RESEARCH PAPER

Swertiamarin Ameliorates NAFLD-Induced Liver Fibrosis via Inhibition of the ASK1/MAPK Signaling Pathway in a Rat Model

Shikha Goswami[1*], Ramesh K Goyal[2]
[1] Department of Pharmacology, School of Pharmaceutical Sciences, DPSRU-110017
[2] School of ITM SLS Baroda University & ITM Medical Hospital, Baroda, India
Corresponding Author*:
Shikha Goswami
Department of Pharmacology, School of Pharmaceutical science, Delhi Pharmaceutical Science and Research University, New Delhi-110017
Email: shikhagoswami14@gmail.com

Abstract

Background: Nonalcoholic fatty liver disease (NAFLD) and liver fibrosis are major health concerns globally, especially in patients with type 2 diabetes mellitus (T2DM). Dysregulated insulin signaling and chronic inflammation are central to disease progression.

Objective: This study evaluates the hepatoprotective and anti-fibrotic efficacy of Swertiamarin (Sw), a secoiridoid glycoside from *Enicostemma littorale*, in a rat model of diet- and toxin-induced liver injury.

Methods: Hepatic injury and fibrosis were induced in Wistar rats using a high-fat diet (HFD) in combination with dimethylnitrosamine (DMN). The protective effects of Sw (100 mg/kg) were compared with the ASK1 inhibitor Selonsertib (50 mg/kg). Key biochemical, histological, and molecular endpoints were evaluated.

Results: Swertiamarin (100 mg/kg) significantly attenuated hepatic steatosis and fibrosis, reduced insulin resistance, and lowered pro-inflammatory cytokine levels. Mechanistically, Sw downregulated the ASK1/MAPK signaling pathway and suppressed the expression of α-SMA and collagen I.

Conclusion: Swertiamarin demonstrates promising anti-fibrotic and anti-inflammatory properties via ASK1/MAPK inhibition, offering a natural alternative to current pharmacological approaches for NAFLD-associated fibrosis.

Keywords: NAFLD, liver fibrosis, Type 2 diabetes mellitus (T2DM), ASK1, Swertiamarin
How to cite this article: Shikha Goswami, RameK Goyal. Swertiamarin Ameliorates NAFLD-Induced Liver Fibrosis via Inhibition of the ASK1/MAPK Signaling Pathway in a Rat Model. Int J Drug Deliv Technol. 2026;16(79s):722-730. DOI: 10.25258/ijddt.16.79s.136

.1. Introduction

Liver fibrosis is a prevalent form of chronic liver disease mostly caused by nonalcoholic steatohepatitis (NASH). NAFLD can lead to hepatic inflammation and advance to NASH, fibrosis, cirrhosis, or hepatocellular carcinoma (HCC) [1]. Approximately 55% of individuals worldwide diagnosed with T2DM are also affected by NAFLD [2]. In addition, almost 37% of individuals with T2DM already exhibit advanced NASH, characterized by the presence of steatosis with hepatocyte ballooning degeneration and possibly fibrosis [3]. There is a strong interconnection between the disorders, and when diagnoses are made in both directions, as expected, there are similar associations. Over 50% of individuals diagnosed with NASH also receive a diagnosis of T2DM. In addition, individuals who do not have diabetes may have either prediabetes or insulin resistance syndrome [4, 5]. Furthermore, it has been documented that a significant proportion of individuals with T2DM also suffer from NAFLD or NASH, which may potentially exacerbate their risk of developing cardiovascular and metabolic disorders[6].

The development of NAFLD and liver fibrosis is intricate, and the molecular mechanisms behind this process are starting to be understood. Over the past decade, many clinical studies have examined the safety and efficacy of NAFLD/NASH medications. These medications include diet tablets, antioxidants, insulin sensitizers, lipid-lowering agents, anti-inflammatory cytokines, cytoprotectants, and intestinal probiotics [6]. Nevertheless, the US Food and Drug Administration (FDA) has not yet granted approval for pharmacotherapy in the treatment of

NAFLD. Insulin resistance is a significant factor that causes the liver to accumulate excessive fat and is crucial in the development and advancement of steatohepatitis and fibrosis [7]. The presence of elevated oxidative stress and impaired antioxidant defense mechanisms has been widely investigated throughout the various phases of NAFLD. These factors are thought to play a role in liver damage and the progression to NASH [8].

This study used a well-established experimental approach to induce NAFLD/NASH in Wistar rats. The approach entailed delivering an HFD to induce steatosis. We also used dimethylnitrosamine (DMN), a chemical known to cause liver cancer and increase hepatic fibrosis [9, 10], in conjunction with the HFD. ASK1 has recently been recognised as a key element in the development of a number of diseases, including liver disorders. This has sparked greater interest in targeting ASK1 for therapeutic purposes. The extended MAPK kinase (MAP3 K) group activates downstream MAPKs such JNKs and p38 MAPKs via ASK1. It controls stress responses like apoptosis, cell death, differentiation, and inflammatory cytokines [11]. ASK1 activates JNK to worsen acetaminophen-induced liver damage. In the NASH model, ASK1 causes liver fibrosis and cell death. Furthermore, overexpressing ASK1 promotes the activation and proliferation of hematopoietic stem cells (HSCs) by increasing p38 and JNK phosphorylation [12]. Given ASK1's proven function in the progression of liver fibrosis, addressing it through pharmaceutical development is critical for effectively treating advanced liver diseases [13]. Selonsertib (Selon), a new small molecule inhibitor of ASK1, has antifibrotic and anti-inflammatory properties. It is currently undergoing clinical trials for the treatment of NAFLD/NASH and liver fibrosis [11].

