Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Qualitative And Hplc Phytochemical Screening Of Salvia Hians Crude Extract For Its Hepato-Protective Potential In Animal Model

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ABSTRACT

Background: *Salvia hians* is traditionally recognized for its therapeutic potential, but comprehensive evaluation of its phytochemical constituents and hepatoprotective properties remains limited. This study aimed to investigate the qualitative phytochemical composition, HPLC profile, acute toxicity, and dose-dependent hepatoprotective efficacy of *Salvia hians* crude extract (SH-Crd) in an animal model of paracetamol-induced liver injury.
Methods: Qualitative screening was conducted to identify key secondary metabolites in the methanolic extract. High-Performance Liquid Chromatography (HPLC) was utilized to quantify major phenolic acids, flavonoids, and diterpenoids, as well as total

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Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

phenolic (TPC) and total flavonoid contents (TFC). Acute toxicity testing was performed in two phases up to 2000 mg/kg body weight over a 24-hour observation period. The hepatoprotective efficacy of SH-Crd (100, 200, and 400 mg/kg) was evaluated against paracetamol toxicity using serum biomarker analysis (SGPT, AST, ALP, and bilirubin) and liver histopathological examination, comparing results with standard Silymarin (500 mg/kg).

Results: Qualitative phytochemical testing revealed a rich presence of flavonoids (+ + +) and phenolic compounds (+ + + +), alongside moderate levels (+ +) of alkaloids, tannins, saponins, terpenoids, glycosides, and carbohydrates. HPLC analysis demonstrated high concentrations of rosmarinic acid (147.44 mg/g extract; 11.9% abundance), luteolin-7-O-glucuronide (118.08 mg/g; 10.2%), and salvianolic acid B (97.67 mg/g; 8.4%), with TPC and TFC values of 151.8 mg/g GAE and 91.6 mg/g QE, respectively. SH-Crd displayed complete safety with zero mortality across all doses (50–2000 mg/kg). In paracetamol-induced hepatotoxicity, SH-Crd treatment yielded dose-dependent restoration of functional serum markers. The 400 mg/kg dose achieved the highest hepatoprotection, significantly reducing SGPT (31.7 ± 1.34 U/L), AST (43.1 ± 2.22 U/L), ALP (116.3 ± 2.36 U/L), and total bilirubin (0.96 ± 0.09 mg/dL) to levels comparable to the Silymarin reference group. Histopathological evaluation confirmed that 400 mg/kg SH-Crd effectively attenuated severe hepatocellular degeneration, inflammation, sinusoidal dilation, and necrosis to preserve near-normal liver architecture.

Conclusion: *Salvia hians* crude extract exhibits a non-toxic profile and strong dose-dependent hepatoprotective activity against paracetamol-induced liver injury. These effects are likely driven by its substantial concentration of antioxidant polyphenols, including rosmarinic acid, salvianolic acid B, and luteolin derivatives.

Keywords: *Salvia Hians*, Phytochemical Screening, High-Performance Liquid Chromatography (HPLC), Hepatoprotective Activity, Paracetamol-Induced Toxicity, Silymarin, Histopathology

Introduction

The liver is a vital metabolic and detoxifying organ that plays a central role in the biotransformation and elimination of endogenous and exogenous substances. Due to these functions, it is constantly exposed to drugs, environmental chemicals, alcohol, nutritional imbalances, and reactive metabolites. Liver injury can occur when such challenges overwhelm its antioxidant defenses and repair mechanisms [1]. Oxidative stress is an important contributor to this damage, as excessive reactive oxygen species may cause lipid peroxidation, protein and DNA damage, mitochondrial dysfunction, inflammation, and hepatocellular apoptosis [1].

