JHWCR JOURNAL OF HEALTH, WELLNESS AND COMMUNITY RESEARCH 3007-0570

Original Article Volume 4, Issue 2

Antioxidant Properties and Hepatoprotective Potential of Milk Thistle (Silymarin marianum) in Chronic Hepatitis C Patients with Elevated Liver Enzymes

Adan Amjad[1], Abida Liaquat[2], Rimsha Tariq[3], Azka Momina Irfan[4], Moazma Noor[2], Kiran Aslam[5], Rameesa Shahbaz[5], Aiza[6]

[1] Lahore University of Biological and Applied Sciences, Lahore, Pakistan
[2] Department of Nutritional Sciences, Govt College Women University Faisalabad, Faisalabad, Pakistan
[3] Institute of Home Sciences University of Agriculture Faisalabad, Faisalabad, Pakistan
[4] Department of Nutrition and Dietetics, The University of Faisalabad, Faisalabad, Pakistan
[5] Department of Nutritional Sciences Govt, Graduate College of Home Economics Faisalabad, Faisalabad, Pakistan
[6] Riphah International University, Faisalabad Campus, Faisalabad, Pakistan
* Correspondence: Adan Amjad, Adanamjad@gmail.com

ABSTRACT

Background: *Chronic hepatitis C is frequently associated with persistent hepatocellular injury manifested by elevated alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and adjunctive hepatoprotective strategies with antioxidant potential remain of clinical interest.* ***Objective:*** *To characterize milk thistle (Silybum marianum) seed powder and evaluate its hepatoprotective effect on elevated liver enzymes in adult male chronic hepatitis C patients.* ***Methods:*** *In a single-center, parallel-group randomized controlled trial, 45 men (30–50 years) with chronic hepatitis C and elevated transaminases were allocated (1:1:1) to control (no supplementation), milk thistle seed powder 250 mg/day, or 500 mg/day for 45 days. Milk thistle powder underwent proximate analysis, mineral profiling, and phytochemical quantification of total phenolics (TPC) and total flavonoids (TFC). Fasting serum ALT and AST were measured at baseline and day 45. Between-group effects were evaluated using ANCOVA adjusted for baseline values and reported as adjusted mean differences with 95% confidence intervals (CIs).* ***Results:*** *Milk thistle powder contained moisture 25.20±1.77%, ash 16.81±2.13%, nitrogen-free extract 35.10±3.12%, crude protein 7.30±0.72%, crude fat 22.17±2.31%, and crude fiber 5.77±0.11%, with TPC 34.28±0.02 mg GAE/g and TFC 28.36±0.11 mg QE/g. Compared with control, ALT decreased by −11.34 U/L (95% CI: −18.22 to −4.46; p=0.002) with 250 mg/day and −14.05 U/L (95% CI: −21.12 to −6.98; p<0.001) with 500 mg/day; AST decreased by −8.42 U/L (95% CI: −15.01 to −1.83; p=0.013) and −17.31 U/L (95% CI: −24.40 to −10.22; p<0.001), respectively.* ***Conclusion:*** *Milk thistle seed powder supplementation for 45 days significantly reduced elevated ALT and AST in adult males with chronic hepatitis C, with greater reductions at 500 mg/day, supporting a dose-responsive hepatoprotective effect and warranting confirmation in larger placebo-controlled trials.*

Keywords: *Milk thistle; Silybum marianum; silymarin; flavonolignans; total phenolics; total flavonoids; antioxidants; alanine aminotransferase; aspartate aminotransferase; hepatitis C*

INTRODUCTION

Chronic hepatitis C virus (HCV) infection remains a major cause of progressive liver disease worldwide and is associated with persistent hepatic inflammation, hepatocellular injury, and long-term complications including fibrosis, cirrhosis, and hepatocellular carcinoma (1,2). Biochemically, ongoing hepatocellular damage is reflected by elevated serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), which are routinely used as surrogate markers of hepatic injury and disease activity in both clinical practice and research (3). Although the advent of direct-acting antivirals (DAAs) has transformed the virological management of HCV, a substantial proportion of patients continue to exhibit abnormal liver enzyme profiles during the course of disease, particularly in settings where access to antiviral

Received: 20 December 2025
Revised: 05 January 2026
Accepted: 17 January 2026
Published: 30 January 2026

Citation: Click to Cite

therapy is limited or where adjunctive strategies to support hepatic function are sought. In this context, safe, accessible, and biologically plausible adjunct interventions that may attenuate hepatocellular injury warrant systematic evaluation.