The most efficacious pharmacotherapies for NAFLD/NASH and liver fibrosis not only prioritise enhancing lipid metabolism, but also emphasise the augmentation of hepatic anti-oxidative and anti-inflammatory actions. Currently, the majority of serious illnesses such as cancer, AIDS, kidney damage, cardiovascular diseases, and others can be effectively treated with medicinal herbs [14]. Enicostemma littorale is a species that is particularly noteworthy for its significant contribution to human healthcare. The plant exhibits antioxidant, hypoglycemic, hepatomodulatory, and hepatoprotective characteristics, and aids in the reduction of obesity [15, 16]. The plant contains several antioxidative phytochemicals, such as alkaloids, glycosides, saponins, sterols, catechins, xanthones, phenolic acid, flavonoids, and triterpinoids [17]. Swertiamarin (Sw), a secoiridoid glycoside, is a key component found in the crude extract of this medicinal plant. Sw has diverse pharmacological properties, with a particular focus on its antioxidant, anti-inflammatory, anti-diabetic, and antihyperlipidaemic effects, as well as its ability to protect against hepatic injury [18]. Although the potential of Sw to improve liver fibrosis induced by DMN has been discussed, no conclusive data has been provided. Thus, this work aimed to investigate the potential of Sw in reducing liver fibrosis generated by DMN. The focus was on examining how Sw inhibits the ASK1/MAPK receptor-mediated apoptotic pathways and understanding the underlying processes *in vivo*.

2. Materials and methods

2.1. Chemicals

Swertiamarin (Purity ≥ 98%, Pub Chem CID: 442435) was extracted and preserved from complete dried *Enicostemma littorale* plant. Selonsertib (Selon) was purchased from Sigma Aldrich, USA. Triglycerides quantification colorimetric assay kit (MAK266-1KT, Sigma-Aldrich, USA) and Shanghai Yuanye Bio-Technology Co., Ltd. (R21819-100T and R21814-100T) were also used.

2.2. Isolation of Swertiamarin from *E. littorale*

The entire botanical specimen of *Enicostemma littorale* was gathered from the state of Tamil Nadu in India. The plant was identified by examining its morphological and microscopical features, as specified in authoritative literature and floras published by the NISCAIR department in New Delhi, India. The process of extracting and analysing Swertiamarin (Sw) was carried out using a method that has been previously described in a published study [19, 20]. Sw was isolated using column chromatography. After being separated, the resultant solid was passed through a filter and then washed with ethyl acetate. Afterwards, Sw was separated and purified using a filter column with ethyl acetate as the mobile phase. The purity of isolated Sw was verified through the use of Mass and NMR spectroscopy methods.

2.3. Animal study

2.3.1. Animals

The experiment was conducted with the previous approval of the Institutional Animal Ethical Committee (IAEC) of Delhi Pharmaceutical Sciences and Research University (DPSRU), situated in New Delhi, India (DPSRU/IAEC/2019/III-R02). A total of thirty-two male Sprague-Dawley rats, aged six weeks and weighing between 150 and 200 grams, were acquired from the animal house facility at the university. The rats were housed under standard laboratory conditions.

2.3.2. Experimental Protocol

The investigation lasted 12 weeks. With 0.5% methylcellulose (MC), Selonsertib (Selon) and Sw were taken orally. In 2010, Jaishree et al[21] found that 100 and 200 mg/kg Sw protected rats against galactosamine-induced liver damage. This information and our first findings led us to choose 100 mg/kg of Sw for the experiment.

8 rats were given equal quantities of saline and 0.5% MC in the normal control group [14]. Twenty-four rats were fed a HFD and DMN for 12 weeks to induce hepatosteatosis and fibrosis. DMN was injected intraperitoneally six times, once every two weeks. The first and second DMN injections were 15 mg/kg, the third and fourth 10 mg/kg, and the fifth and sixth 5 mg/kg [9]. After eight weeks, HFD and DMN-induced rats (n = 24) were randomly divided into three l groups. This comprised the HFD+ DMN, Selon 50 mg/kg [11], and Sw 100 mg/kg groups. In the ninth to twelfth week, animals in the Selon and Sw groups received oral medication once daily [14].
At the conclusion of week 12, the animals in each group were euthanized after being fed the experimental diets. The liver and adipose tissue around the epididymis were promptly removed, weighed, and sliced into sections measuring 3 to 4 mm in thickness, Stored in 10% formalin. Excess liver tissue was frozen and kept at a temperature of -80 °C until it was ready to be processed for biochemical estimation[9].

2.3.3. Biochemical analysis

Blood was obtained by puncturing the abdominal aorta during the sacrifice. Triglycerides (TG), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were quantified using established protocols on an automated analyzer with a commercially available laboratory kit. The Ultra-Sensitive Rat Insulin ELISA Kit (Morinaga) was utilised to analyse insulin. The HOMA-IR scores were computed to test insulin resistance using the formula [9, 22].

