RESEARCH PAPER

Chemoprofiling and Hepatoprotective Potential of Methanolic Extract of Sugar-Free Majoon-e-Dabeed-ul-Ward Against Isoniazid-Induced Hepatotoxicity

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Abstract

Background: Majoon-e-Dabeed-ul-Ward is a traditional Unani polyherbal formulation used for liver-related disorders. Modified Majoon-e-Dabeed-ul-Ward (MMD) refers to a modified form of the classical formulation, while the present study focuses on its sugar-free powdered version, designated as Sugar-Free Majoon-e-Dabeed-ul-Ward (SFMD). Phytochemical characterization and experimental evaluation are important for investigating its chemical profile and hepatoprotective potential.

Objective: To characterize Sugar-Free Majoon-e-Dabeed-ul-Ward (SFMD) using high-performance thin-layer chromatography (HPTLC), quantify ferulic acid, and evaluate its hepatoprotective activity against isoniazid (INH)-induced hepatotoxicity in Wistar rats.

Methods: HPTLC analysis was performed on silica gel 60 F_{254} plates using toluene: ethyl acetate: formic acid (6:3:1) as the mobile phase, with densitometric scanning at 320 nm. Ferulic acid was quantified using a calibration curve, and analytical recovery was assessed. The methanolic extract of SFMD (SFMD-ME) was evaluated in a rat model of INH-induced hepatotoxicity. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, total protein, and albumin were measured, and liver tissues were examined histopathologically.

Results: HPTLC analysis revealed a ferulic acid content of 0.443% (dry weight), an Rf value of approximately 0.48, and an analytical recovery of 95-97%. The methanolic extract of SFMD yielded 40.8% w/w. INH administration produced marked alterations in hepatic biochemical parameters. Treatment with SFMD-ME demonstrated dose-related improvements, with the highest dose (1000 mg/kg) significantly reducing ALT, AST, ALP, and total bilirubin levels while improving total protein and albumin levels compared with the disease-control group. Histopathological examination also indicated progressive improvement in hepatic architecture, with comparatively mild alterations at the highest dose.

Conclusion: SFMD demonstrated preliminary hepatoprotective potential against INH-induced hepatic injury in rats. HPTLC-based quantification of ferulic acid provides a useful approach to its phytochemical characterization, while the animal findings support further investigation of its pharmacological potential. Additional studies incorporating comprehensive phytochemical profiling, mechanistic investigations, and adequately powered experimental designs are warranted.

Keywords: Sugar-Free Majoon-e-Dabeed-ul-Ward; Unani medicine; HPTLC; ferulic acid; hepatoprotective activity; isoniazid-induced hepatotoxicity; Wistar rats.

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Introduction

Drug-induced liver injury (DILI) is a significant adverse effect associated with several therapeutic agents and may compromise treatment outcomes, particularly when the causative drug is essential for managing chronic infectious diseases [1]. Isoniazid (INH), a first-line antitubercular drug, is associated with hepatotoxicity in susceptible individuals. Its hepatic metabolism can generate reactive intermediates, including hydrazine and acetylhydrazine, which contribute to oxidative stress, mitochondrial dysfunction, and hepatocellular injury. The resulting hepatic damage may be reflected by elevations in serum aminotransferases, alterations in other biochemical markers of liver function, and histopathological changes in liver tissue [2,3]. Consequently, experimental approaches to identifying agents with potential hepatoprotective activity remain important

for investigating strategies to mitigate drug-induced hepatic injury.

Traditional systems of medicine, including Unani medicine, have long employed polyherbal formulations for the management of liver-related disorders. Majoon-e-Dabeed-ul-Ward is a classical Unani formulation traditionally used for conditions associated with hepatic dysfunction. Its multiple herbal constituents provide a basis for investigating its phytochemical composition and potential pharmacological effects. However, the conventional formulation contains substantial amounts of sugar and honey, which may limit its suitability for individuals who require restricted sugar intake. To address this limitation, the formulation was modified to prepare a sugar-free powdered version, designated as Sugar-Free Majoon-e-Dabeed-ul-Ward (SFMD) in the present study. The modified formulation is also referred to as Modified Majoon-e-Dabeed-ul-Ward (MMD), reflecting its modified presentation without the conventional sugar-based vehicle. The powdered formulation provides an alternative pharmaceutical presentation suitable for encapsulation.

