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Characterization of Tigogenin Isolated from Solanum Nigrum and it's Hepatoprotective Property

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Abstract

This study evaluates the isolation, structural characterization, and hepatoprotective efficacy of the steroidal sapogenin, Tigogenin (SN-1), obtained from Solanum nigrum. Column chromatography of the plant extract yielded a crystalline solid (SN-1) with a melting point of 203–204°C and an Rf value of 0.45–0.52 (Chloroform:Methanol, 9:1). The identity and high purity of Tigogenin (C27H44O3) were confirmed using elemental analysis, IR spectroscopy (O–H stretching at 3524cm-1), 1H-NMR (δ0.70–1.20 ppm for methyl protons and δ 3.30–3.60 ppm for–CHOH), and Mass Spectrometry [M]+ at m/z 416). Acetylation of the parent compound successfully yielded Tigogenin Acetate (M.P. 204–208°C), verified via spectral studies.
The hepatoprotective potential of the S. nigrum alcoholic extract (100–400 mg/kg) and isolated Tigogenin (10–20 mg/kg) was evaluated against carbon tetrachloride (CCl4) -induced hepatotoxicity in Wistar albino rats over 14 days. Administration of CCl4 alone caused severe hepatic impairment, significantly elevating serum transaminases (ALT, AST, ALP, GGT) and bilirubin, while depleting albumin levels. Co-treatment with S. nigrum extract and Tigogenin demonstrated potent, dose-dependent hepatoprotection. Notably, the extract at 400 mg/kg and Tigogenin at 20 mg/kg exhibited maximum therapeutic efficacy, effectively restoring liver enzymes, bilirubin, and protein synthesis (albumin) toward near-normal baseline levels. These findings conclude that S. nigrum and its active constituent, Tigogenin, act as powerful natural antioxidant and hepatoprotective agents.

Keywords: S. nigrum, Tigogenin, Steroidal sapogenin, Hepatoprotective.

1. Introduction

The global demand for herbal medicines is increasing due to the adverse effects associated with many allopathic drugs1. International organizations such as the World Health Organization have emphasized the need for quality control and standardization of Ayurvedic products using modern scientific methods2. Nature provides a wide

range of medicinal resources in the form of herbs, plants, and algae that help treat many diseases with minimal toxicity2. Herbal medicines are gaining popularity because of their effectiveness, affordability, and fewer side effects. Solanum nigrum is a highly adaptable plant species commonly found in disturbed habitats3. It grows along roadsides, railway tracks, riverbanks, and quaysides. The plant contains steroidal saponins, which include compounds like diosgenin, solasodine, solasonine, and solamargine, known for their anticancer, anti-inflammatory, and immunomodulatory properties4. It also contains alkaloids, primarily nitrogen-containing compounds, contributing to its pharmacological activities5. Flavonoids and polyphenols, such as quercetin and kaempferol derivatives, provide antioxidant and hepatoprotective effects6. Additionally, S. nigrum contains terpenoids, organic acids (like caffeic acid and chlorogenic acid), and carotenoids (β-carotene, xanthophyll), which enhance its protective role against oxidative stress6. Glycosides and other sugar-bound derivatives further augment its bioactivity. The rich and complex phytochemical profile of S. nigrum underlies its widespread use in traditional medicine systems like Ayurveda, offering therapeutic benefits for liver disorders, inflammation, cancer, and immune support7.
Solanum nigrum L. (Black Nightshade) is a well-known medicinal plant traditionally used for the treatment of liver disorders. Numerous pharmacological studies have demonstrated its significant hepatoprotective activity against chemically induced liver damage. The hepatoprotective effect of S. nigrum is mainly attributed to its rich content of steroidal alkaloids, glycoproteins, flavonoids, saponins, and phenolic compounds, which possess strong antioxidant and anti-inflammatory properties8. Experimental studies have shown that aqueous and ethanolic extracts of S. nigrum effectively reduce elevated serum liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin levels in hepatotoxic animal models9,10. The plant also enhances endogenous antioxidant defenses by increasing glutathione (GSH), superoxide dismutase (SOD), and catalase activities while reducing lipid peroxidation and oxidative stress11,12. Histopathological investigations have revealed that treatment with S. nigrum promotes regeneration of damaged hepatocytes and restores normal liver architecture13. Additionally, studies suggest that the plant inhibits inflammatory mediators and fibrotic processes associated with chronic liver injury14. Due to these protective mechanisms, S. nigrum is considered a promising natural hepatoprotective agent and may serve as a potential source for the development of liver-protective herbal formulations15.