2.3.4. Cytokine assay

Recently obtained sera were rapidly extracted from blood samples taken from sacrificed rats. The levels of IFN-γ and TNF-α cytokines were evaluated using the Sandwich ELISA method. The required capture and detection antibodies (R&D system, Minneapolis, MN, USA) were utilised alongside a visualisation system consisting of streptavidin-conjugated HRP and TMB microwell peroxidase substrate (Vector Laboratories, CA, USA)[11].

2.3.5. Western blotting

Following DPBS washing, the liver tissue of each rat was lysed using RIPA buffer. Then, the solution was supplemented with 2 mM Xpert protease and phosphatase inhibitor, and centrifuged for 30 minutes at 15,000 rpm. Using SDS-PAGE, we separated 30 µg of protein lysate, and then we used a wet transfer kit to transfer it to PVDF membrane. Primary antibodies diluted in PBS-T with 5% BSA were then incubated overnight at 4°C after the membrane was blocked with 5% skim milk in PBS and 0.5% Tween 20 (PBS-T). Santa Cruz Biotechnology, Genetex, Sigma-Aldrich, and Cell Signalling Technology were the suppliers of anti-JNK (9252S) and anti-ASK1 (107921). Additionally, we estimated the expression of other markers using standards of anti-SMA (F3777), anti-β-Actin (A5441), and anti-collagen I (ab34710). The blots were visualised using Cell Signalling Technology secondary antibodies and an improved chemiluminescence system from Bio-Rad Laboratories [11].

2.3.6. Histopathological analysis

The liver sections were prepared for paraffin embedding after being stored in a 10% formaldehyde solution and rehydrated to water through a graded series of alcohol in the initial. The sections with a thickness of around 4 µm are then stained with hematoxylin which binds to the acidic components of the cell such as nuclei and imparts a blue-purple color. After rinsing the sections are treated with eosin which stains the cytoplasmic components and the extracellular matrix in pink shades. The stained sections are then dehydrated through graded alcohols, cleared in xylene and mounted with coverslip. Under the microscope fat vacuoles appear as clear spaces within hepatocytes due to the dissolution of fat during tissue processing providing a clear indication of steatosis. Image analysis was used to determine the severity of fibrosis [11].

2.4. Statistical Analysis

Differences in quantitative data, expressed as mean ± standard deviation (SD), between groups were compared by one-way ANOVA followed by Dunnett's comparison tests using Graph Pad Prism 8. P value < 0.05 was considered significant.

3. Results

Sw was extracted from *E. littorale* employing a well-documented isolation procedure. The resultant compound underwent characterization through Mass Spectrometry and Nuclear Magnetic Resonance (NMR) techniques, and its purity was duly validated, as illustrated in Supplementary Fig. 1 and 2. **(SI Fig. 1 & 2).**

3.1. Effect of Sw on metabolic alterations in HFD+DMN-induced steatohepatitis rats

DMN injections and HFD consumption were examined at 12 weeks. The HFD+DMN-treated group had larger body and liver weights than the control group. Rats receiving Sw (100 mg/kg) showed significantly lower body weight and liver

weight ($P \leq 0.05$) compared to the HFD+DMN group (Fig. 1).

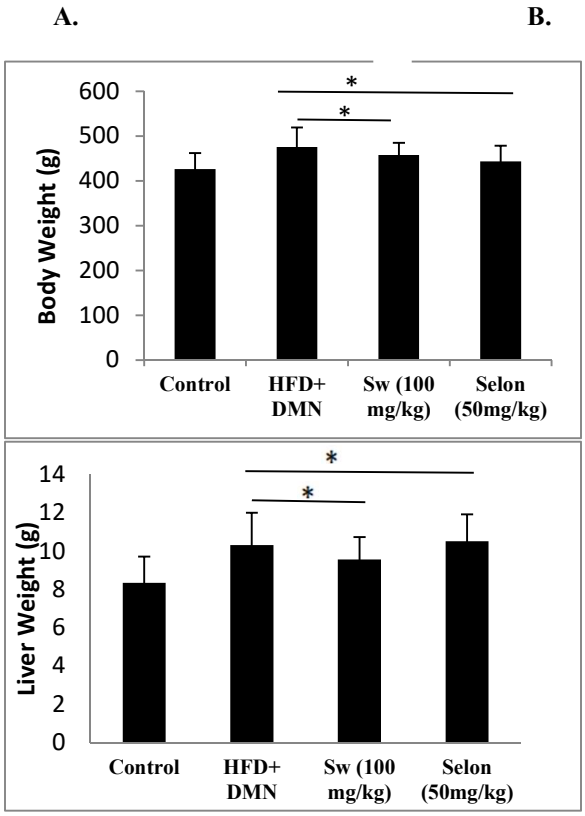

Fig. 1. Effects of Sw on HFD+DMN -induced body and liver weight changes

A). The body weight gain (g) was measured daily. B). Relative liver weight (g/100 g of body weight) was examined after sacrifice. All data are presented as the mean ± SD. (n=8). *p<0.05 versus HFD+DMN group.

After the experiment, the HFD+DMN group had significantly higher serum TG, AST, and ALT levels than the normal control group (Table 1). We measured serum ALT and AST levels to indicate liver damage. Transaminases in the serum rise when the liver is injured due to hepatocyte cell membrane permeability. Compared to rats in the HFD+DMN and standard medication Selon (50 mg/kg) treatment groups, Sw (100 mg/kg) significantly decreased these parameters (Table 1). These findings indicate that Sw decreases HFD+DMN-induced liver hepatotoxicity in treatment group.