Phytochemical characterization is an important component of the quality assessment of polyherbal formulations because it can support the identification and quantification of characteristic chemical constituents. High-performance thin-layer chromatography (HPTLC) is a useful analytical technique for herbal materials owing to its capacity for chromatographic fingerprinting, simultaneous analysis, and quantitative estimation of selected marker compounds [4]. Ferulic acid, a naturally occurring phenolic acid, has been investigated for its antioxidant and anti-inflammatory properties [5]. Its quantification in SFMD may contribute to the analytical characterization of the formulation and provide a basis for subsequent quality-control investigations. Nevertheless, the presence of a particular marker compound does not independently establish the pharmacological activity of a complex herbal formulation.

Alongside chemical characterization, experimental pharmacological evaluation is necessary to investigate the biological effects of SFMD. Animal models of INH-induced hepatotoxicity permit the assessment of treatment-related changes in serum biochemical parameters and liver histology following exposure to a hepatotoxic agent. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, total protein, and albumin provide complementary information regarding hepatic injury and function [6]. Histopathological examination further enables the assessment of structural alterations in liver tissue. Evaluating these endpoints can provide preliminary evidence of the hepatoprotective potential of SFMD under defined experimental conditions.

Although phytochemical analysis and pharmacological evaluation address complementary aspects of herbal formulation research, their integration is valuable for developing a more comprehensive understanding of a formulation. Accordingly, the present study combined HPTLC-based characterization and quantification of ferulic acid with experimental evaluation of the hepatoprotective activity of SFMD against INH-induced hepatotoxicity in Wistar rats. The analytical investigation focused on chromatographic profiling and estimation of ferulic acid, whereas the pharmacological investigation assessed serum biochemical parameters and histopathological changes following treatment with different doses of the methanolic extract of SFMD.

The study aimed to contribute to the phytochemical characterization of SFMD and provide preliminary experimental evidence regarding its potential to attenuate INH-induced hepatic injury. The findings may support further investigations into the formulation's chemical standardization, pharmacological mechanisms, and reproducibility across adequately designed experimental studies.

2. MATERIALS AND METHODS

2.1. Preparation of Sugar-Free Majoon-e-Dabeed-ul-Ward

Sugar-Free Majoon-e-Dabeed-ul-Ward (SFMD) was prepared as a sugar-free powdered formulation using the prescribed herbal ingredients in their predetermined proportions. Unlike the conventional semi-solid formulation, sugar, honey, and other sweetening vehicles were excluded. The resulting homogeneous powder was filled directly into hard capsules without the addition of sugar-containing excipients. The powdered formulation was used for subsequent phytochemical analysis and pharmacological investigation. The composition of the classical formulation is presented in **Table 1,** whereas the experimental material was the sugar-free powder.

2.2. Chemicals and Reference Standard

Methanol and other analytical reagents used for chromatographic analysis were obtained from Merck (Germany). Ferulic acid reference standard was procured from Sigma-Aldrich (Steinheim, Germany) through its authorized Indian supplier. All solvents and reagents were used as specified in the respective analytical procedures.

2.3. Preparation of Sample Extract for HPTLC Analysis

5 g of powdered Sugar-Free Majoon-e-Dabeed-ul-Ward (SFMD) was mixed with 50 mL of methanol and sonicated at 50°C for 20 minutes. The extract was then evaporated to dryness (ETD), and the residue was reconstituted in methanol to obtain a final concentration of 50 mg/mL. The prepared sample solution was used for subsequent HPTLC analysis.

2.4. HPTLC Fingerprinting and Chromatographic Conditions

Chromatographic analysis was performed using precoated silica gel 60 F_{254} HPTLC plates (10 × 10 cm; Merck). Sample solutions and ferulic acid reference standard solutions were applied as 5 mm-wide bands, positioned 15 mm from the lower edge and 15 mm from the side of the plate, using a CAMAG Linomat 5 automated applicator. The application rate was maintained at 150 nL/s under nitrogen flow.

The plates were developed in a CAMAG twin-trough glass chamber pre-saturated with toluene: ethyl acetate: formic acid (6:3:1, v/v/v). Development was carried out to a distance of 8 cm from the origin. After development, the plates were dried and examined under ultraviolet light. Chromatographic fingerprinting was performed to characterize the separated constituents of SFMD.

2.5. Identification and Quantification of Ferulic Acid

Ferulic acid was identified by comparing the chromatographic characteristics of the sample band with those of the reference standard. Densitometric scanning was performed at 320 nm using a CAMAG TLC Scanner 4 equipped with winCATS IV software in absorption-reflection mode. The slit dimensions were 6 mm × 0.45 mm.

