | 0.32±0.01       | 0.32±0.01       | 0.32±0.01       | 0.32±0.01  | 0.32±0.01  | 0.32±0.01  |
| Albumin (g/dl)           | 3.40±0.56       | 3.32±0.58       | 3.32±0.58       | 3.32±0.58  | 3.32±0.58  | 3.32±0.58  |

Data are expressed as mean±SEM and analyzed by one-way ANOVA followed by Dunnett’s posttests. $^*$P<0.01, $^**$P<0.001 as compared with normal group; $^*$P<0.01, $^**$P<0.001 as compared with BLM control group. BLM: Bleomycin; MP: Methylprednisolone, Sild: Sildenafil, SF: SFSE‑G; Standardized fenugreek seed extract‑glycoside base, ALT: Alanine transaminase, AST: Aspartate transaminase, GGT: Gamma-glutamyl transferase.
significant inhibition \((P < 0.001)\) in the BLM-induced increased liver weight to body weight ratio as compared to BLM control group [Table 1].

**Effect of glycosides based standardized fenugreek seed extract on bleomycin-induced alteration in liver function test of rats**

The serum AST, ALT, direct bilirubin, total bilirubin, and GGT levels were increased significantly \((P < 0.01)\) in the BLM control group as compared to the normal and sham group. Administration of SFSE-G (20 and 40 mg/kg) showed significantly decreased \((P < 0.05)\) in the serum AST, ALT and GGT level as compared to BLM control group. Administration of methylprednisolone (10 mg/kg) significantly decreased \((P < 0.05)\) serum ALT and total bilirubin levels as compared to BLM control group. When compared with BLM control group, sildenafil (25 mg/kg) showed significantly decreased \((P < 0.05)\) in serum total bilirubin level [Table 1].

**Effect of glycosides based standardized fenugreek seed extract on bleomycin-induced alteration in serum albumin level of rats**

The serum albumin level was decreased significantly \((P < 0.001)\) in the BLM control group as compared to the normal and sham group. The BLM-induced decreased albumin level was significantly increased \((P < 0.01)\) by SFSE-G (20 and 40 mg/kg) treatment as compared to BLM control rats. Administration of methylprednisolone (10 mg/kg), as well as sildenafil (25 mg/kg) significantly, increased \((P < 0.05)\) serum albumin level as compared to BLM control group [Table 1].

**Effect of glycosides based standardized fenugreek seed extract on bleomycin-induced alteration in of superoxide dismutase, glutathione, malondialdehyde and nitric oxide in liver rats**

BLM control group showed significantly decreased \((P < 0.001 \text{ and } P < 0.01)\) in the liver SOD and GSH level as compared to the normal and sham group. SFSE-G (20 and 40 mg/kg) showed significantly and dose-dependent increase \((P < 0.05 \text{ and } P < 0.01)\) in the liver SOD level as compared to BLM control rats. The decreased liver GSH level was significantly increased \((P < 0.01 \text{ and } P < 0.05)\) by SFSE-G (20 and 40 mg/kg) treatment as compared with BLM control group. When compared with BLM control group, liver GSH level was significantly increased \((P < 0.05)\) in the methylprednisolone (10 mg/kg) as well as sildenafil (25 mg/kg) treatment group as compared to BLM control group [Table 2].

The liver MDA and NO level were increased significantly \((P < 0.001 \text{ and } P < 0.01)\) in the BLM control group as compared to the normal and sham group. This increased liver MDA was significantly and dose-dependently \((P < 0.05, P < 0.05 \text{ and } P < 0.01)\) decreased by 28 days treatment with SFSE-G (10, 20 and 40 mg/kg) as compared to BLM control rats. When compared with BLM control rats, liver MDA was significantly decreased \((P < 0.05)\) in methylprednisolone (10 mg/kg) as well as sildenafil (25 mg/kg) treatment group. However, there was no significant alteration in the liver NO level of SFSE-G (5, 10, 20 and 40 mg/kg), methylprednisolone (10 mg/kg) as well as sildenafil (25 mg/kg) treated rats as compared to BLM control rats [Table 2].