Table 1. Effects of Sw on serum levels of AST & ALT in HFD+DMN-treated rats

Group	TG mmol/L	AST (U/L)	ALT (U/L)
Control	426.5 ± 35.4	71.7 ± 15.1	33.3 ± 4.0
HFD+DMN	465.4 ± 44.1**	102.5 ± 25.5**	64.4 ± 15.0***
Sw (100mg/kg)	452.6 ± 27.5*	74.9 ± 13.8	35.5 ± 6.2
Selon (50 mg/kg)	458.6 ± 34.9**	78.9 ± 13.7	52.0 ± 9.3***

Control, HFD high-fat diet+DMN, Sw 100 mg/kg, Selon 50 mg/kg. One-way ANOVA followed by Dunnett's comparison tests*P < 0.05, **P < 0.01, ***P < 0.001 vs control.

We examined how Sw affected insulin sensitivity in HFD+DMN-induced NASH rats. In rats, HFD+DMN feeding significantly increased blood sugar and insulin levels (Table 1; P < 0.05 and 0.001, respectively), leading to a higher HOMA-IR score.

Table 2. Effects of Sw on insulin resistance in HFD+DMN-treated rats

Group	Blood Sugar (mg/dl)	Insulin (µU/ml)	HOMA-IR
Control	137.0 ± 16.5	15.6 ± 4.6	5.4 ± 2.1
HFD+DMN	162.3 ± 24.8*	44.3 ± 25.8***	18.7 ± 13.0**
Sw (100mg/kg)	147.3 ± 15.6*	18.4 ± 3.6**	7.3 ± 2.4**
Selon (50 mg/kg)	152.0 ± 21.4*	22.3 ± 9.6**	8.6 ± 4.3***

Control, HFD high-fat diet+DMN, Sw 100 mg/kg, Selon 50 mg/kg, HOMA-IR homeostasis model assessment-insulin resistance, One-way ANOVA followed by Dunnett's comparison tests*P < 0.05, **P < 0.01, ***P < 0.001 vs control.

3.2. Sw inhibits fibrotic response by ASK1/MAPK signaling in HFD+DMN- induced liver fibrosis model

ASK1 activates the MAPK signalling cascade, including p38 and JNK, to cause liver disease. Through collagen and other ECM buildup, p38 and JNK drive hepatic fibrosis. Thus, we tested whether Sw would decrease fibrosis-related protein expression via MAPK signalling in treatment group. As seen in Fig. 2, Sw reduced ASK1 phosphorylation and downstream p38/JNK and MAPK signalling compared to control. Sw was tested for its ability to reduce profibrotic mediators such α-SMA and collagen by suppressing ASK1 phosphorylation in an HFD+DMN-induced liver fibrosis model (Fig. 2). The HFD+DMN group exhibited elevated α-SMA and collagen levels. Sw treatment groups had less fibrosis-related indicators. HFD+DMN induction increased ASK1, p38, and JNK expression in control group, but Sw (100 mg/kg) decreased it. Our findings showed that Sw is effective against HFD+DMN-induced liver fibrosis by indicating a significant reduction in protein expressions of fibrotic markers

as comparison to disease control (DC) and standard group (Selon 50 mg/kg).

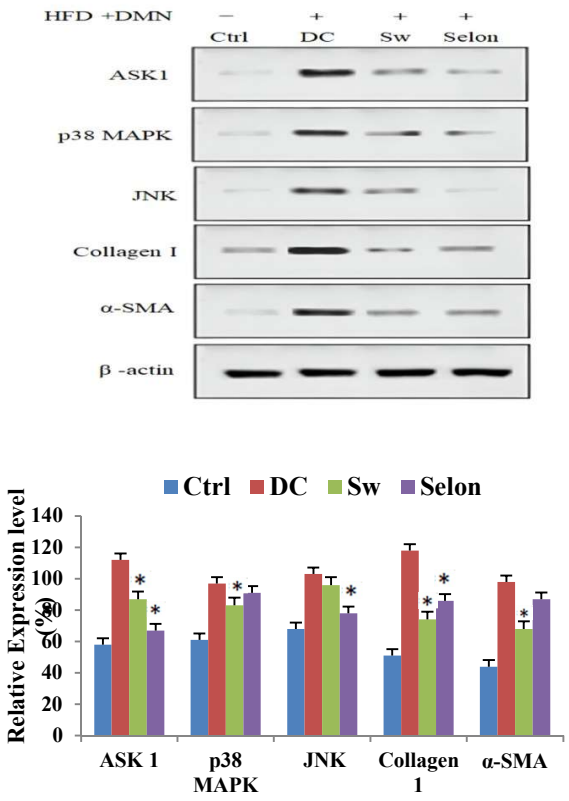

Fig. 2. Effect of Sw (100 mg/kg) on ASK1/MAPK signaling and expression of other fibrogenic proteins such as; β-actin, α-SMA, collagen 1 and JNK. Data represented as the mean ± SD (n=4). *p<0.05 versus the Disease control (DC).