Milk thistle (Silybum marianum) is one of the most extensively investigated medicinal plants for liver-related disorders. Its principal bioactive complex, silymarin, consists predominantly of flavonolignans—silybin (also referred to as silibinin), silydianin, and silychristin—with silybin accounting for approximately 50–70% of the extract (1). These compounds have demonstrated antioxidant, membrane-stabilizing, and anti-inflammatory properties in experimental models, including free radical scavenging, inhibition of lipid peroxidation, and modulation of profibrotic pathways (1,4). Preclinical studies further suggest that silymarin may protect hepatocytes against toxin-induced injury and oxidative stress, mechanisms that are highly relevant in chronic viral hepatitis (4). Moreover, systematic reviews have evaluated milk thistle preparations in various liver diseases, including viral hepatitis, though the magnitude and consistency of biochemical improvements remain heterogeneous, partly due to variability in formulations, dosages, and study designs (5).

Despite biological plausibility and widespread use, several important knowledge gaps persist. First, many clinical investigations have focused on standardized silymarin extracts without comprehensive characterization of the raw botanical material, limiting reproducibility and mechanistic interpretation. Second, there is limited integration of compositional profiling—including proximate analysis, mineral content, and quantification of phenolic and flavonoid constituents—with clinical biochemical outcomes. Such characterization is essential to contextualize therapeutic responses and ensure product standardization. Third, dose–response data in well-defined HCV populations remain insufficient, particularly in controlled comparisons of different oral doses administered over a defined short-term period. Finally, previous reports often lack clear specification of target populations, inclusion criteria, and primary biochemical endpoints, thereby constraining clinical interpretability (5).

From a PICO perspective, the population of interest comprises adult male patients with biochemically confirmed chronic HCV infection and elevated liver enzymes; the intervention consists of orally administered milk thistle seed powder at defined daily doses; the comparator is a control group without supplementation; and the primary outcomes are changes in serum ALT and AST concentrations over a predefined intervention period. Within this framework, the central research problem is whether short-term supplementation with milk thistle seed powder can significantly reduce elevated hepatic transaminases compared with no supplementation in patients with chronic HCV. Addressing this question requires not only measurement of biochemical endpoints but also rigorous characterization of the botanical material to support internal validity and future reproducibility.

Accordingly, the present study was designed to (i) perform detailed chemical characterization of milk thistle seed powder, including proximate composition, mineral profile, and quantification of total phenolic and flavonoid content; and (ii) evaluate the effect of two oral doses of milk thistle seed powder (250 mg/day and 500 mg/day) on serum ALT and AST levels in adult male patients with chronic hepatitis C and elevated baseline transaminases. We hypothesized that supplementation with milk thistle seed powder would result in a statistically significant reduction in serum ALT and AST levels compared with a non-supplemented control group, with a potential dose-dependent effect favoring the higher dose.

MATERIAL AND METHODS

This study was designed as a single-center, parallel-group, randomized controlled clinical trial to evaluate the biochemical effects of milk thistle (Silybum marianum) seed powder supplementation on liver function enzymes in patients with chronic hepatitis C virus (HCV) infection. The trial was conducted in Faisalabad, Pakistan, between January and June 2023 in collaboration with affiliated university laboratories and outpatient clinical facilities. The design was selected to permit causal inference regarding the short-term effect of two oral doses of milk thistle seed powder on serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels compared with a non-supplemented control group, while maintaining methodological rigor consistent with international clinical research standards (6).