**Effect of glycosides based standardized fenugreek seed extract on bleomycin-induced alteration in total antioxidant capacity in liver of rats**

The liver T-AOC of BLM control rats was significantly decreased \((P < 0.001)\) as compared to the normal and sham group. Treatment with SFSE-G (10, 20 and 40 mg/kg) significantly and dose-dependently increased \((P < 0.05, P < 0.01 \text{ and } P < 0.01, \text{ respectively})\) T-AOC of liver as compared to BLM control rats. Methylprednisolone (10 mg/kg) and sildenafil (25 mg/kg) significantly increased \((P < 0.05)\) liver T-AOC as compared to BLM control rats [Figure 1].

**Table 2: Effect of glycosides based standardized fenugreek seed extract on bleomycin-induced alteration in the level of superoxide dismutase, glutathione, malondialdehyde, and nitric oxide in liver of rats (n=4)**

| Parameters | Normal | Sham | BLM control | MP (10) | Sild (25) | SFSE-G (5) | SFSE-G (10) | SFSE-G (20) | SFSE-G (40) |
|------------|--------|------|--------------|---------|----------|------------|------------|------------|------------|
| SOD (U/mg of protein) | 3.95±0.29 | 3.95±0.44 | 0.97±0.16\(^{**}P<0.01\) | 1.24±0.35 | 1.54±0.47 | 1.38±0.19 | 1.89±0.35 | 2.70±0.49* | 3.15±0.28** |
| GSH (µg/mg of protein) | 2.40±0.06 | 2.41±0.08 | 1.78±0.10\(^{**}P<0.01\) | 2.24±0.03* | 2.27±0.12* | 2.08±0.03 | 2.06±0.10 | 2.31±0.07** | 2.20±0.18* |
| MDA (nM/mg of protein) | 0.53±0.05 | 0.44±0.09 | 1.49±0.18\(^{**}P<0.01\) | 0.85±0.07* | 0.80±0.14* | 1.27±0.20 | 0.83±0.21* | 0.77±0.13* | 0.72±0.10** |

NO (µg/mL) | 196.8±15.55 | 203.8±18.00 | 340.6±7.65\(^{**}P<0.01\) | 298.1±33.77 | 261.4±39.80 | 262.3±25.49 | 279.2±15.36 | 265.2±6.96 | 261.6±22.27 |

Data are expressed as mean±SEM and analyzed by one-way ANOVA followed by Dunnett’s posttests. \(^*P<0.01, ^{**}P<0.001\) as compared with normal group, \(^{**}P<0.01, ^{**}P<0.001\) as compared with sham group, and \(^*P<0.05, ^{**}P<0.01\) as compared with bleomycin control group on respective days. BLM: Bleomycin, MP: Methylprednisolone, Sild: Sildenafil, SFSE-G: Standardized fenugreek seed extract-glycoside base, SOD: Superoxide dismutase, GSH: Glutathione, MDA: Malondialdehyde, NO: Nitric oxide, ANOVA: Analysis of variance, SEM: Standard error of mean.
Effect of glycosides based standardized fenugreek seed extract on bleomycin-induced alteration in liver Farnesoid X receptor mRNA expression in rats

When compared with normal and sham group, the liver FXR m-RNA expression was up-regulated significantly \((P < 0.001)\) in BLM control group. Administration of SFSE-G (20 and 40 mg/kg) significantly down-regulates \((P < 0.01)\) liver FXR m-RNA expression as compared to BLM control rats. Methylprednisolone (10 mg/kg), as well as sildenafil (25 mg/kg), did not produce any significant down-regulation in the liver FXR m-RNA expression as compared to BLM control rats [Figure 2].