A ferulic acid stock solution was prepared in methanol at a concentration of 1 mg/mL. Different volumes of the standard solution were applied to the HPTLC plate under the same chromatographic conditions. A calibration curve was constructed by plotting peak area against the applied quantity of ferulic acid. The ferulic acid content of SFMD was estimated using the resulting calibration relationship.

2.6. Assessment of Analytical Recovery

Analytical recovery was assessed by spiking the sample with ferulic acid standard at four different concentration levels. Spiked samples were analyzed in triplicate using the established HPTLC procedure. Percentage recovery was calculated to assess the accuracy of ferulic acid estimation.

2.7. Preparation of Methanolic Extract for Pharmacological Evaluation

The powdered SFMD formulation was used to prepare the methanolic extract. Briefly, 100 g of the powdered material was soaked in 1 L of methanol for 24 h at room temperature. The material was sonicated at 40°C for two cycles of 14 min each, followed by refluxing in a water bath at 50°C for 5 h.

The mixture was filtered, and the residue was washed with fresh methanol. The combined filtrates were concentrated under reduced pressure using a rotary evaporator at a temperature below 50°C. The concentrated extract was allowed to form a solid mass, which was weighed and stored under appropriate conditions. The percentage extraction yield was calculated using the following equation:

Extraction yield (%) = (Weight of dried extract (g) / Weight of starting material (g)) × 100

The resulting methanolic extract of SFMD (SFMD-ME) was used for the animal experiment.

2.8. Experimental Animals and Ethical Considerations

Adult Wistar rats aged approximately 3-4 months and weighing 210-270g were used for the pharmacological evaluation. The animals were maintained under standard laboratory conditions with access to a standard pellet diet and drinking water. The experiment was conducted in accordance with applicable institutional and national guidelines for laboratory animal care and use, following approval from the Institutional Animal Ethics Committee (IAEC), with approval number 2344.

2.9. Experimental Design and Treatment Groups

A total of 28 Wistar rats were randomly allocated into seven experimental groups, with four animals in each group (n = 4). The experimental design comprised normal control, disease control, sham control, three SFMD-ME treatment groups, and a standard-drug group.

The normal-control group received normal saline orally. Hepatotoxicity was induced in the disease-control and treatment groups using isoniazid (INH). The treatment groups received SFMD-ME at doses of 250, 500, and 1000 mg/kg body weight, while the standard-treatment group received silymarin at 100 mg/kg body weight. The sham-control group received SFMD at 1000 mg/kg body weight without INH exposure. The experimental groups and treatment schedule are summarized in **Table 2**.

2.10. Induction of Isoniazid-Induced Hepatotoxicity and Treatment

Experimental hepatotoxicity was induced by administering INH at 200 mg/kg body weight per day through the intraperitoneal route for seven consecutive days [6]. Following the induction phase, the treatment groups received SFMD-ME orally at doses of 250, 500, and 1000 mg/kg body weight for seven days. The standard-treatment group received silymarin at 100 mg/kg body weight orally over the corresponding treatment period.

The normal-control group received normal saline orally throughout the experimental period, whereas the disease-control group received INH followed by normal saline. The sham-control group received SFMD at 1000 mg/kg body weight orally for 14 days without INH administration.

2.11. Biochemical Assessment of Hepatic Function

At the end of the experimental period, overnight-fasted animals were anaesthetized, and blood samples were collected from the retro-orbital plexus. Serum was separated by centrifugation and analyzed using commercially available diagnostic kits.[6]haejiffffffhaad

The biochemical parameters evaluated included:

- Alanine aminotransferase (ALT)
- Aspartate aminotransferase (AST)
- Alkaline phosphatase (ALP)
- Total bilirubin
- Total protein
- Albumin

These parameters were assessed to investigate the effects of SFMD-ME on the biochemical alterations associated with INH-induced hepatotoxicity.

2.12. Histopathological Examination of Liver Tissue

Liver tissues were collected after completion of the experimental period and preserved in neutral buffered formalin for histopathological examination. The tissue sections were examined microscopically to assess hepatic architecture and treatment-associated changes, including hepatocellular degeneration, fatty changes, cytoplasmic vacuolation, pyknosis, inflammatory infiltration, and central vein congestion.

Histopathological findings were evaluated across the experimental groups to assess the extent of hepatic injury and the apparent protective effects of SFMD-ME.

2.13. Statistical Analysis

Biochemical data were expressed as mean ± standard error of the mean (SEM), with four animals per group. Statistical comparisons were performed using one-way analysis of variance (ANOVA), followed by Tukey's multiple-comparison test, using GraphPad Prism version 10. A p-value of less than 0.05 was considered statistically significant.