The search for safer hepatoprotective agents has increasingly focused on medicinal plants and their secondary metabolites. Plant-derived constituents, including phenolic acids, flavonoids, terpenoids, alkaloids, tannins, and saponins, may support liver protection through complementary antioxidant, anti-inflammatory, membrane-stabilizing, and cytoprotective actions. Nevertheless, the therapeutic potential of a

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plant extract cannot be established solely from traditional use or the presence of phytochemical classes. Its composition may vary with species, plant part, geographical origin, extraction solvent, harvesting conditions, and storage. Therefore, phytochemical characterization and extract standardization are essential for preclinical evaluation, while bioavailability, dose optimization, and safety remain important translational challenges [3, 4]

Evidence from related species supports the hepatoprotective potential of the genus. In a rat model of carbon-tetrachloride-induced hepatic injury, an ethanol extract of *Salvia hypoleuca* improved serum bilirubin, protein, alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase measurements, while histological examination showed reduced necrosis and fatty infiltration [3]. Likewise, experimental studies of *Salvia officinalis* and *Salvia hispanic*a have reported improvements in liver enzymes, antioxidant status, or hepatic histology in chemically or drug-induced injury models [5][6][7]. These observations suggest that antioxidant-rich Salvia extracts may influence pathways relevant to hepatic injury, but they represent evidence from other species and cannot substitute for direct evaluation of *S. hians*.

Accordingly, qualitative phytochemical screening and high-performance liquid chromatography (HPLC) are complementary approaches for the preliminary characterization of S. hians crude extract. Qualitative tests can indicate the presence of broad metabolite classes, whereas HPLC provides a more reproducible chromatographic profile and can support the detection or estimation of selected marker compounds. Validated chromatographic approaches in Salvia research have been used to optimize separation, evaluate repeatability and recovery, establish detection and quantification limits, and compare phytochemical fingerprints across plant parts and collection years [8, 9, 10]. The resulting chemical information can then be interpreted alongside biological outcomes rather than treated as an isolated measure of efficacy.

An animal model is necessary to determine whether the chemical constituents of the crude extract are associated with protection against experimentally induced liver injury. Relevant endpoints include serum alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, bilirubin, total protein and albumin; tissue antioxidant or lipid-peroxidation measures may provide additional mechanistic information; and histopathological examination can assess whether biochemical changes correspond to structural preservation of the liver. Studies of plant extracts commonly combine these biochemical and histological endpoints because no single marker fully captures hepatic injury or recovery [11][12].

Despite the broader pharmacological interest in the *Salvia* genus, direct evidence concerning the phytochemical composition and hepatoprotective activity of *Salvia hians* appears limited in the retrieved literature. This knowledge gap provides the rationale for the present study, which investigates the crude extract of *S. hians* through qualitative phytochemical screening, HPLC profiling, and evaluation in an animal model of hepatic injury. By integrating chemical characterization with biochemical and histopathological assessment, the study aims to determine whether *S. hians* possesses measurable hepatoprotective potential and to generate a preliminary evidence base for future isolation, mechanistic, toxicity, and standardization studies.

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Materials and Methods

Collection and authentication of plants

Salvia hians aerial parts (leaves, stems, flowers) were collected in flowering (July-August 2024) in the location: Bagh region, Azad Jammu & Kashmir, Pakistan with coordinates: 34°02'52"N 74°22'26"E with the altitude of 2650 meters above the sea level in habitat *Pinus wallichiana* and *Rhododendron campanulatum.* Authentication was done by identification with Flora of Kashmir (Stewart, 1972), by means of herbarium preparation (voucher specimen no. BSI/KASH/2022/SH-89), by official authentication by Botanical Survey of AJK University Bagh, (Authentication certificate no. BSI/DRC/2022/412). Processing involved shade-drying of plant material in 25±2ºC 21 days, moisture levels were brought to 8.2% (determined using the oven drying technique), powdered using mechanical grinder (particle size 0.5-1.0 mm), powder stored in airtight containers at 4ºC until the extraction [13].

Preparation of Plant Extract for Phytochemical Analysis

The shade-dried aerial portions of *Salvia hians* were coarsely powdered using a mechanical grinder. The powdered plant material was extracted by maceration with 80% methanol at room temperature, with periodic shaking during the extraction process. Following extraction, the mixture was filtered through Whatman No. 1 filter paper, and the solvent was removed under reduced pressure using a rotary evaporator. The resulting crude extract, designated as Sh-Crd, was collected and stored at 4 °C until further analysis [14].