3.3. Effect of Sw on cytokine parameters in HFD+DMN-induced steatohepatitis rats

IFN-γ and TNF-α are crucial pro-inflammatory cytokines that play a significant role in the advancement of liver damage. The levels of IFN-γ (Fig. 3A) and TNF-α (Fig. 3B) in the HFD+DMN group showed a substantial rise ($P \leq 0.05$) at the conclusion of the trial. On the other hand, the administration of Sw (100 mg/kg) resulted in a significant decrease in the levels of IFN-γ and TNF-α compared to the group treated with HFD+DMN. After a duration of 12 weeks, the group of rats treated with HFD+DMN exhibited significant liver injuries in comparison to the normal control group. In certain sections of the liver, the hepatocytes displayed distinct and widespread ballooning degeneration, along with a considerable number of cells with a condensed appearance of the nucleus. These observations were made using a light microscope (Fig. 4).

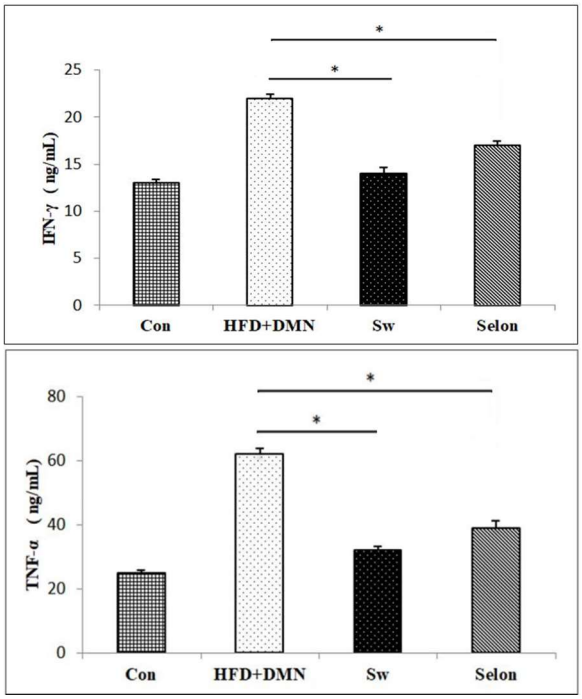

Fig. 3. Effect of Sw (100mg/kg) on IFN-γ and TNF-α level. Con, HFD+DMN, SWL 100mg/kg, Selon 50 mg/kg, (n = 8). Data were analyzed by using One-way ANOVA followed by Dunnett's comparison test. The results are expressed as the mean ± standard deviation (SD), with statistical significance indicated by * ($P < 0.05$), with respect to control group.

3.4. Swertiamarin improves HFD+DMN-induced liver fibrosis in rats

Table 1 & 2 shows how Sw exhibits promise as a major therapeutic agent for enhancing NAFLD and liver fibrosis, providing a more efficacious alternative to current conventional treatments. Animals body and liver weight increased significantly with HFD-DMN treatment compared to control group. The liver fibrosis caused by HFD+DMN in rats was also examined using H&E staining. The control group had normal liver architecture, but the HFD+DMN group had significant hemorrhagic necrosis, tissue rupture, and inflammatory cell infiltration. These changes were greatly reduced in the Sw group. Histopathological results showed that Sw has significantly reduced liver fibrosis, and the results were found to be comparable to the standard drug Selonsertib (50 mg/kg).

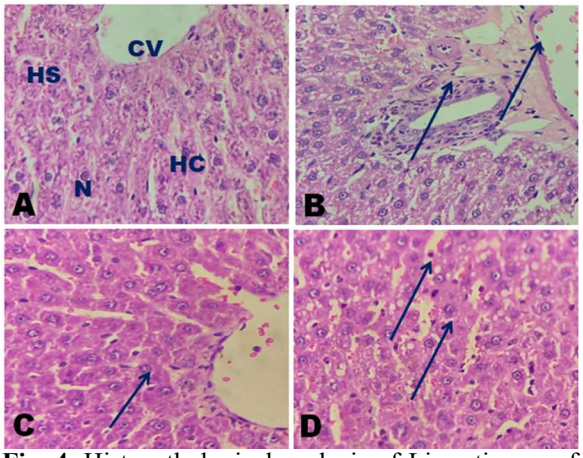

Fig. 4. Histopathological analysis of Liver tissues of Control, Disease control (HFD+DMN), Sw and Selon treated rats; **A)** Control, Group1 treated with vehicle showing normal architecture of hepatic lobule. The central vein (CV) lies at the centre of the lobule surrounded by the hepatocytes (HC) with strongly eosinophilic granulated cytoplasm, and distinct nuclei (N). between the strands of hepatocytes the hepatic sinusoids are shown (HS), B) Group 2, Disease Control (HFD+DMN), rats treated with DMN showing interspersed many dilated vessels, mid and peripheral cloudy degeneration, and granular precipitation in the cytoplasm. **C)** Group 3, a section of liver of DMN intoxicated rat and co-administrated with Sw (100 mg/kg) shows lobule architecture that appear like normal. Notice the few vacuoles (arrowhead), **D)** Group 4, a section of liver of HFD+DMN intoxicated rat and co-administrated with Selon (50 mg/kg) shows few focal necrosis, congested and dilated blood sinusoids (arrowhead)(H & E stain X 300)