Material and Method:

Collection of Medicinal Plant

The medicinal plant species Solanum nigrum was chosen, whole plant including stem, leaf, fruit and root were collected from agricultural fields of Jaunpur district U.P. during May 2023, and plant identified by experts from botanical department Banaras Hindu University, Varanasi, and further compared with herbarium specimen of S. nigrum. All collected plants were washed thoroughly and dried in shade until complete dehydration. Plant parts separated (stem, leaf and fruits, root) were cleaned and dried under shade. The dried plant materials were then ground properly into fine powder. The plant materials were transferred to the laboratory for phytochemical and biological investigation and experimental analytical study.
Preparation of extracts

The powdered plant material of nigrum was used for the preparation of different solvent extracts. A measured quantity of dried powder was subjected to successive extraction using petroleum ether, chloroform and alcohol in increasing order of polarity. Each extraction was carried out separately using a Soxhlet apparatus for 72 hours until complete exhaustion of the plant material. After extraction, the obtained solutions were filtered and concentrated under reduced pressure using a rotary evaporator. The dried extracts were collected, weighed and stored in airtight containers at low temperature. These extracts were further used for phytochemical screening and biological activity studies.

Extraction

The extraction of bioactive compounds from Solanum nigrum typically begins with cleaning and drying the plant parts, which are then ground to a fine powder. This powdered material is subjected to solvent extraction to separate chemical constituents based on polarity. Soxhlet apparatus employed for extraction with solvents of increasing polarity like petroleum ether, chloroform, and methanol/ethanol to remove lipids and extract alkaloids, glycosides, phenolics, and flavonoids16,17.

Chemical and Reagents

Immobilized lipase (triacylglycerol hydrolase, EC 3.1.1.3; Novozym® 435, 10000 PLU/g) from Candida Antarctica, supported on a macroporous acrylic resin with a water content of 3 % (w/w) was purchased from Novo Nordisk A/S (Bagsvaerd, Denmark). Chloroform, n-hexane was obtained from Fisher chemicals. Betulinic acid was purified from Malaysian Callistemon speciosus by previous method. Acetic anhydride was purchased from Acros Organics, Belgium. Ethyl acetate, celite® 545, Na2SO4, K2CO3 and HCl were purchased from Merck, Germany. All chemicals were of analytical reagent grade. Silica gel G & H and other chemicals were purchased from local suppliers Jaunpur and were used further without any adultation.

Instrumentation

Melting points were recorded on stuard melting point apparatus and were in corrected. UV spectra were recorded on UV-cary-100 spectrophotometer in ISSC. Infra Red Spectra (FT-IR) were recorded on Shimadzu FT-IR-8300 spectro photometer in kBR. disc and value are expressed in cm–1. 1H NMR spectra were recorded on a Bruker AC 40w MHz spectrometer using TMS as internal standard in DMSO-d6. Elemental analysis (N, S) was carried out with Euroea-Eiernental analyzer Italia.

Result and Discussion:

Eluents isolated from column 31 to 42 were pooled together and crystalized. Further purification may be carried out using preparative TLC or recrystallization. The isolated SN-1 is then characterized by melting point, TLC Rf, IR spectroscopy, and 1NHMR analysis to confirm purity and identity of tegogenin.

Physical Data of SN-1:

The compound was obtained as a crystalline solid and exhibited a melting point in the range of 203–204°C (200°C -202°C)18,19, which closely matches reported literature values for tigogenin, indicating high purity. Thin-layer chromatographic analysis using chloroform: methanol (9:1) as the mobile phase showed a single well-defined spot with an Rf value between 0.45 and 0.52, comparable to that of the reference standard. Visualization after spraying with anisaldehyde–sulfuric acid reagent produced a characteristic colored spot, further supporting its identity. Elemental

analysis of the isolated compound corresponded well with the theoretical composition of tigogenin (molecular formula $C_{27}H_{44}O_3$). The calculated carbon and hydrogen contents were 74.95% and 10.25%, respectively, while the experimentally found values ranged from 74.60–74.90% for carbon and 10.10–10.30% for hydrogen.

