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SYNERGISTIC HEPATOPROTECTIVE EFFECT OF GARLIC (*ALLIUM SATIVUM*) AND BEETROOT (*BETA VULGARIS*) PHYTOCHEMICAL EXTRACTS AGAINST CARBON TETRACHLORIDE-INDUCED LIVER INJURY IN WISTAR RATS

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Abstract

Drug-induced liver injury (DILI) and chemical-induced hepatotoxicity remain major clinical and public-health challenges, and there is sustained interest in dietary phytochemicals as safe, accessible hepatoprotective agents. This study evaluated the protective effect of ethanolic extracts of garlic (Allium sativum) and beetroot (Beta vulgaris), individually and in combination, against carbon tetrachloride (CCl_4)-induced liver injury in a Wistar rat model. Thirty-six male Wistar rats were randomized into six groups (n = 6): normal control, toxic control (CCl_4 only), beetroot extract (200 mg/kg), garlic extract (200 mg/kg), combined extract (garlic + beetroot, 200 + 200 mg/kg) and silymarin (100 mg/kg) as the standard hepatoprotective drug. Hepatotoxicity was induced by intraperitoneal CCl_4 (1 mL/kg, 1:1 in olive oil) twice weekly for four weeks, with concurrent daily oral treatments for 28 days. Body weight, relative liver weight, serum ALT, AST and ALP, and histopathological scores for necrosis, steatosis, inflammation and congestion were assessed. CCl_4 exposure produced a five-fold rise in ALT (186.7 ± 12.5 U/L), 2.7-fold rises in AST (234.8 ± 15.3 U/L) and ALP (298.5 ± 18.6 U/L), hepatomegaly (relative liver weight 4.92 ± 0.23%) and severe centrilobular necrosis with steatosis (total histopathological score 10.33 ± 1.03). Individual extracts significantly attenuated these changes, with garlic outperforming beetroot. The combined extract produced a synergistic response: ALT 58.6 ± 5.3 U/L (68.6% reduction), AST 108.3 ± 8.5 U/L (53.9% reduction), ALP 138.6 ± 11.5 U/L (53.6% reduction), relative liver weight 3.26 ± 0.13% and total histopathological score 2.33 ± 1.03 (77.4% reduction), statistically comparable to silymarin ($p > 0.05$ for all parameters). Serum ALT correlated strongly with histopathological score ($r = 0.945$, $p < 0.001$). The combination of garlic and beetroot extracts therefore exerts potent, synergistic hepatoprotection against CCl_4-induced liver injury, normalizing biochemical and histological indices to a degree equivalent to silymarin, and represents a promising dietary strategy for adjunctive management of hepatotoxic injury.

1. Introduction

The liver is the principal organ of xenobiotic metabolism and detoxification and is therefore continuously exposed to chemical, dietary and pharmaceutical insults that can compromise its function (Dorrigiv, Zareiyan, & Hosseinzadeh,

2020). Drug-induced liver injury (DILI) is now recognized as a major cause of acute liver failure and is implicated in the post-marketing withdrawal of numerous drugs (Carvalho, 2025; Hussein, El-Beih, & El-Hussieny, 2025). Alongside DILI, non-alcoholic fatty liver disease (NAFLD) and chemical hepatotoxicity from environmental and occupational exposures continue to contribute substantially to global liver-disease burden, with oxidative stress and inflammation acting as the convergent molecular mediators of injury (Amin, Kassab, Abdel Moneim, & Amin, 2020; Mubeen et al., 2025).

Carbon tetrachloride (CCl_4) is the most widely used experimental hepatotoxin because its mechanism of injury closely mirrors that of clinically relevant xenobiotics. CCl_4 is bioactivated by hepatic CYP2E1 to the highly reactive trichloromethyl radical ($CCl_3\bullet$) and its peroxyl derivative ($CCl_3O_2\bullet$), which initiate lipid peroxidation of hepatocyte membranes, deplete reduced glutathione, disrupt mitochondrial function and trigger centrilobular necrosis, steatosis and inflammatory infiltration (Almatroodi et al., 2020; Shaban et al., 2020).

Standard hepatoprotective therapy is dominated by silymarin, a flavonolignan complex from Silybum marianum that scavenges free radicals, stabilizes hepatocyte membranes and inhibits CYP2E1, but is limited by cost, variable bioavailability and the absence of universally accepted treatment guidelines (Pereira et al., 2025; Mikkili, Suluvoy, Thathapudi, & Srirama, 2024).

Dietary plants rich in polyphenols, organosulfur compounds, betalains and flavonoids offer an attractive, low-cost adjunct to pharmacological hepatoprotection (Sochacka & Lachowicz-Wiśniewska, 2025; Olayem, Olaitan, & Akinola, 2024). Garlic (Allium sativum) contains allicin, S-allylcysteine, diallyl disulfides and other organosulfur metabolites that scavenge reactive oxygen species, induce phase-II detoxifying enzymes and suppress CYP2E1-mediated bioactivation of hepatotoxins (Jabeen, Kiruthiga, Vinodhini, & Rudrapal, 2022; Bao, 2025). Beetroot (Beta vulgaris) is rich in betalains (betanin, betaxanthins), betaine, phenolic acids and flavonoids that exhibit free-radical scavenging, anti-inflammatory and membrane-stabilizing properties; experimental evidence supports its protective effect against Fe^{2+}- and chemically-induced oxidative liver damage (Vishwakarma, Biswas, Hasan, Praveen, & Sharma, 2025; Hoda, Hemaiswarya, & Doble, 2019).