Preliminary qualitative phytochemical screening of the *S. hians* crude extract was performed to determine the presence of major classes of secondary metabolites using standard chemical tests reported in the literature. The required reagents were freshly prepared before analysis. Each test was performed separately, and the results were assessed visually on the basis of characteristic colour changes and/or precipitate formation. The intensity of the observed reactions was recorded as weak (+), moderate (++), strong (+++), or very strong (++++), as appropriate [15].

Qualitative Tests for Phytochemical Constituents

Qualitative phytochemical preliminary screening of the *Salvia hians* crude extract was conducted to identify key secondary metabolites using established qualitative chemical assays.

To screen for alkaloids, the extract was subjected to Mayer's and Wagner's reagents, where the formation of a characteristic cream-colored precipitate and a reddish-brown precipitate, respectively, confirmed their presence. Flavonoids were identified through three distinct chemical evaluations: the Shinoda test, where magnesium turnings and concentrated hydrochloric acid produced a magenta-red color; the Alkaline Reagent test, which demonstrated an intense yellow coloration that cleared upon acidification; and the Lead Acetate test, evidenced by a distinct yellow precipitate. Phenolic contents were identified using the Ferric Chloride test based on the appearance of a dark blue-black complex, while tannins were evaluated using both the Gelatin test, marked by a white precipitate, and Braemer's test, indicated by a characteristic green coloration.

The presence of saponins was confirmed via the Froth test upon the formation of

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Online ISSN 3007-3197
Print ISSN 3007-3189
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persistent honeycomb foam following vigorous agitation. Terpenoids and steroids were evaluated using the Salkowski test and the Liebermann–Burchard test, yielding a reddish-brown interface for terpenoids and a green-to-blue color change for steroids and triterpenoids, respectively. Cardiac glycosides were assessed using the Keller–Killiani test, which produced a reddish-brown ring at the interface, and Legal's test, indicated by a pink color reaction. Finally, primary carbohydrate metabolites and reducing sugars were identified using Molisch's test (violet ring formation) and Benedict's test (brick-red precipitate formation), respectively.
The observed qualitative color reactions and precipitate formations were graded according to reaction intensity as weak (+), moderate (++), strong (+++), or very strong (++++) following established methodology [15].

HPLC Analysis
The phytochemical profile of *Salvia hians* crude methanolic extract (Sh-Crd) was analyzed by reverse-phase high-performance liquid chromatography (RP-HPLC) equipped with a UV–Visible detector. The extract was dissolved in HPLC-grade methanol at a concentration of 10 mg/mL, sonicated for 10 min, and filtered through a 0.45 µm membrane filter before injection. Chromatographic separation was achieved on a C18 column (250 × 4.6 mm, 5 µm particle size) using 0.1% formic acid in distilled water (solvent A) and acetonitrile (solvent B) under gradient elution. The flow rate was maintained at 1.0 mL/min, with an injection volume of 20 µL and the column maintained at ambient temperature. Detection was performed at 254, 280, and 330 nm. Phytoconstituents were identified by comparison of retention times and UV absorption spectra with reference standards and published data. Quantification was performed using calibration curves generated from standard solutions and expressed as mg/g of extract and relative peak-area abundance [16].

Acute Toxicity Study
Acute oral toxicity was evaluated according to **OECD Guideline 423 (Acute Toxic Class Method)** following approval from the Institutional Animal Ethics Committee (EC/AWKUM/2024/11). Healthy rats (180–200 g; 8–10 weeks old) were maintained under standard laboratory conditions (22 ± 2°C, 55 ± 10% humidity, 12 h light/dark cycle) with standard pellet diet and water available ad libitum. Animals were acclimatized for 7 days before experimentation.
Swiss albino mice of both sexes, weighing 25–40 g, were selected for the acute oral toxicity assessment. The experiment was performed in two sequential phases to evaluate the safety profile of the synthesized aurone derivatives over a broad dose range. During Phase I, the animals were administered the test compounds orally at doses of 50, 100, and 200 mg/kg. In Phase II, higher doses of 500, 1000, and 2000 mg/kg were administered orally. Following treatment, the animals were observed continuously for any signs of toxicity, with particular attention to mortality during the subsequent 24-hour observation period [17].