4. Discussion

Liver fibrosis is a frequent outcome of NAFLD/NASH, resulting in cirrhosis and hepatocellular cancer. The activation of hepatic stellate cells (HSCs) is crucial in this process, since it is marked by their fibrogenic potential and high rate of proliferation [11, 23] . Research has demonstrated that the ASK1 pathway has a role in facilitating and advancing the development of chronic liver fibrosis caused by NASH. Previous studies have revealed the significance of signalling in the progression of hepatic fibrogenesis by activating hepatic stellate cells (HSCs) and promoting the release of pro-fibrogenic mediators. Consequently, researchers have started studying the suppression of ASK1 signalling in HSCs as a promising approach to cure liver fibrosis [12]. Our work focused on assessing the anti-fibrosis properties of Sw, a secoiridoid glycoside, and investigating the mechanisms by which it acts in liver fibrosis [18]. Furthermore, the obtained data showed that Sw suppressed the ASK1/MAPK signalling pathway and fibrogenesis via reducing extracellular matrix (ECM)-related molecules in liver fibrosis models produced by a HFD and DMN. Our research indicates that Sw is a powerful substance that can prevent the formation of fibrous tissue and inhibit the activation of HSCs by blocking the ASK/MAPK signalling pathway in liver fibrosis. The study investigated the effects of Sw on metabolic changes in rats with HFD+DMN -induced steatohepatitis. The HFD+DMN group showed a remarkable increase in body and liver weight of compared the control group. The Sw-treated group (100 mg/kg) resulted in a notable reduction in body and liver weight relative to the HFD+DMN group (Fig. 1). The analysis of serum biochemical parameters revealed significant differences among the groups. The HFD+DMN group showed elevated serum triglycerides (TG) levels, AST, and ALT compared to the control (Table 1). Sw treatment significantly reduced these parameters, indicating its protective effect against liver injury. Further, insulin sensitivity was examined by measurement of blood sugar and insulin levels and the homeostasis model assessment-insulin resistance score. The HFD+DMN group exhibited increased blood sugar and insulin levels, resulting in a higher HOMA-IR score, indicating insulin resistance. Sw treatment markedly decreased blood sugar, insulin levels, and the HOMA-IR score compared with that in the HFD+DMN group, showing its effect on improving insulin sensitivity. The standard medication, Selonsertib, also showed improvements in these parameters, but Sw exhibited a more pronounced effect (Table 2).

Multiple studies have documented that activated HSCs enhance the production of ECM-related proteins [3, 12]. Consequently, the ECM plays a role in controlling cell proliferation and apoptosis, ultimately leading to the progression of liver fibrosis. α-SMA, fibronectin, and collagen type I are recognised as pro-fibrotic markers that are crucial in the production of collagen and the deposition of ECM [11]. Furthermore, recent research has indicated that mice with a genetic modification that eliminates the ASK1 gene are shielded against abnormal changes in organ structure and the formation of fibrous tissue by suppressing chemicals associated with the ECM. Hence, we investigated the potential of Sw to suppress the expression of pro-fibrotic factors, specifically α-SMA and collagen I (Fig. 2). Sw decreased the expression of these mediators in an animal model of liver fibrosis. Consistent with prior research, the inhibition of ASK1 resulted in decreased expression of fibrotic-related genes, including α-SMA, in pulmonary fibrosis [11]. The pro-inflammatory cytokines IFN-γ

and TNF-α crucially drive the process of progression of liver damage. There was a significant rise in these cytokine levels in the HFD+DMN group, indicating intense inflammation. Sw administration markedly and significantly lowered the levels of these cytokines, again indicative of its anti-inflammatory action (Fig. 3). This suppression of the cytokine levels was proportionately associated with decreased liver injuries, as seen through histopathologic examination, and Sw-treated rats displayed profound protection from hepatic impairments against HFD+DMN-induced liver damage.

Our data showed that Sw suppressed the ASK1/ MAPK signalling pathway by reducing the levels of p-p38 and p-JNK in a liver fibrosis model generated by HFD+DMN. Considering the fact that phosphorylated p38 and JNK have been shown to enhance collagen expression [23], it appears that Sw effectively reduced liver fibrosis by suppressing ECM formation through the suppression of the ASK1/MAPK signalling pathway. Our findings suggest that Sw's ability to reduce fibrosis is achieved through the inhibition of ASK1/MAPK signalling. Moreover, Sw is anticipated to provide anti-fibrotic and anti-inflammatory benefits in several fibrotic disorders. Histopathological examination of liver tissues provided further insights into the protective effects of Sw. The control group showed normal liver architecture, while the HFD+DMN group exhibited severe liver damage characterized by hemorrhagic necrosis, tissue rupture, and inflammatory cell infiltration. Sw treatment significantly mitigated these pathological changes, resulting in a liver architecture comparable to the control group. The histopathological findings corroborate the biochemical and molecular data, reinforcing the potential of Sw as a therapeutic agent for liver fibrosis and steatohepatitis (Fig.4).

To summarize, the present investigation showed that Sw (100 mg/kg) effectively reduced the progression of liver fibrosis compared to the standard treatment Selon (50 mg/kg) by inhibiting the ASK1/MAPK signalling pathway in living organisms. Furthermore, this restraint is crucial for triggering HSC death, resulting in the removal of collagen-producing cells in liver fibrosis. Hence, we propose that administering Sw could be an effective therapeutic strategy for treating chronic hepatic fibrosis by reducing the accumulation of ECM.