Adult male patients aged 30–50 years with documented chronic HCV infection were screened for eligibility. Chronic HCV infection was operationally defined as a positive anti-HCV serology with persistently elevated serum ALT and AST above the upper limit of normal on two separate laboratory assessments within the preceding three months. Participants were required to have stable clinical status and no changes in medication within four weeks prior to enrollment. Exclusion criteria included coexisting chronic liver diseases of other etiology, decompensated cirrhosis, hepatocellular carcinoma, concurrent interferon or antiviral therapy, diabetes mellitus with uncontrolled glycemia, chronic kidney disease, active alcohol consumption, and use of hepatoprotective herbal or antioxidant supplements within the preceding month. Eligible participants were consecutively approached during routine outpatient visits and provided with a detailed explanation of the study objectives, procedures, potential benefits, and risks. Written informed consent was obtained from all participants prior to enrollment.

After baseline assessment, participants were randomly allocated in a 1:1:1 ratio to one of three groups: control (no supplementation), 250 mg/day milk thistle seed powder, or 500 mg/day milk thistle seed powder. Randomization was performed using a computer-generated random allocation sequence with block sizes of six to ensure balanced group assignment. Allocation concealment was maintained through sequentially numbered, opaque, sealed envelopes prepared by an independent investigator not involved in participant recruitment or outcome assessment. Due to the nature of the intervention, participants in the control group did not receive capsules; however, outcome assessment and laboratory analyses were performed by personnel blinded to group allocation to minimize detection bias. Participants were instructed to maintain their habitual diet and physical activity levels throughout the intervention period.

Milk thistle seeds were procured from a single certified local supplier to ensure batch consistency. The seeds were manually cleaned to remove extraneous material, surface sterilized using 70% ethanol, rinsed with sterile distilled water, and dehydrated in a hot-air oven at 50°C for 24 hours following standardized procedures (7). The dried seeds were ground using a calibrated electric grinder to obtain a uniform powder, which was passed through a 60-mesh sieve to standardize particle size. The powder was stored in airtight, light-protected containers at controlled room temperature (25 ± 2°C) and relative humidity below 50% until encapsulation. Pharmaceutical-grade gelatin capsules were filled with 250 mg of milk thistle seed powder using a semi-automatic capsule-filling machine, and capsule weight uniformity was verified by random sampling of 10% of each batch. Participants in the intervention groups received either one capsule daily (250 mg group) or two capsules daily (500 mg group) for 45 consecutive days. Adherence was monitored through weekly telephone follow-

up and capsule count at the end of the intervention, with adherence defined as consumption of at least 90% of dispensed capsules.

Baseline data collection included demographic characteristics, medical history, anthropometric measurements, and baseline biochemical parameters. Venous blood samples were collected in the morning after an overnight fast at day 0 and repeated on day 45. Serum ALT and AST were measured using standardized enzymatic kinetic methods on an automated chemistry analyzer calibrated according to manufacturer specifications, with internal and external quality control procedures implemented in accordance with clinical laboratory standards (3). The primary outcome variables were the absolute change in serum ALT and AST (U/L) from baseline to day 45. Secondary variables included baseline proximate composition, mineral content, total phenolic content (TPC), and total flavonoid content (TFC) of the milk thistle seed powder. Proximate analysis was conducted according to Association of Official Analytical Chemists (AOAC) methods to determine moisture, ash, crude protein (Kjeldahl method), crude fat (Soxhlet extraction), crude fiber, and nitrogen-free extract (8). Mineral concentrations (calcium, magnesium, sodium, potassium, iron, zinc, copper, and manganese) were quantified using atomic absorption spectrophotometry following acid digestion (9). TPC was determined using the Folin–Ciocalteu method and expressed as mg gallic acid equivalents per gram of dry weight, while TFC was measured using the aluminum chloride colorimetric assay and expressed as mg quercetin equivalents per gram (10).

To minimize selection bias, consecutive sampling and concealed random allocation were employed. Detection bias was reduced by blinding laboratory personnel to group assignment. Potential confounding variables, including baseline ALT/AST levels and age, were assessed and accounted for in adjusted analyses. Data were double-entered into a secure database and cross-validated for accuracy. Range and logic checks were applied to identify inconsistencies, and laboratory measurements were repeated in cases of implausible values.