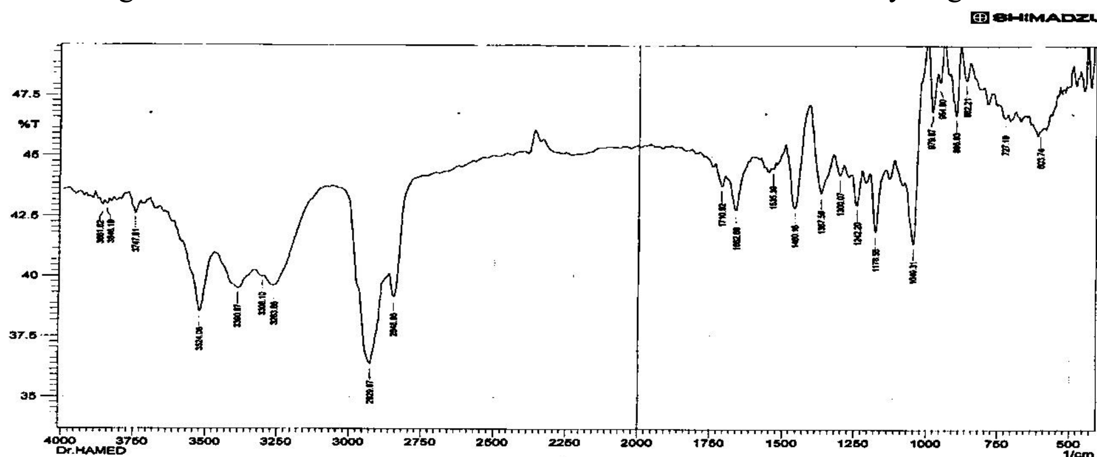

Fig. 1: IR spectra of SN-1

IR spectral analysis of isolated tigogenin showed characteristic absorption bands comparable to the standard. A sharp free O–H stretching band appeared around 3524 cm^{-1} with a broad hydrogen-bonded O–H band. Aliphatic C–H stretching, C–H bending, and C–O stretching vibrations confirmed the steroidal sapogenin structure.

^{1}H NMR spectra of SN-1:

The ^{1}H-NMR spectrum of SN-1 exhibits characteristic signals confirming its steroidal sapogenin structure. Methyl protons appear as singlets and doublets in the region δ 0.70–1.20 ppm, corresponding to angular CH_3 groups of the steroid nucleus. Multiplet signals observed between δ 1.20–2.20 ppm are assigned to methylene and methine protons of the saturated rings. A downfield signal around δ 3.30–3.60 ppm corresponds to the proton attached to the hydroxyl-bearing carbon (–CHOH), confirming the presence of a free hydroxyl group. The overall proton distribution and chemical shifts are consistent with reported ^{1}H-NMR data of Tigogenin isolated from Solanum species, supporting its identity and purity2.

Mass Spectra of SN-1:

Table-1: Mass spectra of SN-1

m/z value	Assignment
416	Molecular ion peak $[M]^+$ ($C_{27}H_{44}O_3$)
398	$[M – H_2O]^+$ (loss of hydroxyl group)
345	Fragment due to side-chain cleavage
301	Steroidal ring fragmentation
255	Cleavage within rings A/B
189	Further breakdown of steroid nucleus
147	Low-mass characteristic fragment
109	Secondary fragmentation ion
69	Alkyl fragment ion
43	Typical hydrocarbon fragment

On the basis of above spectral study we conclude following structure of tigogenin as SN-1.

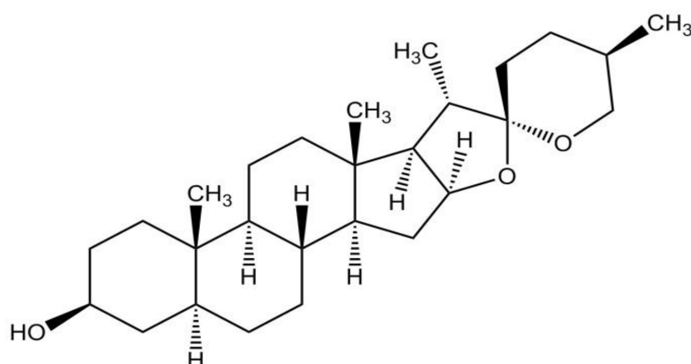

Fig. 2: Structure of Tigogenin

Tigogenin is a steroidal sapogenin with a core structure of a fused cyclopentanoperhydrophenanthrene ring system (the steroid backbone), specifically a (25R)-5α-spirostan-3β-ol, featuring a spiro-fused pyran ring and a hydroxyl group at C-3, making it a key precursor for synthesizing steroid hormones. Its formula is C27H44O3.