5. Conclusions

In conclusion, our study highlights the significant anti-fibrotic potential of Sw, a secoiridoid glycoside, in combating liver fibrosis induced by a high-fat diet and DMN. Sw (100 mg/kg) suppressed the activation of HSCs and the production of associated profibrotic mediators through the inhibition of the ASK1/MAPK pathway. Sw effectively reduced body and liver weights, improved serum biochemical parameters, enhanced insulin sensitivity, inhibited the fibrotic response, and decreased inflammatory cytokine levels. Histopathological analysis further supported these findings, showcasing Sw's ability to preserve liver architecture and reduce fibrosis. By reducing the expression of key fibrogenic markers, such as α-SMA and collagen type I, and suppressing ECM-related molecules, Sw demonstrates its capability to prevent the progression of liver fibrosis. Sw, compared with the standard treatment Selon, further reduced the fibrosis more effectively and promoted apoptosis of collagen-producing cells. These findings suggest that Sw holds promise as a major therapeutic agent for non-alcoholic fatty liver disease (NAFLD) and liver fibrosis, offering a more efficacious alternative to conventional treatments. Further research and clinical trials are required to explore the potential of Sw in human applications.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest in this publication.

Acknowledgement

We express our gratitude to the Indian Council of Medical Research (ICMR), New Delhi, India, for providing research grant (Grant ID: 2020-8387, 2020-2023) for this study. We thank to Vice Chancellor DPSRU for providing excellent infrastructure for this work.

† **Supplementary data:** The supporting information includes spectral data details (NMR and Mass) of Sw.

Abbreviations:

T2DM	Type 2 diabetes mellitus
ASK-1	Apoptosis signal regulating kinase 1
Sw	Swertiamarin
MC	Methyl cellulose
SEL	Selonsertib
ROS	Reactive oxygen species
α-SMA	Alpha smooth muscle actin
JNK1	c-Jun N-terminal kinase 1
MAPK	Mitogen-activated protein kinase
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
HCC	Hepatocellular carcinoma
HFD	High fat diet
DMN	Dimethylnitrosamine
HOMA-IR	Homeostatic model assessment of insulin resistance

References:

[1] S.K. Reddy, O. Hyder, J.W. Marsh, G.C. Sotiropoulos, A. Paul, S. Alexandrescu, H. Marques, C. Pulitano, E. Barroso, L. Aldrighetti, D.A. Geller, C. Sempoux, V. Herlea, I. Popescu, R. Anders, L. Rubbia-Brandt, J.F. Gigot, G. Mentha, T.M. Pawlik, Prevalence of nonalcoholic steatohepatitis among