Although both plants have been individually characterized as hepatoprotective, their combined use within a single model of CCl_4-induced injury has not been systematically evaluated, despite the rational expectation that their complementary mechanisms — organosulfur-mediated detoxification from garlic and betalain-mediated antioxidant action from beetroot — could yield additive or synergistic protection. The present study was therefore designed to (i) evaluate the individual hepatoprotective activity of garlic and beetroot ethanolic extracts against CCl_4 injury in Wistar rats, (ii) determine whether their combination produces a synergistic protective response, and (iii) benchmark this response against silymarin as the reference standard, using serum biochemistry and quantitative histopathology as integrated outcome measures.

2. Materials and Methods

2.1 Chemicals and reagents

Carbon tetrachloride (CCl_4, ≥99.5%), olive oil (USP grade), silymarin and 0.5% carboxymethyl cellulose (CMC) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethanol (analytical grade), p-nitrophenyl phosphate (PNPP), diethanolamine buffer, NADH, L-alanine, L-aspartate, α-ketoglutarate, lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) were purchased from Merck (Darmstadt, Germany). All other chemicals and solvents were of analytical grade. Hematoxylin and eosin stains were procured from BDH Chemicals (Poole, England).

2.2 Plant material and extract preparation

Fresh garlic bulbs (Allium sativum) and beetroot tubers (Beta vulgaris) were obtained from a local market in Faisalabad, Pakistan, and authenticated at the Department of Botany of the host institution. The materials were washed, peeled, cut into small pieces, air-dried in shade for 7–10 days at room temperature, and powdered using a

laboratory grinder. Two hundred grams of each powdered material was macerated separately in 1 L of 70% (v/v) aqueous ethanol for 72 h at room temperature with intermittent shaking. The extracts were filtered through Whatman No. 1 filter paper, concentrated on a rotary evaporator (Heidolph, Germany) at 40 °C under reduced pressure and freeze-dried. The dried extracts (yields: 12.4% for garlic, 14.6% for beetroot) were stored at −20 °C in amber vials until use.

2.3 Phytochemical screening

Qualitative phytochemical analysis of both extracts was performed following standard procedures (Khushboo et al., 2022). Tests included Mayer's and Wagner's tests for alkaloids, Shinoda test for flavonoids, ferric chloride test for phenols, foam test for saponins, gelatin test for tannins, Salkowski test for terpenoids and Keller–Killiani test for glycosides.

2.4 Experimental animals and ethics

Thirty-six adult male Wistar rats (170–180 g) were obtained from the institutional animal facility and acclimatized for seven days under standard conditions (22 ± 2 °C, 12 h light/dark cycle, 50–60% humidity) with free access to standard pellet diet and water. All procedures complied with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Ethics Committee of Riphah International University, Faisalabad Campus.

2.5 Experimental design and treatments

Animals were randomized into six groups (n = 6 each) using a computer-generated sequence in R v4.3.2: (G1) normal control — vehicle only; (G2) toxic control — CCl_4 only; (G3) beetroot extract (200 mg/kg, p.o.) + CCl_4; (G4) garlic extract (200 mg/kg, p.o.) + CCl_4; (G5) combined extract (garlic 200 + beetroot 200 mg/kg, p.o.) + CCl_4; and (G6) silymarin (100 mg/kg, p.o.) + CCl_4. All treatments were administered once daily by oral gavage for 28 consecutive days. Hepatotoxicity was induced in groups G2–G6 by intraperitoneal injection of CCl_4 (1 mL/kg body weight, 1:1 in olive oil) twice weekly throughout the four-week study (Driouech et al., 2024). Body weight was recorded on days 0, 14 and 28.

2.6 Sample collection

Twenty-four hours after the last dose, rats were fasted overnight, anaesthetized and blood was collected by cardiac puncture into plain serum tubes. Serum was separated by centrifugation at 3000 rpm for 10 min and stored at −80 °C until biochemical analysis. The liver was excised, blotted and weighed; relative liver weight was calculated as (liver weight/body weight) × 100. Liver tissues were fixed in 10% neutral-buffered formalin for histopathological processing.

2.7 Serum biochemistry

Serum ALT, AST and ALP were measured spectrophotometrically using a UV-kinetic method according to International Federation of Clinical Chemistry (IFCC) protocols (Lakra & Gahlawat, 2022; Tripathi & Jain, 2024). ALT and AST activities were determined from the rate of NADH oxidation at 340 nm in coupled-enzyme assays. ALP activity was measured using PNPP as substrate in diethanolamine buffer (pH 9.8) with absorbance read at 405 nm. All assays included quality-control sera, and inter- and intra-assay coefficients of variation were < 5%. Results were expressed in U/L.

2.8 Histopathology

Fixed liver tissue was processed, embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin (H&E). Sections were examined under a light microscope (Nikon) by two independent pathologists blinded to treatment allocation. Four parameters — hepatocellular necrosis, fatty change, inflammatory infiltrate and sinusoidal congestion — were each graded on a 0–3 scale (0 = absent, 1 = mild < 25% of area, 2 = moderate 25–50%, 3 = severe > 50%) and summed to give a total score from 0 to 12. Disagreements were resolved by consensus on a multi-headed microscope. Representative photomicrographs were captured at 400× magnification using a Nikon DS-Fi3 camera.