3. RESULTS

3.1. HPTLC Fingerprinting of Sugar-Free Majoon-e-Dabeed-ul-Ward

HPTLC analysis of SFMD was performed using silica gel 60 F_{254} plates (**Figure 1**) and different mobile-phase compositions to obtain satisfactory chromatographic separation. The mobile phase consisting of toluene: ethyl acetate: formic acid (6:3:1, v/v/v) provided the reported chromatographic profile, with densitometric analysis performed at 320 nm. The chromatographic fingerprint displayed multiple separated peaks corresponding to the constituents of the polyherbal formulation (**Fig. 2**).

3.2. Identification and Quantification of Ferulic Acid

Ferulic acid was identified by comparing the chromatographic characteristics of the sample with those of the reference standard. The reported (R_f) value for ferulic acid was 0.48. Quantitative analysis indicated a ferulic acid content of 0.443% on a dry-weight basis. The HPTLC densitograms obtained at different applied quantities of the reference standard demonstrated the chromatographic response used for quantitative estimation.

3.3. Calibration and Analytical Recovery

The calibration curve for ferulic acid was constructed by plotting densitometric peak area against the applied quantity of the reference standard at 320 nm (**Fig. 3**). The calibration showed a correlation coefficient (R) of 0.991214 and a coefficient of variation of 5.38%. The reported ferulic acid content in SFMD was 0.443% w/w, with analytical recovery ranging from 95% to 97%. **Table 3** summarises the HPTLC calibration and analytical parameters**.**

3.4. Percentage Yield of the Methanolic Extract

Extraction of 100 g of powdered SFMD yielded 40.8 g of methanolic extract, corresponding to an extraction yield of 40.8% w/w.

3.5. Effect of SFMD on Serum ALT and AST

Administration of INH at 200 mg/kg produced significant elevations in serum ALT and AST compared with the normal-control group ($p < 0.001$). Treatment with SFMD-ME reduced both enzyme levels, with greater reductions generally observed at higher doses.

Serum ALT decreased from 163 ± 15 in the disease-control group to 152 ± 11, 123 ± 10, and 79 ± 6.6 in the 250, 500, and 1000 mg/kg groups, respectively. A statistically significant reduction was reported at 1000 mg/kg ($p < 0.001$).

Similarly, serum AST decreased from 161 ± 7.8 in the disease-control group to 148 ± 12, 111 ± 5.5, and 67 ± 8.4 in the respective SFMD-ME treatment groups. Significant reductions were reported at 500 mg/kg ($p < 0.01$) and 1000 mg/kg ($p < 0.001$). The corresponding biochemical results are presented in **Figure 4(A) and (B).**

3.6. Effect of SFMD on Serum ALP and Total Bilirubin

Serum ALP increased from 173 ± 17 in the normal-control group to 398 ± 22 in the disease-control group. Treatment with SFMD-ME reduced ALP to 314 ± 14, 225 ± 23, and 174 ± 19 at doses of 250, 500, and 1000 mg/kg, respectively. Significant reductions were reported at 500 and 1000 mg/kg ($p < 0.001$).

Total bilirubin increased from 0.55 ± 0.12 mg/dL in the normal-control group to 1.50 ± 0.087 mg/dL in the disease-control group. Following SFMD-ME treatment, the corresponding values were 1.40 ± 0.16, 1.00 ± 0.12, and 0.83 ± 0.11 mg/dL. A statistically significant reduction was reported at 1000 mg/kg ($p < 0.01$). The results for ALP and total bilirubin are presented in **Figure 4(C) and (D),** respectively.

3.7. Effect of SFMD on Total Protein and Albumin

Total protein decreased from 7.4 ± 0.065 g/dL in the normal-control group to 5.4 ± 0.20 g/dL in the disease-control group. Treatment with SFMD-ME at 250, 500, and 1000 mg/kg resulted in total protein values of 5.9 ± 0.20, 6.7 ± 0.16, and 6.7 ± 0.18 g/dL, respectively. Significant increases were reported at 500 and 1000 mg/kg ($p < 0.001$).

Serum albumin decreased from 5.0 ± 0.096 g/dL in the normal-control group to 2.8 ± 0.13 g/dL in the disease-control group. Following SFMD-ME treatment, albumin values were 3.0 ± 0.30, 3.7 ± 0.35, and 4.0 ± 0.18 g/dL at the respective doses. A statistically significant increase was reported at 1000 mg/kg ($p < 0.05$). The results for total protein and albumin are presented in **Figure 4(E) and (F),** respectively.

