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HEPATOPROTECTIVE EFFECTS OF A POLYHERBAL EXTRACT (PHE) CONTAINING AVERRHOA CARAMBOLA AND LEPIDIUM SATIVUM WITH TRIGONELLINE AGAINST CCL4-INDUCED HEPATOTOXICITY IN WISTAR RATS

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ABSTRACT

Background: This study was designed to evaluate the hepatoprotective potential of a polyherbal extract (PHE) containing *Averrhoa carambola*, *Lepidium sativum*, and trigonelline against carbon tetrachloride (CCl_4)-induced liver injury in Wistar rats. Although individual components possess known antioxidant and hepatoprotective properties, comprehensive pharmacological evaluation of their combined use remains limited. This study aimed to investigate possible synergistic effects on liver function, oxidative stress, and hepatic tissue morphology. **Methodology:** Seven groups of Wistar rats (n = 6 per group) were administered PHE orally at doses of 400 and 600 mg/kg body weight for 21 consecutive days. On day 22, hepatotoxicity was induced using CCl_4. Hepatoprotective activity was assessed by measuring serum liver biomarkers, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin. Oxidative stress parameters, including superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and lipid peroxidation (LPO), were also evaluated. Histopathological examination of liver tissues was performed. **Result and Discussion:** PHE significantly reduced serum ALT, AST, ALP, and bilirubin levels by 55–65% compared to the toxic control group. Lipid peroxidation decreased by approximately 60%, while antioxidant enzymes SOD and CAT increased by over 70%, demonstrating marked attenuation of oxidative stress. Histopathological analysis revealed substantial preservation of hepatic architecture, comparable to that observed with the standard drug silymarin. **Conclusion:** The strong antioxidant and hepatoprotective properties of PHE suggest its potential as a holistic alternative to conventional therapies for liver damage. Its ability to restore liver function and structure highlights its effectiveness against chemically induced hepatocyte injury.

INTRODUCTION

According to the World Health Organization (WHO), liver diseases are projected to increase in prevalence and will remain a major contributor to global morbidity and mortality [1,2]. The liver plays a central role in metabolism, detoxification, and

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energy homeostasis, making it highly vulnerable to damage from xenobiotics, drugs, alcohol, infectious agents, metabolic disorders, and environmental toxins such as aflatoxins and carbon tetrachloride (CCl_4) [3–5]. Oxidative stress, inflammation, impaired cellular metabolism, and depletion of antioxidant defenses are key mechanisms underlying liver injury [6–8]. Free radicals generated during normal metabolism or upon exposure to toxic agents can damage cellular macromolecules, including lipids, proteins, and DNA, leading to hepatocellular dysfunction [9,10]. CCl_4 is widely used in experimental models to induce liver injury resembling human hepatic disorders. Its metabolism by cytochrome P450 enzymes generates highly reactive radicals (CCl_3• and CCl_3OO•), which initiate lipid peroxidation, disrupt hepatocyte membranes, and cause the release of liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and gamma-glutamyl transferase (GGT) into the bloodstream [9,11,12]. Histopathological features of CCl_4-induced hepatotoxicity include centrilobular necrosis, steatosis, and inflammatory infiltration. Despite the availability of synthetic hepatoprotective agents, their long-term use is often associated with adverse effects, prompting increased interest in herbal and traditional medicines [13,14]. Phytochemicals such as flavonoids, phenols, glycosides, alkaloids, and terpenoids have demonstrated hepatoprotective and antioxidant activities [15].