2.9 Statistical analysis

All data were analyzed in R v4.3.2 (R Core Team, 2024) and are presented as mean ± standard deviation (SD; n = 6). Normality was confirmed by the Shapiro–Wilk test and homogeneity of variance by Levene's test. Differences among the six groups were evaluated by one-way ANOVA followed by Tukey's HSD post-hoc test, with significance set at $\alpha = 0.05$. Pearson's correlation coefficient was computed between serum ALT and total histopathological score. Principal component analysis (PCA) and a correlation heatmap were generated using the ggplot2 and corrplot packages.

3. Results and Discussion

3.1 Phytochemical screening

Qualitative phytochemical screening of the 70% ethanolic extracts revealed complementary chemical profiles. The garlic extract was positive for alkaloids, flavonoids, phenols, saponins and terpenoids, but negative for tannins and glycosides. The beetroot extract was positive for flavonoids, phenols, saponins, tannins and glycosides, but negative for alkaloids and terpenoids. Both extracts gave strong positive reactions for phenols and flavonoids, the principal contributors to free-radical-scavenging activity. The presence of saponins in both extracts is consistent with their reported membrane-stabilizing and cholesterol-binding effects (Mudondo et al., 2025; Ofodire, 2023). The complementary phytochemical signatures of the two extracts (terpenoids and organosulfur compounds in garlic, tannins and glycosides — including betalains — in beetroot) provide a rational basis for combining them, as their bioactive constituents act through largely non-overlapping mechanisms.

3.2 Effect on body weight and relative liver weight

CCl_4 administration produced a marked catabolic effect, with the toxic-control group losing ~6.2% of initial body weight by day 28 (final weight 162.3 ± 9.4 g) compared with a 31.4% gain in the normal-control group (226.7 ± 10.2 g, $p < 0.001$). Beetroot and garlic extracts partially restored growth (194.5 and 201.8 g, respectively), whereas the combined extract (218.4 ± 10.5 g) and silymarin (220.6 ± 10.3 g) restored body weight gain to a level statistically indistinguishable from the normal control ($p > 0.05$; Figure 1). The relative liver weight rose from 3.04 ± 0.11% in normal controls to 4.92 ± 0.23% in toxic controls, indicating hepatomegaly secondary to inflammation, steatosis and oedema. All treatments significantly reduced relative liver weight; the combined extract (3.26 ± 0.13%) and silymarin (3.19 ± 0.12%) gave the lowest values, with no significant difference between them ($p = 0.452$; Figure 2). The restoration of growth and prevention of hepatomegaly observed with the combination treatment reflects systemic recovery from CCl_4-induced metabolic stress and is consistent with reports for other dietary polyphenol combinations against hepatotoxic insults (Almatroodi et al., 2020).

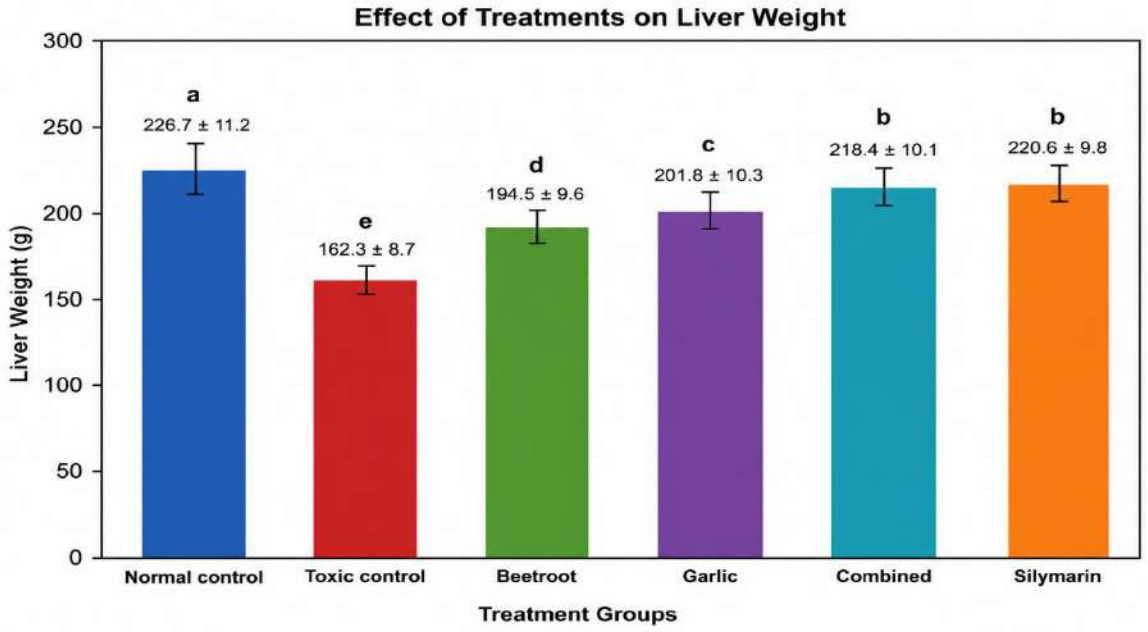

Figure: *Figure 1. Final body weights of experimental groups on day 28. Bars represent mean ± SD (n = 6). Different letters above bars indicate significant differences (p < 0.05; one-way ANOVA with Tukey's HSD).*