patients with resectable intrahepatic cholangiocarcinoma, Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract, 17 (2013) 748-755, https://doi.org/10.1007/s11605-013-2149-x.
[2] M. Rinella, M. Charlton, The globalization of nonalcoholic fatty liver disease: Prevalence and impact on world health, Hepatology (Baltimore, Md.), 64 (2016) 19-22, https://doi.org/10.1002/hep.28524.
[3] Z.M. Younossi, P. Golabi, L. de Avila, J.M. Paik, M. Srishord, N. Fukui, Y. Qiu, L. Burns, A. Afendy, F. Nader, The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis, Journal of hepatology, 71 (2019) 793-801, https://doi.org/10.1016/j.jhep.2019.06.021.
[4] L. Cao, Y. An, H. Liu, J. Jiang, W. Liu, Y. Zhou, M. Shi, W. Dai, Y. Lv, Y. Zhao, Y. Lu, L. Chen, Y. Xia, Global epidemiology of type 2 diabetes in patients with NAFLD or MAFLD: a systematic review and meta-analysis, BMC medicine, 22 (2024) 101, https://doi.org/10.1186/s12916-024-03315-0.
[5] P. Dongiovanni, E. Paolini, A. Corsini, C.R. Sirtori, M. Ruscica, Nonalcoholic fatty liver disease or metabolic dysfunction-associated fatty liver disease diagnoses and cardiovascular diseases: From epidemiology to drug approaches, European journal of clinical investigation, 51 (2021) e13519, https://doi.org/10.1111/eci.13519.
[6] X. Xie, Y. Liao, Z. Lin, H. Luo, G. Wei, N. Huang, Y. Li, J. Chen, Z. Su, X. Yu, L. Chen, Y. Liu, Patchouli alcohol alleviates metabolic dysfunction-associated steatohepatitis via inhibiting mitochondria-associated endoplasmic reticulum membrane disruption-induced hepatic steatosis and inflammation in rats, International immunopharmacology, 138 (2024) 112634, https://doi.org/10.1016/j.intimp.2024.112634.
[7] S. Raza, S. Rajak, A. Upadhyay, A. Tewari, R. Anthony Sinha, Current treatment paradigms and emerging therapies for NAFLD/NASH, Frontiers in bioscience (Landmark edition), 26 (2021) 206-237, https://doi.org/ 10.2741/4892.
[8] A. Gottlieb, W. Mosthael, J.P. Sowa, A. Canbay, Nonalcoholic-Fatty-Liver-Disease and Nonalcoholic Steatohepatitis: Successful Development of Pharmacological Treatment Will Depend on Translational Research, Digestion, 100 (2019) 79-85, https://doi.org/10.1159/000493259.
[9] A. Naiki-Ito, H. Kato, T. Naiki, R. Yeewa, Y. Aoyama, Y. Nagayasu, S. Suzuki, S. Inaguma, S. Takahashi, A novel model of non-alcoholic steatohepatitis with fibrosis and carcinogenesis in connexin 32 dominant-negative transgenic rats, Archives of toxicology, 94 (2020) 4085-4097, https://doi.org/10.1007/s00204-020-02873-5.
[10] G. Akbari, S.A. Mard, F. Savari, B. Barati, M.J. Sameri, Characterization of diet based nonalcoholic fatty liver disease/nonalcoholic steatohepatitis in rodent models: Histological and biochemical outcomes, Histology and histopathology, 37 (2022) 813-824, https://doi.org/10.14670/hh-18-462.
[11] Y.C. Yoon, Z. Fang, J.E. Lee, J.H. Park, J.K. Ryu, K.H. Jung, S.S. Hong, Selonsertib Inhibits Liver Fibrosis via Downregulation of ASK1/ MAPK Pathway of Hepatic Stellate Cells, Biomolecules & therapeutics, 28 (2020) 527-536, https://doi.org/10.4062/biomolther.2020.016.
[12] A.J. Kovalic, S.K. Satapathy, N. Chalasani, Targeting incretin hormones and the ASK-1 pathway as therapeutic options in the treatment of non-alcoholic steatohepatitis, Hepatology international, 12 (2018) 97-106, https://doi.org/10.1007/s12072-018-9854-1.
[13] Y. Zhang, T. Ye, P. Zhou, R. Li, Z. Liu, J. Xie, T. Hua, Q. Sun, Exercise ameliorates insulin resistance and improves ASK1-mediated insulin signalling in obese rats, Journal of cellular and molecular medicine, 25 (2021) 10930-10938, https://doi.org/10.1111/jcmm.16994.
[14] Y. Yang, J. Li, C. Wei, Y. He, Y. Cao, Y. Zhang, W. Sun, B. Qiao, J. He, Amelioration of nonalcoholic fatty liver disease by swertiamarin in fructose-fed mice, Phytomedicine : international journal of phytotherapy and phytopharmacology, 59 (2019) 152782, https://doi.org/10.1016/j.phymed.2018.12.005.
[15] M. Vigneswaran, G. Prem Kumar, S. Sivakumar, G. Siva, T. Nandakumaran, A. Lakshmi Prabha, N. Jayabalan, Compendious review of Enicostemma littorale Blume. Panacea to several maladies, International Journal of Scientific & Engineering Research, 8 (2017) 1817-1836.
[16] M.M.C. Rebellow, Biomedical Applications of Enicostemma littorale Leaves, in: Encyclopedia of Green Materials, Springer, 2022, pp. 1-7, https://doi.org/10.1007/978-981-16-4921-9_77-1.
[17] V. Sanmugarajah, A review on therapeutic and pharmacognostic properties of vellarugu (enicostemma littorale blume), International Journal of Ayurveda and Pharma Research, (2020) 47-67, https://doi.org/10.47070/ijapr.v8i4.1448.
[18] T.P. Patel, K. Rawal, S. Soni, S. Gupta, Swertiamarin ameliorates oleic acid induced lipid accumulation and oxidative stress by attenuating gluconeogenesis and lipogenesis in hepatic steatosis, Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie, 83 (2016) 785-791, https://doi.org/10.1016/j.biopha.2016.07.028.

[19] S. Vishwakarma, M. Rajani, M. Bagul, R. Goyal, A rapid method for the isolation of swertiamarin from Enicostemma littorale, Pharmaceutical biology, 42 (2004) 400-403, https://doi.org/ 10.1080/13880200490885095.
[20] H. Vaidya, A. Prajapati, M. Rajani, V. Sudarsanam, H. Padh, R.K. Goyal, Beneficial effects of swertiamarin on dyslipidaemia in streptozotocin-induced type 2 diabetic rats, Phytotherapy research : PTR, 26 (2012) 1259-1261, https://doi.org/10.1002/ptr.3708.
[21] V. Jaishree, S. Badami, Antioxidant and hepatoprotective effect of swertiamarin from Enicostemma axillare against D-galactosamine induced acute liver damage in rats, Journal of ethnopharmacology, 130 (2010) 103-106, https://doi.org/ 10.1016/j.jep.2010.04.019.
[22] N. Rodríguez-Hernández, M.L. Lazo-de-la-Vega-Monroy, Y. Ruiz-Noa, M.D. Preciado-Puga, J.R. Garcia-Ramirez, B. Jordan-Perez, S. Garnelo-Cabañas, L.D. Ibarra-Reynoso, Predictive Models for Non-Alcoholic Fatty Liver Disease Diagnosis in Mexican Patients with Gallstone Disease: Sex-Specific Insights, Diagnostics (Basel, Switzerland), 14 (2024), https://doi.org/10.3390/diagnostics14141487.
[23] L.A. Amos, F.Y. Ma, G.H. Tesch, J.T. Liles, D.G. Breckenridge, D.J. Nikolic-Paterson, Y. Han, ASK1 inhibitor treatment suppresses p38/JNK signalling with reduced kidney inflammation and fibrosis in rat crescentic glomerulonephritis, Journal of cellular and molecular medicine, 22 (2018) 4522-4533, https://doi.org/10.1111/jcmm.13705.