Experimental Animals and Grouping
Animals used were Wistar rats (180-200gm) aged 10-12 weeks, males (to avoid hormonal variations), housed in polypropylene cages with 6 rats per cage under

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conditions of 22±2°C, 55±10% humidity, 12h light/dark cycle, with standard pellet diet (Amrut Lab, India) and water ad libitum, and acclimatization for 7 days [18].
Grouping included seven groups (n=4 each): Normal Control receiving vehicle (0.5% CMC, 10 mL/kg); Toxic Control receiving paracetamol 1000 mg/kg (single dose); Standard receiving silymarin 100mg/kg along with paracetamol; Sh.crd-100 receiving *S. hians* extract 100mg/kg+paracetamol; Sh.crd-200 receiving *S. hians* extract 200 mg/kg + paracetamol; Sh.crd 400 recives *S. hians* extract 400mg/kg+paracetamol.

The in vivo Hepatoprotective activity
Experimental Design
The hepatoprotective activity of the crude extract of the plant *Salvia hians* (Sh.Crd) was tested by using a paracetamol (300mg/kg) induced hepatotoxicity test in male Wistar rats. The experimental subjects were randomly allocated to control, toxic control, standard and treatment cohorts. Sh.Crd was orally transferred at doses of 100, 200, and 400mg/kg/BW daily within 28 consecutive days. Hepatoprotective reference was Silymarin. Day 1-28, animals were presented to Day-specific oral interventions (vehicle, silymarin, or Sh.Crd) through oral gavage. Biochemical markers of hepatic injury were assessed by the blood samples 24 hours after administration of paracetamol under light ether anaesthesia. Animals were euthanized and liver tissues were harvested on Day 28 and assessed biochemically.

Sample Collection and Processing (Liver)
Cardiac puncture was conducted under light ether anaesthesia and blood was collected by cardiac puncture and serum was isolated by centrifugation at 3000 rpm during 15 min of centrifugation at 4ºC after the formation of a clot. Biochemical analysis was performed on serum aliquots that were stored at 4-8°C.

Liver Function Tests
The serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin and direct bilirubin were examined using an automated Erba Mannheim analyzer. The ALT and AST were conducted using the IFCC method, AMP buffer method was used on ALP, and bilirubin fractions was done using diazo method [18].

Histopathological examination of paracetamol-induced liver injury:
Twenty-eight male rats were randomly divided into four groups of seven animals each: normal control, paracetamol-treated, paracetamol-plus-Sh.Crd , and Silymarin treated group. The paracetamol group received 2 g/kg paracetamol intraperitoneally to induce acute hepatic injury. The Sh.Crd-only group received 100, 200, 400 mg/kg Sc.Crd, while control animals received distilled water and saline and Standard group received Silymarin. At the end of the experimental period, the animals were euthanized and blood and liver samples were collected. The excised livers were examined for gross pathological changes and representative tissue samples were obtained. Liver tissues were fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin wax, and sectioned at approximately 3–5 µm.

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Online ISSN 3007-3197
Print ISSN 3007-3189

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Sections were stained with hematoxylin and eosin and examined under a light microscope for hepatocellular degeneration, necrosis, congestion, vacuolation, hemorrhage, and inflammatory-cell infiltration [19]

Statistical Analysis

Graphpad Prism version 5.0 was used to analyse the statistics. Continuous variables were given in the form of mean + standard error of the mean (SEM), whereas the categorical variables were given in the form of frequencies (percentages). Multiple group comparison was performed by one way analysis of variance (ANOVA) and post hoc test which is Dunnett's test. A significance level of $p \leq 0.001$ was used. The number of animals was n=4 in each of the experimental group

Results:

Qualitative Analysis of Crude Extracts of *Salvia hians*

Phytochemical profile of the methanolic extract was rich and broad boasted of strong presence of flavonoids and phenolic compounds, moderate levels of alkaloids, terpenoids and tannins, and traceable levels of saponins, glycosides, and carbohydrates. The high presence of phenolic and flavonoid compounds indicates a significant antioxidant ability, which is supported by the biological activities. The qualitative phytochemical analysis clearly shows that the methanolic extract of the *Salvia hians* is a source of a broad range of bioactive secondary metabolites. The positive reaction of considerable intensity to phenolic compounds and high level of positivity to flavonoids do signify that these compounds are prevailing in the extract as shown in table 1. Phenolics and flavonoids have been widely known to have free-radical scavenging, anti-inflammatory and organ-protective effects and so explains the antioxidant and protective effects that were witnessed in later biological studies.