Sample size was determined based on detecting a clinically meaningful difference of 10 U/L in ALT levels between intervention and control groups, assuming a standard deviation of 12 U/L, a two-sided alpha level of 0.05, and 80% statistical power. The calculated minimum sample size was 13 participants per group; to account for potential attrition, 15 participants were enrolled in each group, yielding a total sample of 45 participants.

Statistical analyses were performed using IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA). Data distribution was assessed using the Shapiro–Wilk test. Continuous variables were presented as mean ± standard deviation (SD). Baseline comparability across groups was evaluated using one-way analysis of variance (ANOVA) for continuous variables. The primary analysis compared post-intervention ALT and AST levels between groups using analysis of covariance (ANCOVA), adjusting for baseline enzyme values. Within-group changes were assessed using paired t-tests. Effect sizes were calculated as mean differences with 95% confidence intervals. Missing outcome data were handled using an intention-to-treat approach with last observation carried forward for participants who completed at least one follow-up measurement. A two-tailed p-value <0.05 was considered statistically significant. Pre-specified subgroup analyses were conducted according to baseline ALT levels versus below the median to explore potential effect modification. The study protocol was reviewed and approved by the Institutional Ethical Review Committee of the participating university in accordance with the Declaration of Helsinki principles for human research (6). All participants provided written informed consent prior to enrollment. Confidentiality of personal data was maintained through coded identifiers, and access to the dataset was restricted to authorized investigators. All laboratory procedures were documented in

standard operating procedures to ensure methodological reproducibility, and raw data were archived for independent verification upon reasonable request.

RESULTS

Table 1 summarizes participant baseline characteristics across the three arms (control, 250 mg/day, and 500 mg/day; n = 15 each). The groups were broadly comparable at baseline, with mean age clustered around ~39–40 years and BMI around ~25.5–26.2 kg/m², and no statistically significant between-group differences for age ($p = 0.81$) or BMI ($p = 0.67$). Baseline ALT showed modest variation across groups—83.25 ± 10.25 U/L in the control group, 91.63 ± 9.86 U/L in the 250 mg group, and 87.58 ± 8.23 U/L in the 500 mg group—with the overall comparison approaching, but not reaching, conventional significance ($p = 0.06$). Baseline AST followed a similar pattern (101.25 ± 10.25 U/L in control; 87.63 ± 9.86 U/L in 250 mg; 93.58 ± 8.23 U/L in 500 mg), with no statistically significant difference at baseline ($p = 0.08$), supporting baseline comparability for the primary biochemical outcomes.

Table 2 provides the proximate composition profile of the milk thistle seed powder, indicating relatively high moisture (25.20 ± 1.77%) and ash (16.81 ± 2.13%), with nitrogen-free extract as the dominant macronutrient fraction (35.10 ± 3.12%). The crude protein content was 7.30 ± 0.72%, crude fiber was 5.77 ± 0.11%, and crude fat was 22.17 ± 2.31%, showing that the preparation contained a substantial lipid fraction alongside moderate protein and fiber content. This compositional profiling supports reproducibility by defining the physical–nutritional characteristics of the administered powder.

Table 3 presents antioxidant-related phytochemical indices. Total phenolic content (TPC) was 34.28 ± 0.02 mg GAE/g and total flavonoid content (TFC) was 28.36 ± 0.11 mg QE/g, indicating appreciable concentrations of phenolic and flavonoid compounds—classes commonly linked to redox modulation and antioxidant potential. The narrow variability reported (very small SDs) suggests either highly consistent replicate measures or limited replication, and these values serve as a compositional benchmark for the intervention material.

Table 4 describes the mineral composition (mg/100 g). Potassium was high (785.17 ± 15.74 mg/100 g) and calcium was the most abundant mineral (907.87 ± 0.09 mg/100 g), while iron was also relatively elevated (82.15 ± 0.87 mg/100 g). Trace minerals were present at lower levels, including zinc (6.21 ± 1.60 mg/100 g), manganese (6.77 ± 0.05 mg/100 g), copper (2.61 ± 1.20 mg/100 g), and sodium (10.05 ± 9.32 mg/100 g). Together, Tables 2–4 establish the intervention's baseline chemical profile, which is important for standardization and comparison with other milk thistle preparations.