Acetylation of Tigogenin: Tigogenin acetate can be synthesized directly from the parent compound, tigogenin. Tigogenin is reacted with acetic anhydride in pyridine, often with heating (e.g., on a steam bath) for approximately 0.5 to 1 hour. M.P. of Tigogenin acetate is 204–208 °C20.

Table-2: Characterization of SN-1 Derivatives

IR cm-1	1H NMR δ (ppm)
1721–1735	~3.5–4.5, m, 1H (Typical spirostan pattern)
Ester Carbonyl,	~1.5–2.5, m, 1H (Ring A methylene adjacent)
1240–1250	~1.0–2.0, m, 2–4H (Alkyl protons)
Acetate C-O, 984,	~0.8–2.5, m, multiple (Ring protons and methylenes)
920, 902, 860	~0.9–1.1, d, 3H (Secondary methyl)
Spirostane Ring,	~0.8–1.0, s, 3H (Tertiary methyl)
2835–2974	~0.7–0.9, s, 3H (Tertiary methyl)
Aliphatic CH	~2.02–2.15, s, 3H (Characteristic acetate singlet)

On the basis of above spectral study the proposed derivative is Tigogenin Acetatec.

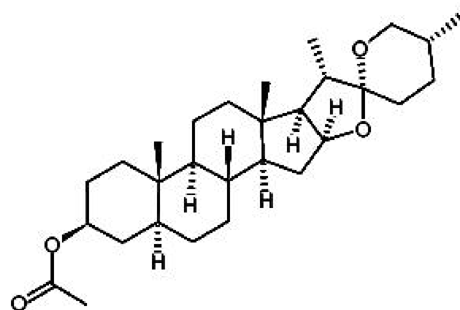

Fig.3: Structure of Tigogenin Acetate

Hepatoprotective properties of S.nigrum & tegogenin:

S. nigrum has been shown to possess significant hepatoprotective activity through multiple experimental models, particularly against chemical-induced liver injury. The aqueous leaf extract of S. nigrum significantly reversed carbon tetrachloride (CCl_4)-induced hepatotoxicity in albino rats by lowering elevated liver enzymes (ALT, AST) and restoring antioxidant markers such as glutathione (GSH), superoxide dismutase

(SOD), and catalase (CAT) to near-normal levels. Active compound tegogenin demonstrated potent mitigation of CCl_4-induced oxidative stress and liver injury in rats, with decreases in serum transaminases and improvements in tissue antioxidant status21. Supplementation of S. nigrum also showed protective effects against fatty liver and lipid peroxidation in high-fat diet models, indicating its role in reducing oxidative stress and maintaining hepatic function22. Additionally, earlier studies have reported that S. nigrum extracts reduce the severity of histopathological lesions and biochemical markers in chemically induced liver damage, further supporting its protective efficacy. Alcoholic extract of plant and tegogenin act as a natural hepatoprotective agent, likely due to its rich antioxidant phytochemicals23.

Experimental Design:

40 male Wistar albino rats were randomly divided into eight groups (I-VIII) of five animals in each group.

Table 3 - Experimental protocol

Group	Treatment
Group 1	Distilled water only for 14 days
Group 2	2.0 ml/kg bw CCl_4 alone for a single administration
Group 3	2.0 ml/kg bw CCl_4 + 100 mg/kg S.nigrum alcoholic extract for 14 days
Group 4	2.0 ml/kg bw CCl_4 + 200 mg/kg S.nigrum alcoholic extract for 14 days
Group 5	2.0 ml/kg bw CCl_4 + 300 mg/kg S.nigrum alcoholic extract for 14 days
Group 6	2.0 ml/kg bw CCl_4 + 400 mg/kg S.nigrum alcoholic extract for 14 days
Group 7	2.0 ml/kg bw CCl_4 + 10 mg/kg tegogenin solution for 14 days
Group 8	2.0 ml/kg bw CCl_4 + 20 mg/kg tegogenin solution for 14 days

For hepatoprotective enzyme such as Alkaline phosphatase (ALP), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Gamma glutamyl transferase (GGT), Albumin, BIL Bilirubin(ALB) examined in serum of albino rat given in table.

Table-4: Effect of S.nigrum extract and solasodine on liver enzyme.