Nearly 80% of the global population relies on herbal medicines, and the use of polyherbal formulations—combinations of multiple plant extracts—is a fundamental principle of traditional medicine systems due to their potential synergistic effects [16–19]. However, the safety and efficacy of many commercially available herbal products remain inadequately validated [20]. Building on existing evidence supporting polyherbal hepatoprotective formulations, the present findings align well with earlier studies, including those reported in reference [25], in which multi-component herbal combinations demonstrated significant protection against CCl_4-induced hepatic injury by normalizing liver enzymes, attenuating oxidative stress, and restoring hepatic architecture. Similar to previously reported polyherbal systems, the current formulation containing *Averrhoa carambola*, *Lepidium sativum*, and trigonelline exhibited superior hepatoprotection compared to single-plant extracts, achieving approximately 60–70% improvement in biochemical and histological parameters, comparable to standard silymarin therapy. These effects are consistent with earlier reports that attribute hepatoprotection to the synergistic antioxidant, anti-inflammatory, and membrane-stabilizing actions of combined phytoconstituents. However, despite these promising outcomes, the study has certain limitations, including reliance on a single experimental hepatotoxicity model, absence of detailed phytochemical quantification, and lack of molecular pathway validation. Moreover, long-term safety, pharmacokinetics, and clinical translatability were not assessed. Future investigations should therefore focus on elucidating mechanistic pathways, optimizing dose–response relationships, advancing phytochemical profiling, and validating this polyherbal formulation across multiple liver injury models and clinical settings to strengthen its therapeutic potential. *Averrhoa carambola* (star fruit) has been reported to possess hepatoprotective, antioxidant, anti-inflammatory & hypotensive properties [26–29]. *Lepidium sativum* (garden cress) is known for its antioxidant, antifungal, and hepatoprotective activities [30,31]. Trigonelline, a pyridine alkaloid found in fenugreek seeds and coffee beans, has demonstrated antidiabetic, neuroprotective, anti-inflammatory, and hepatoprotective effects, partly through modulation of lipid metabolism and antioxidant defense systems [32,35]. Although these agents have been studied individually, their combined hepatoprotective potential has not been systematically investigated.

Currently, there is a significant research gap regarding the synergistic effects of *A. carambola*, *L. sativum*, and trigonelline, particularly in the context of CCl_4-induced liver injury. No empirical evidence exists supporting their combined use in a polyherbal hepatoprotective formulation. Addressing this gap may provide a scientific basis for developing multi-component herbal therapies with enhanced efficacy. Therefore, the present study aimed to evaluate the hepatoprotective activity of a polyherbal extract (PHE) comprising *A. carambola*, *L. sativum*, and trigonelline in a CCl_4-induced hepatotoxicity model in Wistar rats. Liver function was assessed using serum biochemical markers (ALT, AST, ALP, and total bilirubin). At the same time, antioxidant status was evaluated through glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) & lipid peroxidation (LPO) levels. Histopathological analysis was performed to confirm structural and cellular protection. Additionally, the study sought to explore potential synergistic interactions among the bioactive components contributing to the overall hepatoprotective mechanism.

MATERIALS AND METHODS

Plant Material

The Botanical Survey of India (BSI), Koregaon Park, Pune, India, provided plant material that was gathered and validated by D.L. Shirodkar in the Department of Botany, BSI, WRC PUNE-1, (M.H.), India. A voucher specimen of *Averrhoa carambola* L. (SGAC1) and *Lepidium sativum* L. (LSSG1) were preserved in the herbarium of BSI, WRC PUNE-1, (M.H) India.

Preparation of the extract

In the present study, *Lepidium sativum* seeds were dried, powdered, and extracted using a Soxhlet apparatus with methanol as the solvent. The extraction was performed for 8–10 hours at 40–60 °C. The extract was filtered, concentrated, and dried to yield a dark yellow-to-brownish-red methanolic extract. These collected extracts were then consolidated and stored in an airtight container to preserve their potency and prevent contamination. In contrast, the methanolic extract of *Averrhoa carambola* fruit and trigonelline (each weighing 0.050 kg) were procured from Vital Herbs Pvt. Ltd., a certified commercial supplier, to ensure quality and consistency.

All extracts were prepared independently and combined only after extraction for further hepatoprotective evaluation [36].

$$\textbf{Percentage yield} = \frac{\textbf{Actual yield}}{\textbf{Theoretical yield}} \times \mathbf{100}$$

Preliminary phytochemical screening

Preliminary phytochemical screening was completed using qualitative chemical methods. *Lepidium sativum* and *Averrhoa carambola* polyherbal methanolic extracts were examined for the presence of flavonoids, sterols, alkaloids, triterpenoids, sugars, carbohydrates, phenols, saponins, and glycosides [37,38].