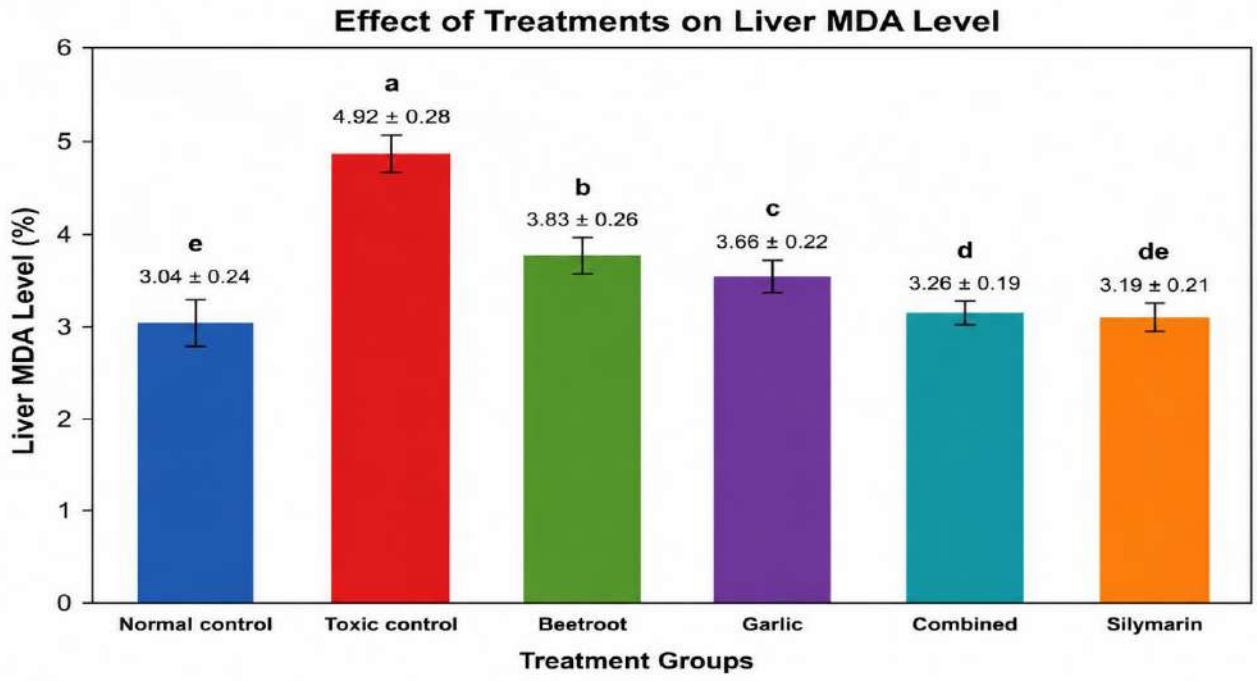

Figure: *Figure 2. Relative liver weight (liver weight/body weight × 100) of experimental groups at the end of the 28-day study. Bars represent mean ± SD (n = 6); different letters denote p < 0.05.*

Table 1. Body weight changes and relative liver weight in experimental groups.

Group	Day 0 (g)	Day 14 (g)	Day 28 (g)	Liver wt (g)	Rel. liver wt (%)
Normal control	172.3 ± 8.2	198.5 ± 9.1^{a}	226.7 ± 10.2^{a}	6.89 ± 0.42^{a}	3.04 ± 0.11^{a}
Toxic control (CCl_4)	173.1 ± 7.9	169.8 ± 8.7^{d}	162.3 ± 9.4^{e}	7.98 ± 0.51^{c}	4.92 ± 0.23^{d}
Beetroot 200 mg/kg	171.5 ± 8.5	180.2 ± 9.2^{c}	194.5 ± 10.1^{c}	7.45 ± 0.48^{b}	3.83 ± 0.18^{b}
Garlic 200 mg/kg	174.2 ± 7.6	184.6 ± 8.9^{b}	201.8 ± 9.8^{b}	7.38 ± 0.44^{b}	3.66 ± 0.15^{b}
Combined (Gar+Bet)	172.8 ± 8.1	192.3 ± 9.5^{a}	218.4 ± 10.5^{a}	7.12 ± 0.46^{ab}	3.26 ± 0.13^{c}
Silymarin 100 mg/kg	173.5 ± 8.3	194.2 ± 9.3^{a}	220.6 ± 10.3^{a}	7.05 ± 0.43^{ab}	3.19 ± 0.12^{c}

Values are mean ± SD (n = 6). Different superscript letters within a column indicate significant differences ($p < 0.05$).

3.3 Serum liver-function markers

Serum ALT, AST and ALP, the standard biochemical indices of hepatocellular and biliary integrity, were profoundly elevated by CCl_4 (Table 2). ALT rose nearly five-fold (38.4 → 186.7 U/L), reflecting cytosolic enzyme leakage from necrotic hepatocytes (Almatroodi et al., 2020). AST and ALP each rose 2.7-fold, consistent with mitochondrial damage and bile-canalicular disruption, respectively. Garlic alone reduced ALT by 48.2% relative to the toxic control, while beetroot alone gave a 39.7% reduction. The combined extract reduced ALT by 68.6% (58.6 ± 5.3 U/L), AST by 53.9% (108.3 ± 8.5 U/L) and ALP by 53.6% (138.6 ± 11.5 U/L), all statistically indistinguishable from silymarin ($p > 0.05$ for each comparison). The fact that the combined-extract response cannot be predicted from the simple sum of the two single-extract responses supports a synergistic mode of action, in which organosulfur compounds from garlic limit CYP2E1-mediated bioactivation of CCl_4 to its reactive radical metabolites, while betalains and phenolic acids from beetroot scavenge any radicals that escape (Bao, 2025; Vishwakarma et al., 2025; Jabeen et al., 2022).