Groups	ALP (mg/dl)	ALT (U/L)	AST (U/L)	GGT (mg/dl)	ALB (mg/dl)	BIL (mg/dl)
1	60.13±1.64	92.92±2.26	69.47±1.37	7.65±0.21	54.43±2.18	30.02±1.77
2	97.1 ± 1.79	138.8±3.64	108.79±3.26	10.7±0.45	26.24±0.88	55.27±1.91
3	62.4 ± 2.16	95.61±2.23	72.5 ± 1.47	7.9 ± 0.4	50.12±1.57	32.57 ± 1.3
4	82.76±3.38	120.07±3.18	90.21 ± 2.41	10.39±0.55	30.4 ± 0.92	45.89±2.19
5	71.27±2.54	100.94 ± 2.3	84.46 ± 2.17	8.84 ± 0.67	38.93±1.27	39.43±1.44
6	64.99±1.39	93.83 ± 2.16	75.57 ± 1.64	8.22 ± 0.59	49.65±1.52	33.38±1.35
7	81.85±3.29	121.24±3.10	91.72 ± 2.9	9.41 ± 0.56	28.14±0.81	35.12±2.36
8	68.36±1.23	94.41 ± 2.29	78.21 ± 1.71	7.85 ± 0.69	52.12±1.61	32.2 ± 1.37