The biochemical findings for all experimental groups, including ALT, AST, ALP, total bilirubin, total protein, and albumin, are summarized in **Table 4**.

3.8. Histopathological Examination of Liver Tissue

Histopathological examination revealed normal hepatic architecture in the normal-control and sham-control groups, characterized by polygonal hepatocytes arranged in trabeculae, clear cytoplasm, and normal nuclei.

The disease-control group exhibited substantial hepatic alterations, including fatty changes, cytoplasmic vacuolation, pyknosis, inflammatory infiltration, and central vein congestion. The SFMD-ME 250 mg/kg group showed severe residual hepatic changes, whereas the 500 mg/kg group exhibited moderate alterations. The 1000 mg/kg group showed comparatively mild changes, including slight vacuolation, fatty changes, pyknosis, and inflammatory infiltration, without apparent central vein congestion.

The silymarin-treated group showed minimal histological alterations, with negligible vacuolation and pyknosis and mild inflammatory infiltration. Overall, the histopathological observations showed a pattern of progressively reduced hepatic alterations with increasing SFMD-ME dose. Representative liver sections from the experimental groups are presented in **Figure 5(A-G)**.

4. DISCUSSION

The present study investigated the phytochemical characteristics and hepatoprotective potential of Sugar-Free Majoon-e-Dabeed-ul-Ward (SFMD) by integrating HPTLC analysis with an experimental model of isoniazid (INH)-induced hepatotoxicity. The findings provide preliminary evidence of the biological activity of the modified formulation and extend the investigation of traditional Majoon-e-Dabeed-ul-Ward (MDW) to a different hepatotoxicity model.

Previous studies by Shakya and Shukla (2011) and Shakya et al. (2012) demonstrated the hepatoprotective effects of traditional MDW against acetaminophen- and carbon tetrachloride-induced liver injury, respectively [7,8]. These studies reported dose-dependent improvements in biochemical and histopathological parameters, while the latter also demonstrated improvements in oxidative stress-related markers, including lipid peroxidation and reduced glutathione. In the present study, SFMD-methanolic extract (SFMD-ME) similarly produced dose-related improvements in hepatic biochemical parameters following INH exposure. ALT decreased from 163 ± 15 in the disease-control group to 79 ± 6.6 at 1000 mg/kg, while AST decreased from 161 ± 7.8 to 67 ± 8.4. Improvements in ALP, total bilirubin, total protein and albumin were also observed, particularly at the medium and high doses. These findings are broadly consistent with earlier reports, although differences in formulation and hepatotoxicity models prevent direct comparison of efficacy.

The biochemical findings were supported by liver histopathology. The disease-control group exhibited marked hepatic abnormalities, whereas comparatively milder changes were observed in the high-dose SFMD-ME group. This pattern agrees with the dose-dependent histopathological improvement reported in the earlier MDW studies [9]. However, the present histopathological assessment was qualitative; therefore, the extent of tissue protection requires confirmation through quantitative scoring.

HPTLC analysis identified ferulic acid at an approximate (R_f) value of 0.48 and reported its content as 0.443% w/w, with recovery values of 95-97%. This provides a preliminary basis for using ferulic acid as a marker for the chemical characterization of SFMD [10]. Unlike the earlier hepatoprotective studies of traditional MDW, the present investigation also incorporated marker-compound estimation. Nevertheless, the contribution of ferulic acid to the observed biological effects cannot be established from the current findings, as the study did not investigate its specific activity or directly correlate its concentration with hepatoprotection.

5. LIMITATIONS AND FUTURE PERSPECTIVES

The present study has certain limitations, including the small number of animals per group, qualitative histopathological assessment and limited phytochemical characterization. Furthermore, the HPTLC method requires additional validation, and the contribution of ferulic acid to the observed hepatoprotective effects remains undetermined.

Future studies should include adequately powered experimental designs, quantitative histopathological scoring, comprehensive phytochemical profiling and further validation of the HPTLC method. Investigations of oxidative stress and inflammatory biomarkers, together with studies examining the relationship between chemical composition and biological activity, would help clarify the potential mechanisms underlying the observed effects of SFMD.