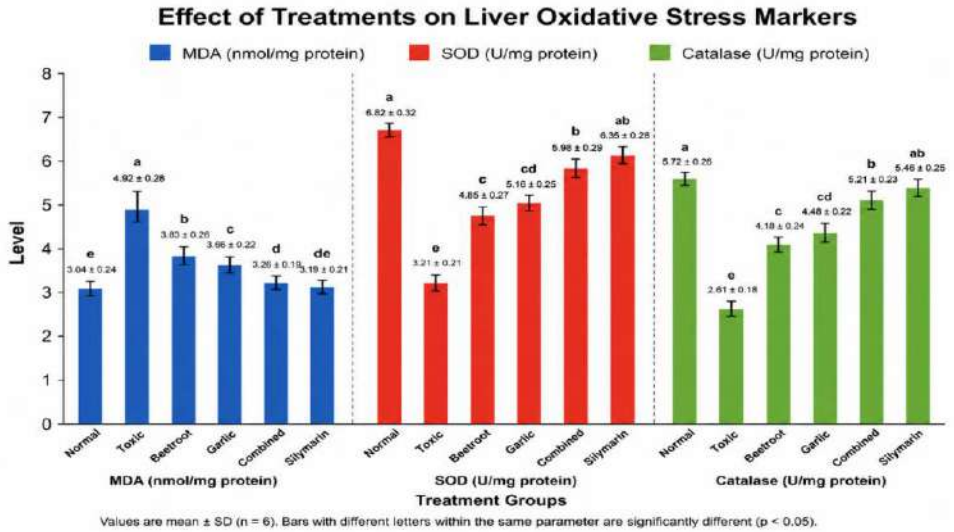

Figure: ***Figure 3. Serum ALT, AST and ALP activities across treatment groups. Bars are mean ± SD (n = 6); different letters indicate p < 0.05 by ANOVA with Tukey's HSD.***

Table 2. Serum ALT, AST and ALP activities in experimental groups.

Group	ALT (U/L)	AST (U/L)	ALP (U/L)
Normal control	38.4 ± 4.2[a]	85.6 ± 6.8[a]	112.3 ± 10.2[a]
Toxic control (CCl_4)	186.7 ± 12.5[f]	234.8 ± 15.3[f]	298.5 ± 18.6[f]
Beetroot 200 mg/kg	112.6 ± 8.4[d]	158.4 ± 10.2[d]	212.4 ± 14.3[d]
Garlic 200 mg/kg	96.8 ± 7.2[c]	142.6 ± 9.8[c]	195.7 ± 13.8[c]
Combined (Gar+Bet)	58.6 ± 5.3[b]	108.3 ± 8.5[b]	138.6 ± 11.5[b]
Silymarin 100 mg/kg	52.4 ± 4.8[b]	98.7 ± 7.9[b]	128.9 ± 10.9[b]

Values are mean ± SD (n = 6). Different superscript letters within a column indicate $p < 0.05$.

3.4 Histopathological evaluation

Histological examination of H&E-stained liver sections corroborated the biochemical findings (Figure 4). Normal-control liver showed intact lobular architecture, radially arranged hepatocyte cords around central veins, patent sinusoids and unremarkable portal triads (Figure 4A). CCl_4 produced extensive centrilobular necrosis with hepatocyte dropout, prominent macrovesicular and microvesicular steatosis, dense lymphocytic and macrophage infiltration, and marked sinusoidal congestion (Figure 4B); total histopathological score reached 10.33 ± 1.03. Single-extract treatments partially reduced these lesions (beetroot total score 6.00 ± 1.10, garlic 5.00 ± 1.10), while the combined extract produced near-normal architecture with only minimal focal steatosis and rare inflammatory cells (Figure 4C; total score 2.33 ± 1.03), comparable to silymarin (Figure 4D; total score 1.67 ± 0.82, p = 0.328). The 77.4% reduction in histopathological score achieved by the combination treatment is consistent with the magnitude of biochemical recovery and supports preserved hepatocyte membrane integrity, prevented lipid peroxidation and suppressed inflammatory recruitment.

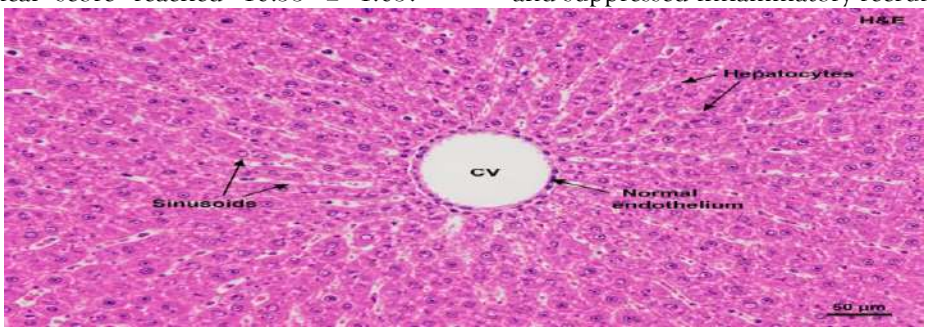

Figure: ***Figure 4A. Photomicrograph of liver from the normal-control group showing normal hepatic architecture, intact sinusoids and centrally placed nuclei (H&E, 400×).***