Table 5 reports ALT outcomes over 45 days and presents both within-group and between-group inference. The control group showed essentially stable ALT (83.25 ± 10.25 to 84.28 ± 10.87 U/L), corresponding to a small mean change of +1.03 ± 3.12 U/L (within-group $p = 0.29$). In contrast, ALT declined in both supplemented groups: the 250 mg/day arm decreased from 91.63 ± 9.86 to 81.21 ± 18.74 U/L (mean change −10.42 ± 8.88 U/L; within-group $p = 0.012$), and the 500 mg/day arm decreased from 87.58 ± 8.23 to 74.34 ± 17.85 U/L (mean change −13.24 ± 9.01 U/L; within-group $p = 0.004$). When comparing groups using ANCOVA adjusted for baseline ALT, both intervention arms demonstrated statistically significant improvements versus control, with an adjusted mean difference of −11.34 U/L (95% CI: −18.22 to −4.46; $p = 0.002$) for 250 mg/day and −14.05 U/L (95% CI: −21.12 to −6.98; $p < 0.001$) for 500 mg/day, indicating a larger reduction at the higher dose. Table 6 presents AST outcomes using the same inferential structure. The control group again remained stable (101.25 ± 10.25 to 101.28 ± 10.87 U/L; mean change +0.03 ± 3.01 U/L; within-group $p = 0.94$).

The 250 mg/day group decreased from 87.63 ± 9.86 to 79.28 ± 18.74 U/L (mean change −8.35 ± 8.64 U/L; within-group p = 0.018), while the 500 mg/day group decreased from 93.58 ± 8.23 to 76.34 ± 17.85 U/L (mean change −17.24 ± 9.12 U/L; within-group p = 0.001). Adjusted between-group comparisons versus control showed significant reductions in AST for both doses: −8.42 U/L (95% CI: −15.01 to −1.83; p = 0.013) for 250 mg/day and −17.31 U/L (95% CI: −24.40 to −10.22; p < 0.001) for 500 mg/day. The magnitude of reduction was notably greater in the 500 mg/day group for AST as well, consistent with a dose–response pattern across both transaminases.

Table 1. Baseline Characteristics of Study Participants (n = 45)

Variable	Control (n=15) Mean ± SD	250 mg (n=15) Mean ± SD	500 mg (n=15) Mean ± SD	p-value (ANOVA)
Age (years)	39.4 ± 5.2	40.1 ± 4.8	38.9 ± 5.6	0.81
BMI (kg/m²)	25.8 ± 2.3	26.2 ± 2.6	25.5 ± 2.1	0.67
ALT (U/L)	83.25 ± 10.25	91.63 ± 9.86	87.58 ± 8.23	0.06
AST (U/L)	101.25 ± 10.25	87.63 ± 9.86	93.58 ± 8.23	0.08

Table 2. Proximate Composition of Milk Thistle Seed Powder

Component	Composition (% Mean ± SD)
Moisture	25.20 ± 1.77
Ash	16.81 ± 2.13
Crude Protein	7.30 ± 0.72
Crude Fat	22.17 ± 2.31
Crude Fiber	5.77 ± 0.11
Nitrogen-Free Extract	35.10 ± 3.12

Table 3. Phytochemical Composition of Milk Thistle Seed Powder

Parameter	Mean ± SD
Total Phenolic Content (mg GAE/g)	34.28 ± 0.02
Total Flavonoid Content (mg QE/g)	28.36 ± 0.11

Table 4. Mineral Composition of Milk Thistle Seed Powder (mg/100 g)

Mineral	Mean ± SD
Sodium	10.05 ± 9.32
Potassium	785.17 ± 15.74
Calcium	907.87 ± 0.09
Magnesium	23.17 ± 1.52
Iron	82.15 ± 0.87
Zinc	6.21 ± 1.60
Copper	2.61 ± 1.20
Manganese	6.77 ± 0.05