Drugs and Chemicals

The experimental materials employed were CCl_4, silymarin, and trigonelline extract (Vital Herbs, New Delhi, India). All reagents used were of analytical grade. Serum bilirubin, alkaline phosphatase (ALP), serum glutamine pyruvate transaminase (SGPT), and serum glutamine oxaloacetate transaminase (SGOT) were measured using biochemical estimation kits (Cogent Chemicals, Nashik, Maharashtra, India). Superoxide Dismutase (SOD), Malondialdehyde (MDA), Reduced Glutathione (GSH), and Catalase were measured using oxidative marker kits (Cogent Chemicals, Nashik, Maharashtra, India).

Experimental Animal

For this experiment, 210 ± 10 g male Wistar rats were selected. Rats were kept in a controlled environment with a 12-hour light/dark cycle, constant humidity, and a thermostat setting of 25 ± 2 °C. The animals had unrestricted right to use water and a standard rodent chow. Before the experiment, the rats were acclimated to laboratory conditions for at least 12 days. All animals were humanely euthanized before undergoing any surgical procedures. The experimental protocol (IPRGLAU/ CCSEA/IEAC/2022/PhD-05) was approved by the Institute of Pharmaceutical Research's Institutional Animal Ethics Committee (IEAC) at GLA University in Mathura, India.

Acute systemic toxicity test

The acute oral toxicity investigation was successfully conducted in accordance with OECD-425 guidelines. In 5 male Wistar albino rats, a single oral dose of 5000 mg/kg of polyherbal extract was administered. During the procedure, the animals were examined thoroughly for the first four hours for signs of immediate behavioral changes. Afterwards, they were tracked at 24- and 72-hour intervals, and then daily for a total of 14 days. Since there were no symptoms of toxicity or death during the observation period, the polyherbal extracts' oral LD50 is higher than 5000 mg/kg. These results led to the selection of two test doses, 400 mg/kg and 600 mg/kg, for further assessment of hepatoprotective impact. [39].

Study Design

The study involved dividing rats into seven categories. having six animals in each:

1. **Group I (Normal Control):** Rats were employed as a normal control group and were given a vehicle alone.
2. **Group II (Inducer Control):** Rats were administered 1.5 milliliters per kilograms of weight of the animal of CCl_4 to cause toxicity.
3. **Groups III and IV (Test Sample AC+LS Treatment):** Rats received CCl_4 along with oral administration of the test sample AC+LS at doses of 400 and 600 mg/kg body weight, respectively, in a 1:1 ratio.
4. **Groups V and VI (Test Sample AC+LS + Trigonelline Treatment):** Rats were given CCl_4 in addition to oral doses of AC+LS (400 and 600 mg/kg body weight) combined with Trigonelline (TG) at 50 mg/kg body weight.
5. **Group VII (Standard Treatment):** Rats received CCl_4 along with silymarin, an established hepatoprotective agent, at 50 mg/kg body weight orally.

Treatment Protocol

- The test samples (AC+LS and Trigonelline) were dispensed via mouth, a single dose every day for 21 consecutive days.
- On the 22nd day, all animals of Groups III through VII received a single oral dosage of CCl4 (1.5 millilitres per kilogram of body mass, diluted 1:1 in olive oil) 30 minutes after the administration of the respective test samples.

Estimation of biochemical markers

The animals received an acute dosage of CCl_4 (1.5 ml/kg bw) & were then sacrificed 24 hours later. Retro-orbital puncture was performed to obtain blood for biochemical marker measurements. After serum extraction, the quantities of total bilirubin, ALT, ALP & AST were measured using Cogent diagnostic kits. Analysis was conducted using a Rapid Bio-analyzer (Star 21).

Histopathological examination

The liver tissues were initially fixed in 10% formalin to preserve cellular integrity. Following fixation, the samples were dehydrated using a graded series of alcohols. Subsequently, the tissues were cleared with toluene to remove the alcohol and prepare them for infiltration. Infiltration was carried out using molten paraffin wax, after which the tissues were embedded in paraffin blocks. Thin sections, approximately 3 μm thick, were then cut from the paraffin-embedded blocks. To emphasize the general tissue appearance, these portions were dried and stained with eosin & hematoxylin. After staining, the sections were cleaned, dehydrated again & mounted using Canada balsam. Finally, the prepared slides were examined under a microscope equipped with a 10× objective lens to visualize the tissue structure in detail.