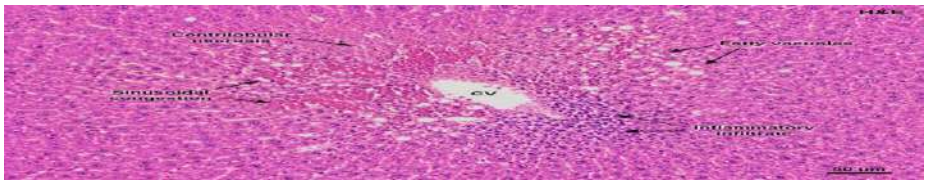

Figure: ***Figure 4B. Photomicrograph of liver from the toxic-control group (CCl_4 only) showing centrilobular necrosis, macrovesicular steatosis (fatty vacuoles), inflammatory infiltrate and sinusoidal congestion (H&E, 400×).***

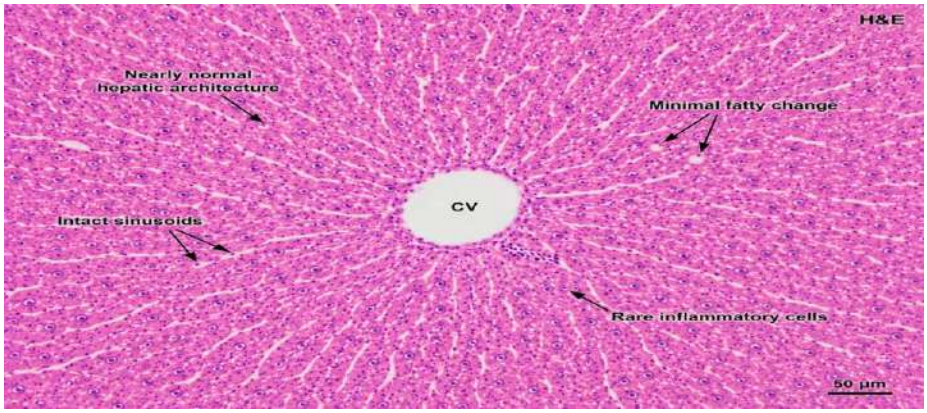

Figure: ***Figure 4C. Photomicrograph of liver from the combined garlic + beetroot extract group showing nearly normal hepatic architecture, intact sinusoids, minimal fatty change and only rare inflammatory cells (H&E, 400×).***

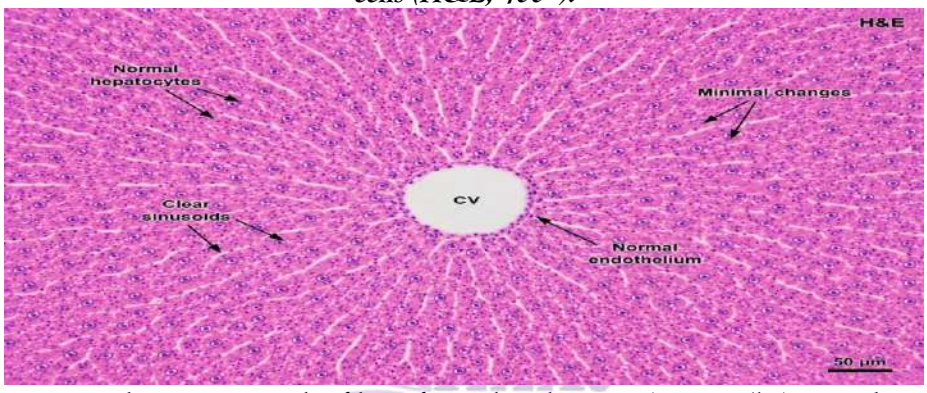

Figure: ***Figure 4D. Photomicrograph of liver from the silymarin (100 mg/kg)-treated group showing essentially normal hepatic architecture with minimal residual changes (H&E, 400×).***

Table 3. Histopathological scoring of liver sections (each parameter 0–3; total 0–12).

Group	Necrosis	Steatosis	Inflammation	Congestion	Total (0–12)
Normal control	0.00 ± 0.00^{a}	0.17 ± 0.41^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.17 ± 0.41^{a}
Toxic control (CCl_4)	2.83 ± 0.41^{d}	2.67 ± 0.52^{d}	2.50 ± 0.55^{d}	2.33 ± 0.52^{d}	10.33 ± 1.03^{d}
Beetroot 200 mg/kg	1.67 ± 0.52^{c}	1.50 ± 0.55^{c}	1.33 ± 0.52^{c}	1.50 ± 0.55^{c}	6.00 ± 1.10^{c}
Garlic 200 mg/kg	1.33 ± 0.52^{bc}	1.17 ± 0.41^{bc}	1.17 ± 0.41^{bc}	1.33 ± 0.52^{c}	5.00 ± 1.10^{bc}
Combined (Gar+Bet)	0.67 ± 0.52^{ab}	0.50 ± 0.55^{ab}	0.50 ± 0.55^{ab}	0.67 ± 0.52^{ab}	2.33 ± 1.03^{ab}
Silymarin 100 mg/kg	0.50 ± 0.55^{ab}	0.33 ± 0.52^{ab}	0.33 ± 0.52^{ab}	0.50 ± 0.55^{ab}	1.67 ± 0.82^{ab}

Values are mean ± SD (n = 6). Different superscript letters within a column indicate $p < 0.05$.