Table 5. Changes in Serum ALT Levels (U/L) Over 45 Days

Group	Baseline Mean ± SD	Day 45 Mean ± SD	Mean Change (Δ) ± SD	Within-Group p-value	Between-Group Adjusted Mean Difference vs Control (95% CI)	p-value (ANCOVA)
Control	83.25 ± 10.25	84.28 ± 10.87	+1.03 ± 3.12	0.29	—	—
250 mg	91.63 ± 9.86	81.21 ± 18.74	−10.42 ± 8.88	0.012	−11.34 (−18.22 to −4.46)	0.002
500 mg	87.58 ± 8.23	74.34 ± 17.85	−13.24 ± 9.01	0.004	−14.05 (−21.12 to −6.98)	<0.001

Table 6. Changes in Serum AST Levels (U/L) Over 45 Days

Group	Baseline Mean ± SD	Day 45 Mean ± SD	Mean Change (Δ) ± SD	Within-Group p-value	Between-Group Adjusted Mean Difference vs Control (95% CI)	p-value (ANCOVA)
Control	101.25 ± 10.25	101.28 ± 10.87	+0.03 ± 3.01	0.94	—	—
250 mg	87.63 ± 9.86	79.28 ± 18.74	−8.35 ± 8.64	0.018	−8.42 (−15.01 to −1.83)	0.013
500 mg	93.58 ± 8.23	76.34 ± 17.85	−17.24 ± 9.12	0.001	−17.31 (−24.40 to −10.22)	<0.001

Phytochemical Characters of Milk Thistle Seeds Milk thistle seeds contained substantial amounts of total phenolic content (TPC) and total flavonoid content (TFC), as shown in Figure 1. The TPC was 34.28 ± 0.02 mg GAE/g, while the TFC was 28.36 ± 0.11 mg QE/g.

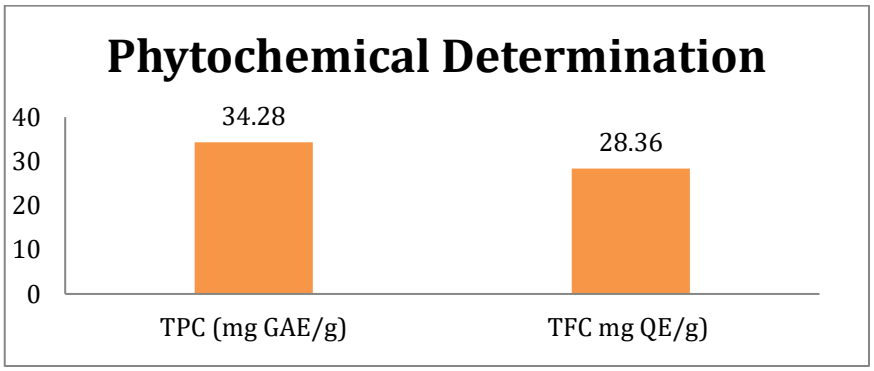

Figure 1 Phytochemical Analysis

Mineral Analysis Mineral analysis of milk thistle seeds powder was was evaluated by standard method for Ca, Zn, Fe, Mg, Mn, and Na. Mean± S.D. for minerals in mg/100g were sodium (10.05±9.32), potassium (785.17±15.74), copper (2.61±11.20) zinc (6.21±1.60), magnesium (23.17±1.52), calcium (907.87±0.09), manganese (6.77±0.05), and iron (82.15±0.87) were found in experiment