Table 1 : Qualitative Phytochemical Analysis of *Salvia hians* Methanolic Extract

Phytoconstituents	**Test performed**	**Result**
Alkaloids	Mayer's Test	++
Flavonoids	Shinoda Test	+++
Phenolic compounds	Ferric Chloride Test	++++
Tannins	Braymer's Test	++
Saponins	Froth Test	++
Terpenoids	Salkowski Test	++
Steroids	Liebermann–Burchard Test	+
Glycosides	Keller–Killiani Test	++
Carbohydrates	Molisch's Test	++

HPLC Analysis of *Salvia hians* Crude Extract

HPLC profiling of the *Salvia hians* crude extract (SH-Crd) revealed a diverse range of phenolic acids, flavonoids, and diterpenoids. Among the identified compounds**, rosmarinic acid was the most abundant constituent,** with a concentration of **147.44 mg/g extract** and a relative abundance of **11.9%,** followed by **luteolin-7-O-glucuronide (118.08 mg/g; 10.2%)** and **salvianolic acid B (97.67 mg/g; 8.4%).**

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Online ISSN 3007-3197
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Other notable constituents included carnosic acid (68.10 mg/g), apigenin (55.34 mg/g), carnosol (45.76 mg/g), hispidulin (38.87 mg/g), caffeic acid (38.31 mg/g), chlorogenic acid (32.88 mg/g), and cirsimaritin (26.34 mg/g). Overall, the extract showed appreciable levels of phenolic and flavonoid constituents, with **total phenolic content of 151.8 mg/g GAE** and **total flavonoid content of 91.6 mg/g QE.** The presence of these bioactive phytoconstituents, particularly rosmarinic acid, salvianolic acid B, luteolin-7-O-glucuronide, and diterpenoids, indicates a chemically rich extract that may contribute to the observed pharmacological and antioxidant properties of *S. hians.*

Table 2: HPLC Quantification of Major Bioactive Compounds in *Salvia hians* Crude Extract (SH-Crd)

Compound	**Compound Class**	**Retention Time (min)**	**Concentration (mg/g extract)**	**Relative Abundance (%)**
Rosmarinic acid	Phenolic Acids	19.53	147.44	11.9
Salvianolic acid B		26.08	97.67	8.4
Caffeic acid		12.44	38.31	4.02
Chlorogenic acid		16.04	32.88	3.1
Luteolin-7-O-glucuronide	Flavonoids	22.21	118.08	10.2
Apigenin		31.26	55.34	5.2
Hispidulin		27.67	38.87	4.1
Cirsimaritin		33.02	26.34	1.9
Carnosic acid	Diterpenoids	36.46	68.1	6.3
Carnosol		35.06	45.76	4.5
Total Quantification	Phenolic Content as of GAE		151.8 mg/g	
	Flavonoid Contents as of QE		91.6 mg/g	

Relative abundance was calculated in percentage. Values are expressed as Mean. GAE: Gallic Acid Equivalent; QE: Quercetin

Acute Toxicity:

The Sh.Crds were determined to be safe at both phases as shown in table 3. In phase –l and Phase-2, all the test compound at doses of 50, 100 and 200 mg/kg and higher doses 500, 1000 and 2000mg/kg body weights were safe while observing them for 24 hours. All the animals remained in a normal state and exhibited no signs of toxicity throughout the 24-hour assessment period as shown in Table 3.