Effect of glycosides based standardized fenugreek seed extract on bleomycin-induced alteration in liver histology of rats

Microscopic examination of liver tissues from normal and the sham group showed the normal histological architecture; there was no infiltration of inflammatory cells, congestion or necrosis [Figure 3a and b]. Intratracheal administration of BLM resulted in mild perivascular fibrous tissue proliferation, hepatocellular necrosis (black arrow, Grade 3), inflammatory infiltration (red arrow, Grade 2) with reduced sinusoidal spaces [Figure 3c]. Liver tissue from methylprednisolone (10 mg/kg) as well as sildenafil (25 mg/kg) treated rats showed the presence of minimal degenerative changes in hepatocytes (black arrow, Grade 2) along with infiltration of inflammatory cells (red arrow, Grade 2) [Figure 3d and e]. Liver tissue from SFSE-G (20 and 40 mg/kg) treated group showed a reduction in BLM-induced hepatic injury [Figure 3h and i]. However, SFSE-G (5 and 10 mg/kg) failed to show any protective effects against the BLM-induced liver damage [Figure 3f, g and Table 3].

**DISCUSSION**

Chronic hepatic damage along with the accumulation of extracellular matrix proteins is a characteristic feature of liver fibrosis. The release of cytokines (TNF-α and IL’s), as well as growth factors (TGF-β1), appears to be a key mediator of hepatic fibrogenesis.\(^{[36]}\) The BLM-induced release of these pro-inflammatory cytokines and growth factors via elevated ROS through the formation of redox complexation of DNA/Fe2+/BLM complex thus resulting in initiation of fibrosis.\(^{[37]}\) In the present investigation, intratracheal administration of BLM caused significantly decreased body weight and increased liver injury index reflecting the compromised health status of BLM-induced animals as compared to normal animals. However, treatment with SFSE-G significantly attenuated BLM-induced alteration in body weight and liver injury index.

Liver enzymes such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are found in the cells and its elevated level in serum serve as a hallmark
Kandhare, et al.: Fenugreek seed extract ameliorates BLM-induced liver fibrosis

The elevated levels of these enzymes can be measured in serum when hepatocytes undergo necrosis or membrane damage. As ALT had a significant role in the conversion of alanine to pyruvate and glutamate which represent almost 90% of total enzymes present in the body, thus ALT serves as the better index of liver injury than AST. The elevated levels of serum ALT and AST thus improved its hepatoprotective potential.

It has been reported that release of alkaline phosphatase (ALP) occurred from bile canaliculi cell during hepatotoxicity that resulted in increased biliary pressure. Moreover, serum total bilirubin, as well as direct bilirubin, serves as a gold standard for hepatic diseases. In liver parenchyma cells, glucuronidation occurred where bilirubin formed conjugates with glucuronic acid in the presence of catalytic converting enzyme glucuronyltransferase. The resultant

Table 3: Effect of glycosides based standardized fenugreek seed extract on bleomycin-induced alteration in liver histology of rats

| Treatment       | Inflammatory cell infiltration | Peri-vascular fibrous tissue proliferation | Vascular congestion | Hepatocellular necrosis |
|-----------------|--------------------------------|-------------------------------------------|---------------------|------------------------|
| Normal          | 0                              | 0                                         | 0                   | 0                      |
| Sham            | 0                              | 0                                         | 0                   | 0                      |
| BLM control     | ++                             | ++                                        | ++                  | +++                    |
| MP (10)         | ++                             | ++                                        | +                   | ++                     |
| Sild (25)       | ++                             | +                                         | +                   | +                      |
| SFSE-G (5)      | ++                             | ++                                        | +                   | ++                     |
| SFSE-G (10)     | ++                             | +                                         | +                   | +                      |
| SFSE-G (20)     | ++                             | +                                         | +                   | +                      |
| SFSE-G (40)     | 0                              | ++                                        | +                   | +                      |

0: No abnormality detected, +: Damage/active changes up to <25%, ++: Damage/active changes up to <50%, +++: Damage/active changes up to <75%, BLM: Bleomycin, MP: Methylprednisolone, Sild: Sildenafil, SFSE-G: Standardized fenugreek seed extract-glycoside base

Figure 3: Effect of glycosides based standardized fenugreek seed extract on bleomycin-induced alteration in liver histology of rats. Photomicrograph of sections of liver of normal (a), sham control (b), bleomycin control (c), methylprednisolone (10 mg/kg) treated (d), sildenafil (40 mg/kg) treated (e), glycosides based standardized fenugreek seed extract (5 mg/kg) treated (f), glycosides based standardized fenugreek seed extract (10 mg/kg) treated (g), glycosides based standardized fenugreek seed extract (20 mg/kg) treated (h) and glycosides based standardized fenugreek seed extract (40 mg/kg) treated (i) rats (Lung H and E, ×40)
product is excreted into the bile. Thus, an elevated level of total bilirubin and direct bilirubin in serum reflected damage to hepatic parenchymal cells.\(^{[40]}\)

However, administration of SFSE-G failed to produce any significant decreased the level of total bilirubin and direct bilirubin.