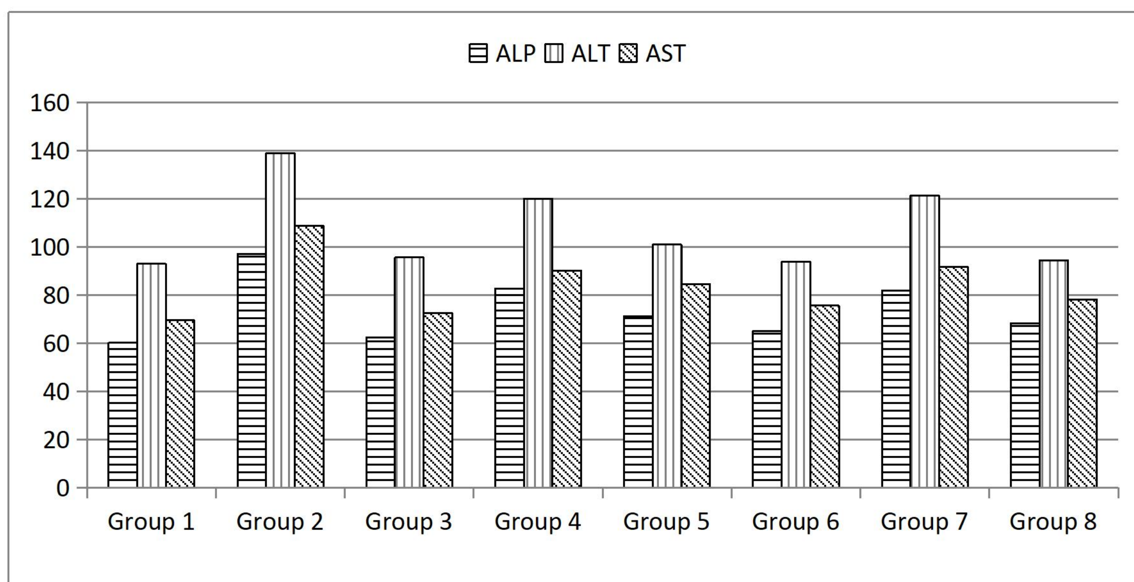

Fig 4: Effect of S.nigrum extract and solasodine on ALP, ALT & AST

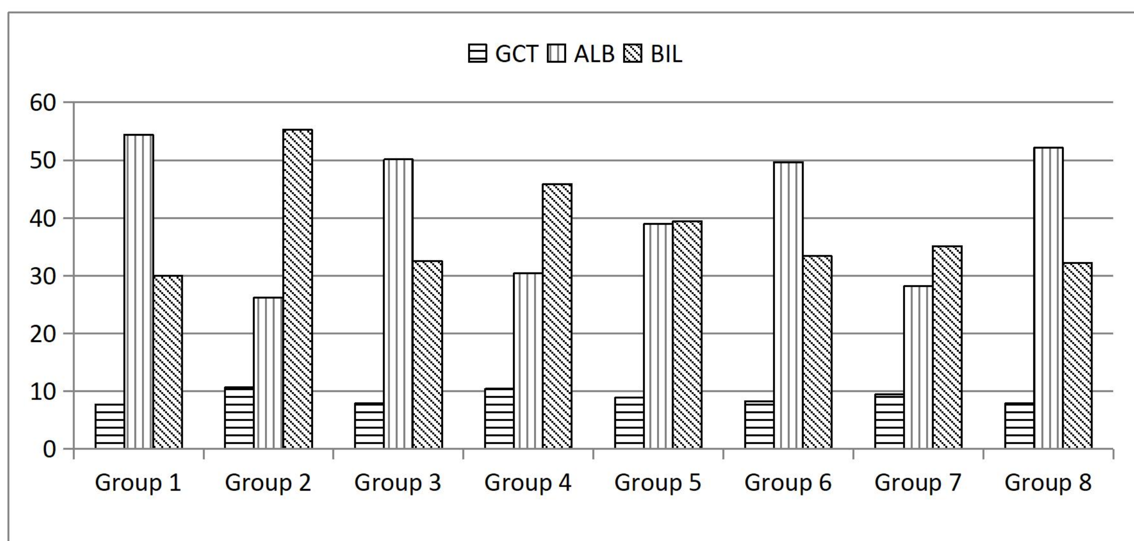

Fig 5: Effect of S.nigrum extract and tigogenin on ALP, ALT & AST

The table summarizes liver biochemical markers (ALP, ALT, AST, GGT), a synthetic-function marker (albumin), and an excretory marker (bilirubin) across eight concentrations, reported as mean ± SD. Overall, Group 2 shows the most pronounced biochemical disturbance, with the highest ALP (97.1), ALT (138.8), AST (108.79), GGT (10.7) and bilirubin (55.27), alongside the lowest albumin (26.24), suggesting maximal hepatic injury with impaired synthetic capacity24.

Group 1 represents the best biochemical profile in this dataset, with comparatively lower enzyme activities (ALP 60.13; ALT 92.92; AST 69.47; GGT 7.65) and bilirubin (30.02), and the highest albumin (54.43), consistent with minimal hepatocellular damage and preserved protein synthesis. Relative to Group 2, all other concentrations (3–8) show partial normalization: transaminases and cholestatic enzymes decline and albumin rises, indicating varying degrees of hepatoprotection or recovery25,26.

Among the “improved” groups, Group 3 and 6 appear closest to the healthier pattern because ALT/AST are relatively lower (95.61/72.5 and 93.83/75.57, respectively)

while albumin is relatively higher (50.12 and 49.65) and bilirubin remains near the lower end of the dataset (32.57 and 33.38). Group 4 and 5 show intermediate injury, with higher bilirubin (45.89 and 39.43) and moderate enzyme elevations, implying incomplete restoration of hepatocyte integrity and bile handling. Interestingly, Group 7 shows a moderate enzyme profile but relatively low albumin (29.34), which may indicate that synthetic function lags behind enzymatic recovery or reflects nutritional/protein status effects that can influence albumin independent of acute enzyme release27,28.

Discussion:

In experimental hepatotoxicity models, hepatoprotection is inferred when treatment prevents the toxin-induced rise in serum ALT and AST (hepatocellular leakage enzymes) and reduces ALP/GGT (cholestatic/biliary enzymes), while normalizing bilirubin and restoring albumin/synthetic function29-31. Recent evidence supports Solanum nigrum (S. nigrum) extract as a hepatoprotective intervention32,33: Study on methanolic leaf extract reported that CCl_4 increased ALT and AST, while S. nigrum treatment significantly reduced these activities and improved liver histology, consistent with membrane stabilization and antioxidant defense34. Similarly, experimental study with aqueous S. nigrum extract found that CCl_4 elevated ALT, ALP and total bilirubin, whereas S. nigrum significantly limited the rise of these markers and improved histopathology35.

For solasodine (a steroidal alkaloid from Solanum spp.), in vivo work indicates hepatoprotective potential against CCl_4 toxicity, reporting restoration of hepatocyte architecture and protective effects when solasodine was administered (including in combination regimens), which aligns with lowering of injury-related enzyme leakage and oxidative stress in hepatoprotection studies33. When interpreting solasodine treatment, dose and safety remain important because some datasets flag hepatotoxicity risk signals in predictive toxicity assessments, so enzyme monitoring (ALT/AST, bilirubin) is essential during efficacy evaluation. Overall, S. nigrum extract (and potentially solasodine under controlled dosing) can be discussed as hepatoprotective by improving serum enzyme patterns and functional indices alongside histological recovery36.

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