Table 3: Acute toxicity assay results of *Salvia hians* in general

Phases	**mg/kg**	**Mortality %**	**Survival %**
Phase I	50	0	100
	100	0	100
	200	0	100
Phase II	500	0	100
	1000	0	100
	2000	0	100

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Print ISSN 3007-3189

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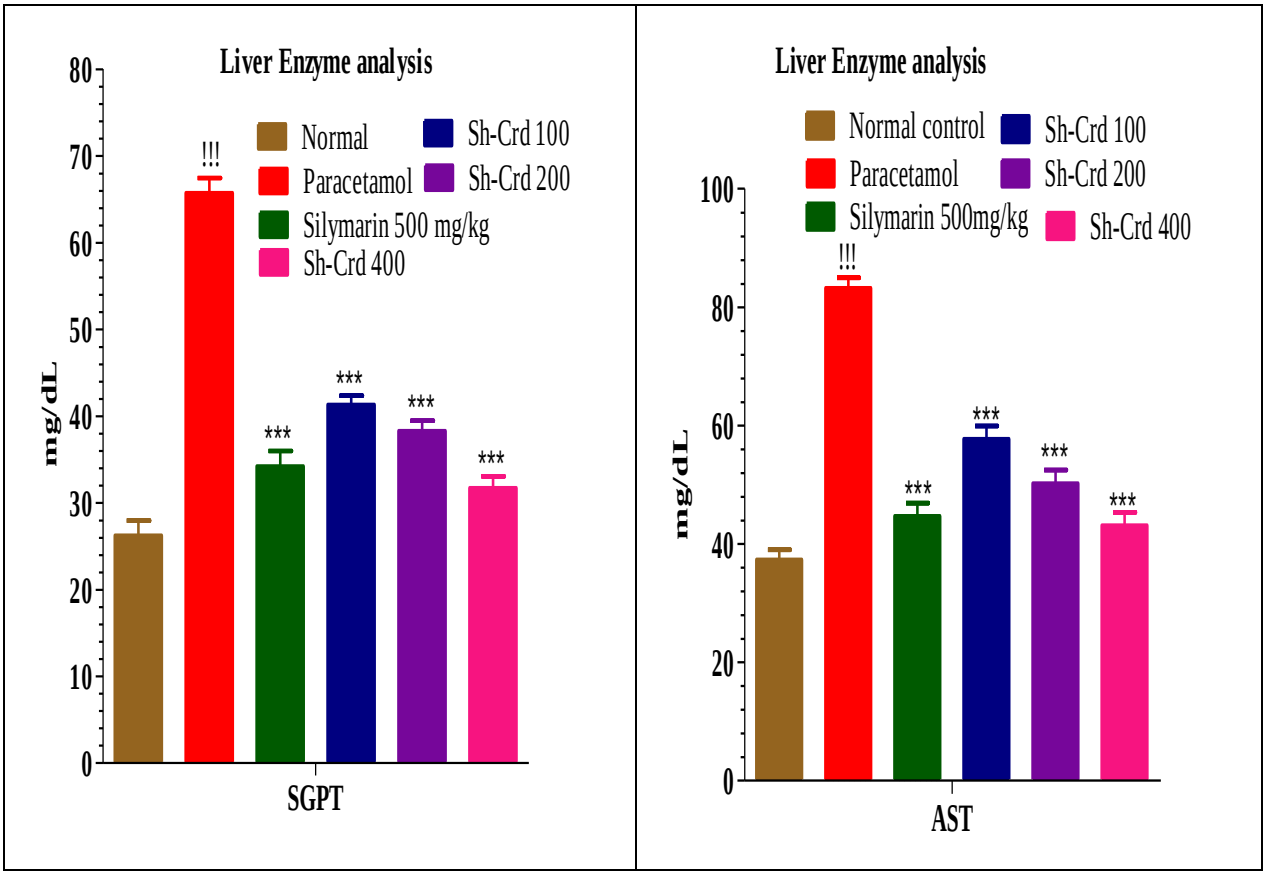
Effect of *Salvia hians* Crude Extract on Liver Function

These results support the claim that *Salvia hians* crude extract provides a substantial protection against hepatotoxicity caused by paracetamol in a dose-dependent mode, probably by stabilization of the hepatocellular membranes and maintenance of the hepatic functional capacity.