6. CONCLUSION

The present study integrated HPTLC-based phytochemical characterization with the evaluation of the hepatoprotective potential of Sugar-Free

Majoon-e-Dabeed-ul-Ward (SFMD). HPTLC analysis enabled the identification and quantification of ferulic acid in the formulation, with a reported content of 0.443% w/w and recovery values of 95-97%. In the experimental model of isoniazid-induced hepatotoxicity, the methanolic extract of SFMD demonstrated dose-related improvements in serum biochemical parameters, particularly at 500 and 1000 mg/kg, supported by improvements in liver histopathological features. These findings provide preliminary evidence of the hepatoprotective potential of SFMD and demonstrate the usefulness of combining chromatographic characterization with biological evaluation. However, the limited sample size, the need for further validation of the HPTLC method, and the absence of detailed mechanistic investigations warrant cautious interpretation. Further studies involving comprehensive phytochemical profiling, validated analytical methods, mechanistic assessment, and larger experimental groups are required to substantiate these findings.

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AUTHOR CONTRIBUTIONS

Conceptualization and Supervision: SA, MM; Original draft preparation and writing: JA; Draft revision and editing: JA, RPM, MM, SA; Final approval: JA, RPM, MM, SA. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this article.

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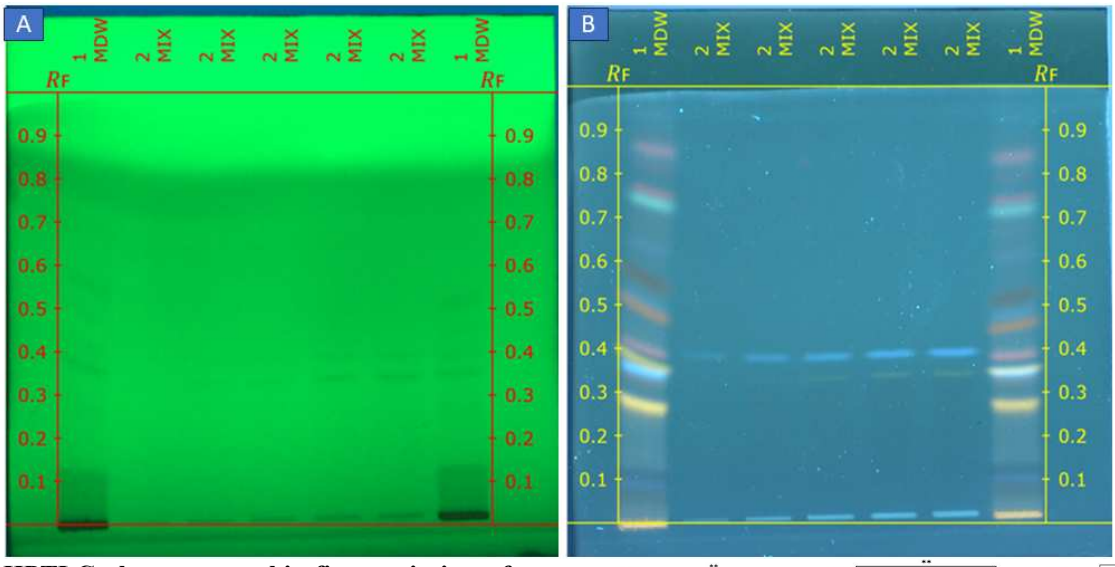


Figure 1. HPTLC chromatographic fingerprinting of Sugar-Free Majoon-e-Dabeed-ul-Ward (SFMD) at different wavelengths: (A) densitogram at 254 nm and (B) densitogram at 366 nm, showing the chromatographic profiles of the formulation.

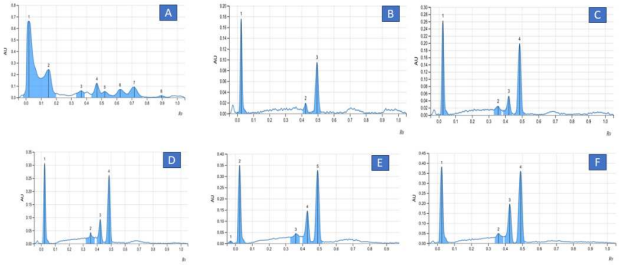


Figure 2. HPTLC densitograms showing the chromatographic fingerprint of Sugar-Free Majoon-e-Dabeed-ul-Ward (SFMD) and the standard mixture at 320 nm: (A) SFMD sample showing eight distinct peaks (1-8); at application volumes of (B) 0.4 µL, (C) 0.8 µL, (D) 1.2 µL, (E) 1.6 µL, and (F) 2.0 µL, each showing four distinct peaks (1–4).