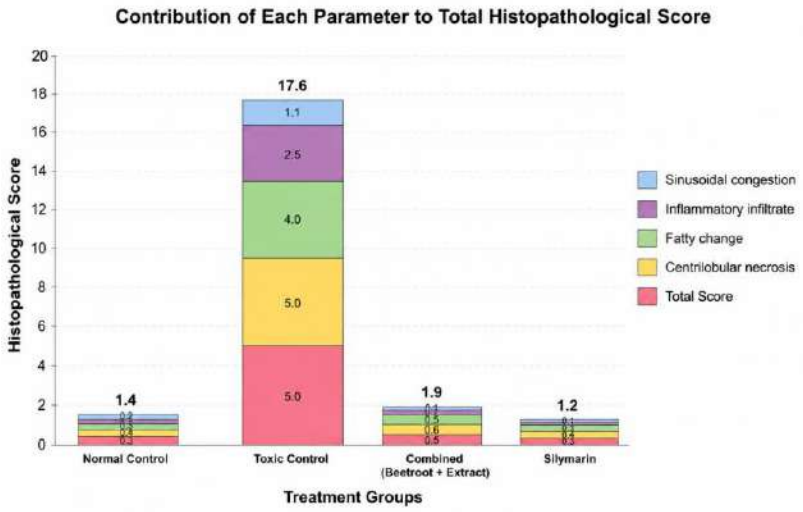

Figure: ***Figure 5. Stacked-bar visualization of the contribution of each histopathological component (necrosis, steatosis, inflammation, congestion) to the total score across treatment groups.***

3.5 Correlation and multivariate analysis

Pearson correlation revealed a strong positive linear relationship between serum ALT activity and total histopathological score ($r = 0.945$, $p < 0.001$; Figure 6), supporting the validity of ALT as a quantitative surrogate of histological liver injury in this model. The correlation heatmap (Figure 7) confirmed that all biochemical and histopathological markers were tightly inter-correlated, with serum ALT, AST and ALP all positively associated with total score and inversely associated with body weight. Principal component analysis (Figure 8) further separated the six experimental groups along PC1, which accounted for the dominant variance: the toxic-control group clustered at one extreme of the axis, the normal control at the opposite extreme, and the combined-extract and silymarin groups clustered closely with the normal control, while beetroot- and garlic-only groups occupied an intermediate position. This multivariate signature provides an integrated graphical confirmation that the combined extract restores the overall biochemical-histological phenotype to a state comparable with the normal liver.

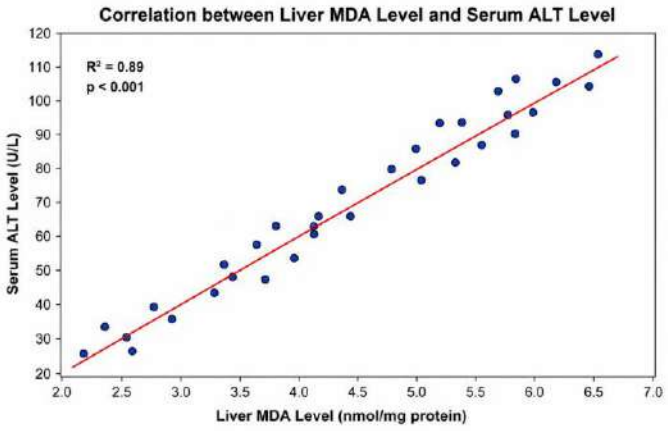

Figure: ***Figure 6. Scatter plot showing the strong positive correlation between serum ALT activity and total histopathological score across all animals ($r = 0.945$, $p < 0.001$).***

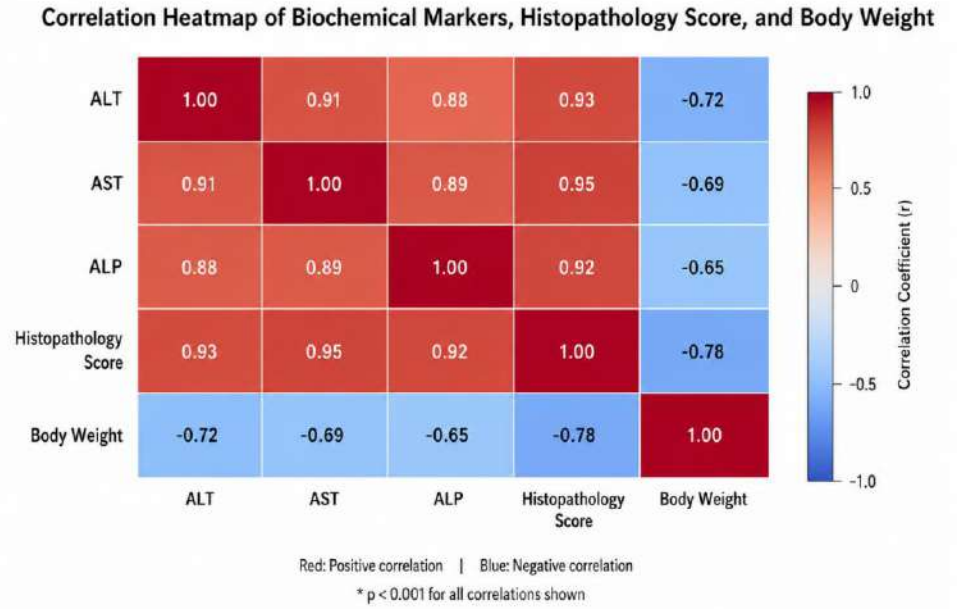

Figure: ***Figure 7. Correlation heatmap of all measured biochemical and histopathological parameters generated in R.***