Paracetamol administration markedly impaired liver function, as reflected by significant elevations in **SGPT, AST, ALP, and serum bilirubin** compared with the normal group. Treatment with *Salvia hians* crude extract (SH-Crd) produced a dose-dependent improvement in these biochemical parameters. At **100 mg/kg,** SGPT, AST, ALP, and bilirubin decreased to 41.3, 57.7, 129.1 U/L, and 1.02 mg/dL, respectively. The **200 mg/kg dose produced further improvement,** while the **400 mg/kg dose showed the greatest overall protection**, reducing SGPT to **31.7 ± 1.34 U/L**, AST to **43.1 ± 2.22 U/L,** ALP to **116.3 ± 2.36 U/L,** and serum bilirubin to **0.96 ± 0.09 mg/dL**, approaching the values observed in the normal and silymarin-treated groups. Notably, the highest dose produced biochemical responses comparable to **silymarin (500 mg/kg),** particularly for SGPT and AST. These findings indicate that SH-Crd substantially attenuated paracetamol-induced hepatic dysfunction, with the protective effect becoming more pronounced at higher doses. The hepatoprotective activity may plausibly be associated with the phenolic acids, flavonoids, and diterpenoids identified in the HPLC profile, although mechanistic confirmation would require further investigation.



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Print ISSN 3007-3189

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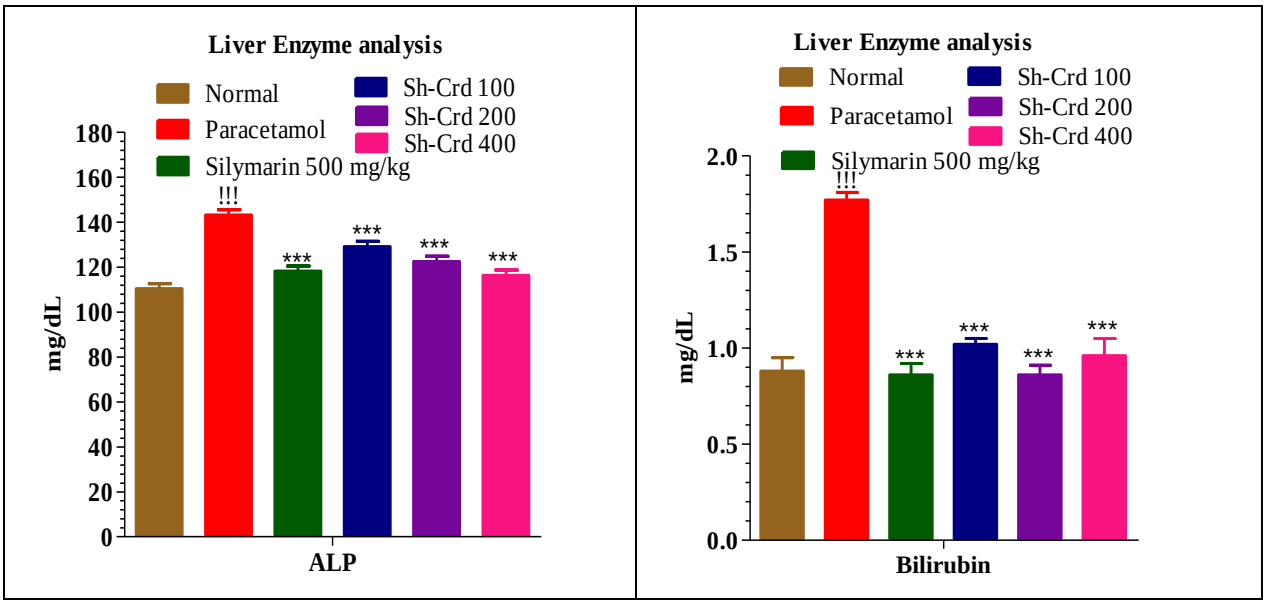

Figure 4. 1: Liver function test.
The data is expressed as mean ± SEM (n=6) one-way ANOVA, was applied followed by Dunnet's multiple comparison tests. $^{!!!}P < 0.001$ versus normal group. ***P < 0.001, **P < 0.01, and *P< 0.05 versus paracetamol group. ALP, alkaline phosphatase; AST, Aspartate transaminase; SGPT, Serum Glutamic Pyruvic Transaminase.

Histopathological Analysis of *Salvia hians* Crude Extract on Liver

The histopathological findings demonstrate a **clear dose-dependent hepatoprotective effect of Sh.Crd**. Paracetamol produced extensive hepatocellular degeneration and vacuolization, inflammatory-cell infiltration, sinusoidal dilation, central-vein congestion and necrosis. Silymarin markedly restored hepatic architecture. Treatment with Sh.Crd produced progressive improvement at **100, 200 and 400 mg/kg,** with the **400 mg/kg dose producing histological features approaching those of the normal and silymarin-treated groups.** This dose-dependent pattern supports the hepatoprotective potential of *S. hians* against paracetamol-induced hepatic injury.