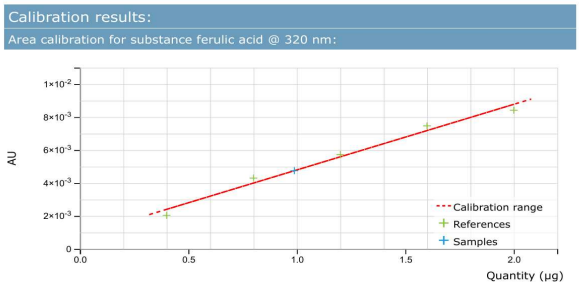


Figure 3. HPTLC calibration curve of ferulic acid at 320 nm, showing the linear regression of peak area against applied quantity (0.4-2.0µg/spot).

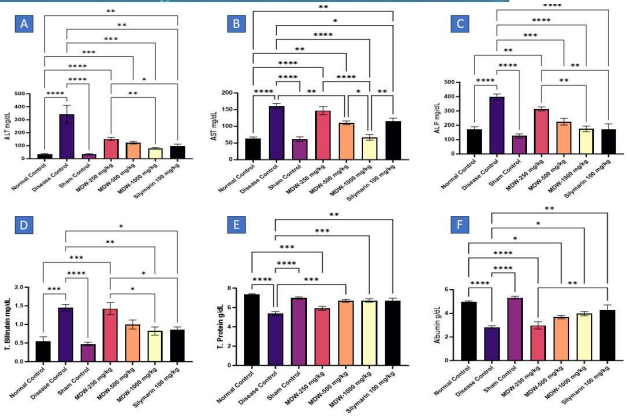


Figure 4. Effects of Sugar-Free Majoon-e-Dabeed-ul-Ward (SFMD) on serum biochemical parameters in isoniazid (INH)-induced hepatotoxicity in rats: (A) alanine aminotransferase (ALT), (B) aspartate aminotransferase (AST), (C) alkaline phosphatase (ALP), (D) total bilirubin, (E) total protein, and (F) albumin. Data are expressed as mean ± SEM (n = 4). Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer multiple-comparison test.

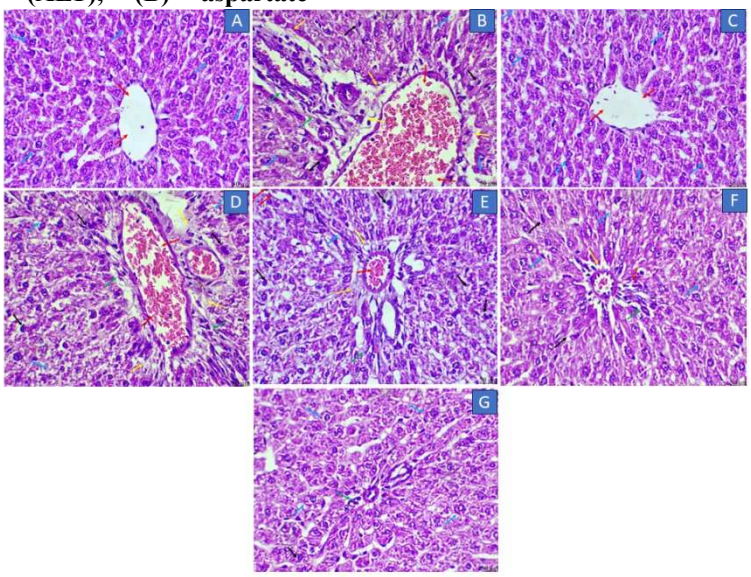


Figure 5. Representative photomicrographs of liver sections from rats subjected to different treatments in the INH-induced hepatotoxicity model: (A) normal control, showing normal hepatic architecture; (B) disease control, showing severe hepatocellular damage, fatty changes, vacuolation, pyknosis, inflammatory infiltration, and central vein congestion; (C) sham control (SFMD 1000 mg/kg), showing normal hepatic architecture; (D) SFMD-ME 250 mg/kg, showing severe hepatic alterations; (E) SFMD-ME 500 mg/kg, showing moderate hepatic alterations; (F) SFMD-ME 1000 mg/kg, showing mild to minimal histopathological changes; and (G) silymarin 100 mg/kg, showing mild to minimal hepatic alterations. Arrows indicate the respective histopathological features as follows: blue, hepatocytes; red, central vein/congestion; yellow, fatty changes; white, vacuolation; black, pyknosis; and green, inflammatory infiltrates. Hematoxylin and eosin (H&E) staining, 400× magnification.