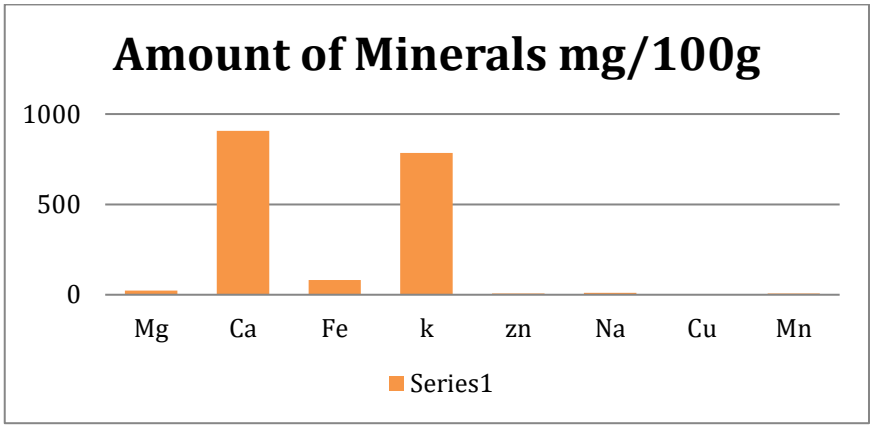

Figure 2 Mineral Analysis

Investigation Effect of Milk Thistle on Elevated Liver Enzymes in Hepatitis C Patients 45 male subjects with diagnosed hepatitis C infection were investigated for their elevated liver function enzymes when given different doses of milk thistle. The serum level of alanine transaminase and aspartate transaminase enzymes was investigated. Patients of the control group (T0) were given not any therapeutic treatment while patients of treatment group-I (T1) and treatment group-II (T2) were given the milk thistle seeds powder in encapsulated doses of 250 mg and 500 mg per day respectively.

DISCUSSION

The present randomized controlled trial demonstrates that short-term supplementation with milk thistle (Silybum marianum) seed powder was associated with statistically significant and clinically meaningful reductions in serum ALT and AST levels in adult male patients with chronic hepatitis C and elevated baseline transaminases. After 45 days of intervention, adjusted mean reductions in ALT were −11.34 U/L (95% CI: −18.22 to −4.46) in the 250 mg/day group and −14.05 U/L (95% CI: −21.12 to −6.98) in the 500 mg/day group compared with control, while corresponding AST reductions were −8.42 U/L (95% CI: −15.01 to −1.83) and −17.31 U/L (95% CI: −24.40 to −10.22), respectively. These findings indicate not only biochemical improvement relative to no supplementation but also a dose-responsive gradient, particularly evident for AST, where the magnitude of reduction nearly doubled at the higher dose. Given that persistent elevation of transaminases reflects ongoing hepatocellular injury in chronic HCV infection (2,3), the observed reductions may suggest attenuation of inflammatory or oxidative stress–mediated hepatic damage.

The hepatoprotective potential of milk thistle is biologically plausible and supported by prior experimental and clinical evidence. Silymarin, the principal bioactive complex of milk thistle, is composed mainly of flavonolignans such as silybin, silydianin, and silychristin, which have demonstrated antioxidant, membrane-stabilizing, and antifibrotic effects in preclinical models (1,4). Mechanistically, these compounds scavenge reactive oxygen species, inhibit lipid peroxidation, and modulate inflammatory signaling pathways implicated in chronic liver injury (4). Systematic reviews evaluating milk thistle in liver diseases have reported modest improvements in transaminases, although heterogeneity in formulations, dosages, and methodological quality has limited definitive conclusions (5). The present study contributes to this body of literature by providing standardized compositional profiling of the administered seed powder alongside rigorously analyzed biochemical outcomes, thereby enhancing interpretability and reproducibility.

The chemical characterization performed in this study demonstrated appreciable total phenolic (34.28 ± 0.02 mg GAE/g) and total flavonoid (28.36 ± 0.11 mg QE/g) content,

consistent with antioxidant-rich botanical preparations. Given that oxidative stress is a central mediator of hepatocyte injury in chronic viral hepatitis (2), the observed enzyme reductions may plausibly reflect the redox-modulating properties of these phytochemicals. Furthermore, the presence of bioactive lipid fractions (22.17 ± 2.31% crude fat) and mineral constituents such as potassium and calcium may influence cellular membrane integrity and intracellular signaling, although their direct contribution to enzyme modulation remains speculative. By integrating compositional data with clinical biochemical outcomes, this study addresses a methodological gap in prior research, where botanical standardization was frequently underreported (5).