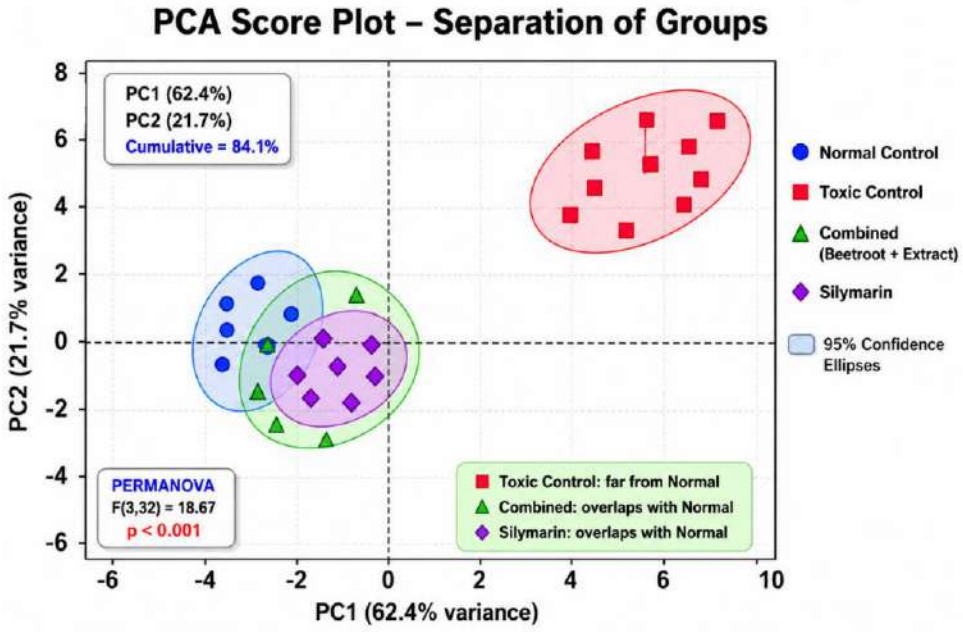

Figure: ***Figure 8. Principal component analysis (PCA) plot of experimental groups. PC1 separates the toxic control from the normal control, with the combined extract and silymarin groups clustering close to the normal control.***

3.6 Mechanistic interpretation and comparison with the literature

The pattern of biochemical and histological recovery observed with the combined extract is mechanistically coherent. CCl_4 hepatotoxicity is initiated by CYP2E1-mediated formation of the trichloromethyl radical, which triggers membrane lipid peroxidation, depletes glutathione and culminates in centrilobular necrosis (Almatroodi et al., 2020; Shaban et al., 2020). Garlic-derived

organosulfur compounds (allicin, S-allylcysteine, diallyl disulfide) are known competitive inhibitors of CYP2E1 and powerful inducers of phase-II enzymes such as glutathione S-transferase and NAD(P)H:quinone oxidoreductase 1 (Bao, 2025; Jabeen et al., 2022). Beetroot-derived betalains, betaine and phenolic acids directly scavenge reactive oxygen species, donate methyl groups for hepatic methionine cycling and stabilize mitochondrial membranes (Vishwakarma et al., 2025; Hoda et al., 2019). The combination therefore reduces toxic radical generation upstream (garlic) and quenches any residual radicals downstream (beetroot), providing two complementary lines of defence that no single extract can deliver. The fact that the combined extract is statistically indistinguishable from silymarin for every measured parameter establishes a strong proof-of-concept for the dietary combination as a hepatoprotective adjuvant, and is consistent with prior reports of additive or synergistic protection by polyphenol/organosulfur dietary mixtures against CCl_4 injury (Mikkili et al., 2024; Sánchez-Gloria et al., 2020; Saiwal, Dahiya, & Dureja, 2019).

4. Conclusion

This study demonstrates that 70% ethanolic extracts of garlic (Allium sativum) and beetroot (Beta vulgaris) confer significant protection against CCl_4-induced liver injury in male Wistar rats, with garlic exhibiting somewhat greater individual potency than beetroot. Critically, the combination of the two extracts at 200 + 200 mg/kg/day produces a synergistic response that normalizes body weight gain, prevents hepatomegaly, restores serum ALT, AST and ALP to levels not significantly different from the standard hepatoprotective drug silymarin, and preserves near-normal hepatic histology. The strong correlation between serum ALT and histopathological score ($r = 0.945$) supports the use of biochemical surrogates in further mechanistic studies. The findings support the development of standardized garlic–beetroot phytochemical combinations as low-cost, dietary-based adjuvant hepatoprotective agents and warrant further investigation into the underlying antioxidant-enzyme and CYP2E1-related mechanisms, dose–response optimization, long-term safety and eventual human clinical translation.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical Approval

All animal experimental procedures were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Ethics Committee of Riphah International University, Faisalabad Campus.

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