Table 4: Hepatoprotive effect of Salvia hians on Histopathological parameters of Liver

Group	Dose	Hepatocellular degeneration/ vacuolization	Inflammatory cell infiltration	Sinusoidal/vascular changes	Necrosis	Overall hepatic architecture	Hepatoprotective effect
Normal contro	—	None	None	Normal, intact sinusoids	None	**Normal/well preser**	—

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Print ISSN 3007-3189

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l				and central vein		**ved**	
Paracetamol toxic control	—	**Severe**	**Severe**	**Severe dilation & congestion**	**Severe**	**Markedly disrupted**	**Absent**
Silymarin + paracetamol	400 mg/kg	Minimal/none	Mild	Near normal	None/minimal	**Almost normal**	**Strong**
Sh.Crd + paracetamol	100 mg/kg	Moderate	Mild–moderate	Some abnormalities	Mild	**Partially improved**	**Moderate**
Sh.Crd + paracetamol	200 mg/kg	Mild	Mild	Improved	Minimal	**Significantly improved**	**Strong**
Sh.Crd + paracetamol	400 mg/kg	Minimal	Minimal	Near normal	Minimal/absent	**Almost normal**	**Very strong**

Discussion

The observed strong reactions for phenolics and flavonoids are consistent with evidence from other *Salvia* species. Methanolic extracts of *S. eigii*, *S. hierosolymitana*, and *S. viridis* contained phenolic acids and flavonoids, including rosmarinic acid, salvianolic acid B, luteolin derivatives, and quercetin, and exhibited radical-scavenging and iron-chelating activity [20]. Similarly, methanolic extracts of several *Salvia* species showed antioxidant activity associated with phenolic constituents such as rosmarinic acid, kaempferol, apigenin, and luteolin [21]. Reviews likewise identify polyphenols, flavonoids, and terpenes as important contributors to the antioxidant and anti-inflammatory potential of the genus [22]. However, qualitative intensity does not establish compound concentrations or antioxidant potency, and the supplied sources provide no *S. hians*-specific validation. The remaining sources were omitted as less directly relevant.

In the reported experiment, paracetamol-associated increases in SGPT, AST, ALP, and bilirubin were progressively reduced by SH-Crd, with the 400 mg/kg dose producing values close to normal and silymarin-treated animals. This indicates substantial biochemical protection and supports the proposed preservation of hepatic

Online ISSN 3007-3197
Print ISSN 3007-3189

function. However, the supplied sources do not independently assess Salvia hians, its HPLC constituents, membrane stabilization, or the statistical significance of the stated dose response; therefore, mechanistic and comparative conclusions remain provisional. Indirect support comes from Cheedella et al., who found that a 400 mg/kg plant extract nearly restored paracetamol-elevated AST, ALT, ALP, and bilirubin to normal and produced activity comparable to silymarin [23]. Similarly, Mane et al. reported that a plant extract reduced paracetamol-induced liver enzyme elevations and preserved hepatic architecture, although silymarin was slightly more effective [24]. Other supplied sources were omitted because they evaluated different extracts or silymarin rather than Salvia hians.

Comparable histopathological recovery with silymarin, including reduced necrosis and inflammatory infiltration, has been documented in paracetamol-induced liver injury models [25]. In addition, silymarin treatment restored hepatic architecture toward normal after paracetamol exposure, supporting the use of near-normal histology as evidence of hepatoprotection [26]. The progressive improvement from 100 to 400 mg/kg therefore strengthens the interpretation of a dose-related effect. However, the cited study investigated *S. hians*, the extract-specific conclusion confirmed direct phytochemical, biochemical, and histopathological studies of *S. hians* using the stated doses for hepatoprotective potential.

It is concluded that the current study revealed the quantitave and HPLC phytochemical analysis of *Salvia hians alongwith invivo* Hepatoprotive exploration with promising results.

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