Table 1. Composition of Classical Majoon Dabeed-ul-Ward

S. No.	Unani Name	Botanical Name	Part Used	Weight (g)
1	Sumbul-ut-Teeb	Nardostachys jatamansi DC.	Root	10
2	Mastagi	Pistacia lentiscus Linn.	Resin	10
3	Zafran	Crocus sativus Linn.	Flower (stigma)	10
4	Tabasheer	Bambusa bambos (L.) Voss.	Resin	10
5	Darchini	Cinnamomum zeylanicum Blume	Stem bark	10
6	Izkhar	Cymbopogon jwarancusa (Jones) Schult.	Whole plant	10
7	Asaroon	Asarum europaeum Linn.	Root	10
8	Qust Shireen	Saussurea hypoleuca Spreng.	Root	10
9	Gul-e-Ghafis	Gentiana olivieri Griseb	Flowers	10
10	Tukm-e-kasoos	Cuscuta reflexa Roxb.	Seed	10
11	Majeeth	Rubia cordifolia Linn.	Root	10
12	LukMaghsool	Coccus lacca	Secretion by leaves insect	10
13	Tukm-e-Kasni	Cichorium intybus Linn.	Seed	10

14	Tukm-e-Karafs	Apium graveolens Linn.	Seed	10
15	Zara wand Taweel	Aristolochia indica Linn.	Root	10
16	Ood-e-Balsan	Commiphora opobalsamum Linn.	Stem	10
17	Ood-e-Hindi	Aquilaria agalocha Roxb.	Stem	10
18	Qaranful	Syzygium aromaticum (Linn.) Merr. & Perry	Dried buds	10
19	Heel Khurd	Elettaria cardamomum Maton.	Dried fruit	10
20	Waaraq-e-Gul-e-Surkh	Rosa damascena Mill.	Petals of Rose	200
21	Qand Safed /Asl Khalis	Granular Sugar/Honey		600

Table 2. Experimental Groups and Treatment Schedule

Group	Experimental group	Treatment	Duration
I	Normal Control	Normal saline (1 mL/kg body weight, p.o.)	14 days
II	Disease Control	Isoniazid (INH) 200 mg/kg/day, i.p. for 7 days, followed by Normal saline (1 mL/kg body weight, p.o.) for the next 7 days	14 days
III	Sham Control I	SFMD 1000 mg/kg, p.o. for 14 days	14 days
IV	Treatment Group I – Low dose	INH 200 mg/kg/day, i.p. for 7 days, followed by SFMD 250 mg/kg, p.o. for the next 7 days	14 days
V	Treatment Group II – Medium dose	INH 200 mg/kg/day, i.p. for 7 days, followed by SFMD 500 mg/kg, p.o. for the next 7 days	14 days
VI	Treatment Group III – High dose	INH 200 mg/kg/day, i.p. for 7 days, followed by SFMD 1000 mg/kg, p.o. for the next 7 days	14 days
VII	Standard Drug	INH 200 mg/kg/day, i.p. for 7 days, followed by Silymarin 100 mg/kg, p.o. for the next 7 days	14 days
MDD: Modified Majoon Dabeed-ul-Ward; INH: Isoniazid			

Table 3. HPTLC calibration and analytical parameters for ferulic acid

Parameter	Result
Reference standard	Ferulic acid
Detection wavelength	320 nm
Regression equation	$y = 3.983 \times 10^{-9}x + 8.105 \times 10^{-4}$.
Correlation coefficient (R)	0.991214
Coefficient of variation (CV)	5.38%
Number of calibration reference points	5
Ferulic acid content in SFMD	0.443% w/w
Analytical recovery	95-97%

Table 4. Effect of SFMD on Biochemical Parameters in INH-induced Hepatotoxicity in Rats

Experimental group/ Parameter	Normal Control	Disease Control	Sham Control	SFMD-250 mg/kg	SFMD-500 mg/kg	SFMD-1000 mg/kg	Silymarin 100 mg/kg
ALT (IU/L)	37± 3.9	163 ±15	34± 5.1	152 ±11	123 ±10	79± 6.6	98± 14
AST (IU/L)	63± 5.0	161 ±7.8	62± 7.2	148 ±12	111 ±5.5	67± 8.4	115 ±8.9

ALP (IU/L)	173 ±17	398 ±22	126 ±15	314 ±14	225 ±23	174 ±19	171 ±39
T Bilirubin (mg/dL)	0.55 ±0.12	1.5 ±0.087	0.48 ±0.048	1.4 ±0.16	1.0 ±0.12	0.83±0.11	0.85 ±0.087
T Protein (g/dL)	7.4 ±0.065	5.4 ±0.20	7.0± 0.13	5.9 ±0.20	6.7 ±0.16	6.7 ±0.18	6.7± 0.29
Albumin (g/dL)	5.0 ±0.096	2.8 ±0.13	5.3± 0.13	3.0 ±0.30	3.7 ±0.15	4.0 ±0.18	4.3± 0.41