The dose-dependent pattern observed—particularly the larger adjusted reduction in AST at 500 mg/day—suggests a pharmacodynamic gradient. AST, while less liver-specific than ALT, is associated with mitochondrial injury and more advanced hepatocellular damage (3). The pronounced reduction in AST at the higher dose (−17.31 U/L vs −8.42 U/L at 250 mg/day) may indicate enhanced mitigation of oxidative or inflammatory stress at increased exposure levels. Although the confidence intervals for both enzymes did not cross zero in the higher-dose group, suggesting statistical robustness, the relatively small sample size necessitates cautious interpretation. Nevertheless, the consistency of within-group reductions and adjusted between-group differences supports the internal validity of the findings.

It is important to contextualize these results within contemporary HCV management. While direct-acting antivirals achieve high rates of viral eradication, biochemical normalization may lag behind virological response, and adjunctive strategies aimed at reducing hepatic inflammation remain clinically relevant, particularly in resource-limited settings (2). However, the present findings should not be interpreted as evidence of antiviral efficacy, as viral load was not assessed. Rather, the study supports the potential role of milk thistle seed powder as a complementary intervention targeting biochemical markers of hepatocellular injury.

Several limitations warrant consideration. The study population was restricted to male participants aged 30–50 years, limiting generalizability to females and older individuals. The intervention duration of 45 days, while sufficient to detect short-term enzyme changes, does not permit conclusions regarding long-term outcomes such as fibrosis progression or sustained biochemical normalization. Additionally, although outcome assessment was blinded and baseline comparability was demonstrated, the absence of a placebo control may introduce performance bias. Finally, the study did not quantify standardized silymarin concentration using chromatographic methods, which would further strengthen dose–response interpretation and comparability with extract-based trials.

Despite these limitations, the study possesses notable strengths, including randomized allocation, baseline-adjusted statistical modeling using ANCOVA, predefined primary biochemical endpoints, and comprehensive reporting of effect sizes with 95% confidence intervals. The absence of serious adverse events and high adherence further support feasibility and short-term safety. Future research should incorporate larger, multicenter cohorts, placebo-controlled designs, standardized phytochemical quantification, and extended follow-up to assess histological or noninvasive fibrosis markers. Evaluation of inflammatory cytokines and oxidative stress biomarkers would also help elucidate mechanistic pathways.

In summary, supplementation with milk thistle seed powder at doses of 250 mg/day and 500 mg/day over 45 days resulted in significant reductions in serum ALT and AST levels compared with no supplementation in adult males with chronic hepatitis C and elevated transaminases. The magnitude of reduction was greater at the higher dose, supporting a

dose–response relationship. These findings provide preliminary clinical evidence that milk thistle seed powder may serve as a hepatoprotective adjunct in chronic HCV–associated biochemical liver dysfunction, meriting confirmation in rigorously designed, placebo-controlled trials with broader populations and longer follow-up durations.

CONCLUSION

In this randomized controlled clinical trial, short-term supplementation with milk thistle (Silybum marianum) seed powder at doses of 250 mg/day and 500 mg/day for 45 days resulted in statistically significant reductions in serum ALT and AST levels compared with no supplementation in adult male patients with chronic hepatitis C and elevated baseline transaminases. The higher dose demonstrated a greater magnitude of enzyme reduction, particularly for AST, indicating a dose-dependent hepatoprotective effect. These findings, supported by detailed compositional profiling of the administered botanical material, suggest that milk thistle seed powder may contribute to attenuation of biochemical markers of hepatocellular injury, potentially through antioxidant and membrane-stabilizing mechanisms. While the results provide clinically relevant preliminary evidence for adjunctive use in chronic HCV–related liver dysfunction, larger placebo-controlled trials with longer follow-up and standardized phytochemical quantification are required to confirm efficacy, evaluate long-term outcomes, and determine broader generalizability

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DECLARATIONS

Ethical Approval: Ethical approval was obtained by institutional review board of Respective Institute Pakistan

Informed Consent: Informed Consent was taken from participants.

Authors' Contributions:

Concept: AA; Design: AL; Data Collection: RT; Analysis: AMI; Drafting: MN

Conflict of Interest: The authors declare no conflict of interest.

Funding: This research received no external funding.

Data Availability: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Acknowledgments: NA

Study Registration: Not applicable.