Insult to some susceptible amino acids of proteins by ROS played an important role in the induction of hepatotoxicity. GGT has an important role in synthesis and degradation of GSH in gamma-glutamyl cycle. Elevated ROS caused activation of GGT to induced degradation of GSH thus resulted in diminished antioxidant potential.\(^{[35,41-43]}\) Elevated serum GGT level is a representative of induction of liver disorders. An elevated level of β and γ globulins caused the production of IgG and IgM that resulted in decreased serum albumin level and it is most common in liver diseases.\(^{[44]}\) In the present investigation, BLM administration resulted in elevated GGT level followed by hypoalbuminemia and treatment with SFSE-G significantly reduced GGT level thus increased serum albumin level.

In the pathogenesis of liver fibrosis, elevated ROS played a vital role via increased oxidative stress thus decreasing AOC. SOD and GSH are endogenous antioxidant enzymes played an essential role in detoxification of toxic oxygen radicals (Kandhare et al., 2011a, 2013a; Kumar et al., 2014). T-AOC is another important measure of determination of tissue oxidative stress level.\(^{[17]}\) There was a subsequent increase in lipid peroxidation (MDA) with increased production of free radicals (Kandhare et al., 2012c; Raygude et al., 2012a, 2012b; Sarkar et al., 2015). BLM-administration caused a marked reduction in tissue antioxidant level reflected by decreased SOD and GSH level along with T-AOC of the liver from BLM control rats. Whereas, administration of SFSE-G showed elevated levels of SOD and GSH thus increased T-AOC of the liver which may be by virtue of its antioxidant property. Findings of our previous investigation support these finding where treatment with SFSE-G improved anti-oxidant status in BLM insulted animals.\(^{[17]}\)

It has been reported that elevated NO production played a vital role in the induction of various inflammatory diseases including liver fibrosis.\(^{[45]}\) Tissue injury occurred when NO react with superoxide and form peroxynitrites (Honmore et al., 2015; Kandhare et al., 2012a; Visnagri et al., 2012, 2014). Thus, it is necessary to inhibit NO production that ameliorates tissue injury (Kandhare et al., 2012, 2013, 2015c). In the present investigation, BLM caused significant induction of liver fibrosis via increased in the production of NO. However, administration of SFSE-G failed to ameliorate BLM-induced NO production.

FXR is a member of the nuclear receptor superfamily of ligand-activated transcription factors plays a pivotal role in the synthesis and transport of bile acids.\(^{[46,47]}\) Chenodeoxycholic acid is active ligand formed when FXR bound and activated by bile acid resulted in an alteration in the concentration of bile acids within hepatocytes via forming interacting with the 9-cis-retinoic acid receptor.\(^{[48,49]}\) Thus, alteration in FXR level resulted in inappropriate excretion of endogenous toxic compounds, such as bile acids and bilirubin.\(^{[50]}\) Moreover, various in vitro and in vivo studies of liver fibrosis showed that FXR serves as an important regulator of cytokines and collagen-I synthesis.\(^{[51]}\) SFSE-G administration significantly restored FXR mRNA expression thus reduced BLM-induced liver fibrosis.

**CONCLUSION**

SFSE-G exerts its hepatoprotective potential via inhibition of oxido-nitrosative stress and modulation of FXR mRNA expression to ameliorate BLM-induced liver fibrosis.

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**Conflicts of interest**

There are no conflicts of interest.

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Kandhare, et al.: Fenugreek seed extract ameliorates BLM-induced liver fibrosis

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