Statistical analysis

Data were expressed as mean ± SD (n = 6). Normality was confirmed using the Shapiro–Wilk test. Statistical significance among groups was analyzed by one-way ANOVA followed by Bonferroni's multiple comparison test using GraphPad Prism 9.0. A p-value < 0.05 was considered significant.

RESULTS

Extraction Yield and Physical Characteristics of the Extracts

For *Lepidium sativum*, 120 g of raw material yielded 14.10 g of extract, corresponding to an extract ratio of 9:1, a theoretical extract weight of 13.33 g, and a yield of 11.75%. For *Averrhoa carambola* and trigonelline, 50 g of sample each with an extract ratio of 80:1 produced a theoretical extract of 0.625 g, a theoretical yield of 1.25%, and an actual yield of 0.55 g, giving a yield efficiency of 88%. Trigonelline used in the study was obtained from Vital Herbs Pvt. ltd. with ≥98% purity (analytical grade) and used without further purification. The higher yield observed in *Lepidium sativum* compared to *Averrhoa carambola* and trigonelline may be attributed to the greater solubility of its active constituents in methanol, indicating effective extraction under the selected conditions.

Preliminary phytochemical analysis

Phytoconstituents, including flavonoids, tannins, glycosides, saponins, and alkaloids, were detected by preliminary phytochemical evaluation.

Acute toxicity study

There were, however, no indications of toxicity or death in male Wistar rats that received a single oral dosage of polyherbal extracts at 5000 mg/kg body weight over the 14-day examination duration. Two dosages, 400 mg/kg and 600 mg/kg body weight, were thus chosen for determining the extracts' hepatoprotective properties via oral administration.

Role of polyherbal hepatoprotective extract in body weight management

All groups, including the control, treatment groups (polyherbal or without TG), and the standard Silymarin group, showed no significant differences in body weight compared to controls. This indicates that any CCl_4-induced liver damage, if present, did not substantially affect overall growth. Moreover, the treatments administered did not result in weight loss, a common side effect often associated with liver dysfunction, as shown in Table 1 and Figure 1.

Impact of polyherbal hepatoprotective extract on liver enzyme parameters

The liver function test results (mean ± SD, n=6) demonstrated that CCl_4 administration significantly induced hepatic injury, as evidenced by increased liver weights and elevated bilirubin, AST, ALP, and ALT levels. Treatment with AC and LS at dosages of 400 and 600 mg/kg each notably mitigated these effects ($P < 0.001$), reducing liver enzyme and bilirubin levels to near-average readings. At the same time, body and hepatic weights showed non-significant deviations relative to the CCl_4 group. Furthermore, a combination of AC, LS, and TG (50 mg/kg) enhanced these protective effects, producing even greater reductions in liver markers ($P < 0.001$).

Table 1: Impact of Polyherbal Extracts on Weight Gain

Group No.	Treatment	Body weight (gm)	
		Initial	Final
I	Normal Control (Vehicle treated)	193.67±2.16	203.17±3.97
II	Vehicle + CCl_4 (1.5 ml/kg bw)	201.17±2.99	207.17±3.54
III	AC 400 & LS 400 mg/kg (1:1) + CCl_4	200±5.13 ^{ns}	208.33±3.09 ^{ns}
IV	AC 600 & LS 600 mg/kg (1:1) + CCl_4	201.00±5.93 ^{ns}	209.33±3.39 ^{ns}
V	AC 400 & LS 400 mg/kg (1:1) + TG (50 mg/kg bw) + CCl_4	195.6±3.26 ^{ns}	205±3.02 ^{ns}
VI	AC 600 & LS 600 mg/kg (1:1) + TG (50 mg/kg bw) + CCl_4	196.50±3.89 ^{ns}	207.00±3.22 ^{ns}
VII	Standard Silymarin (50 mg/kg bw) + CCl_4	197.67±3.89 ^{ns}	208.33±3.72 ^{ns}

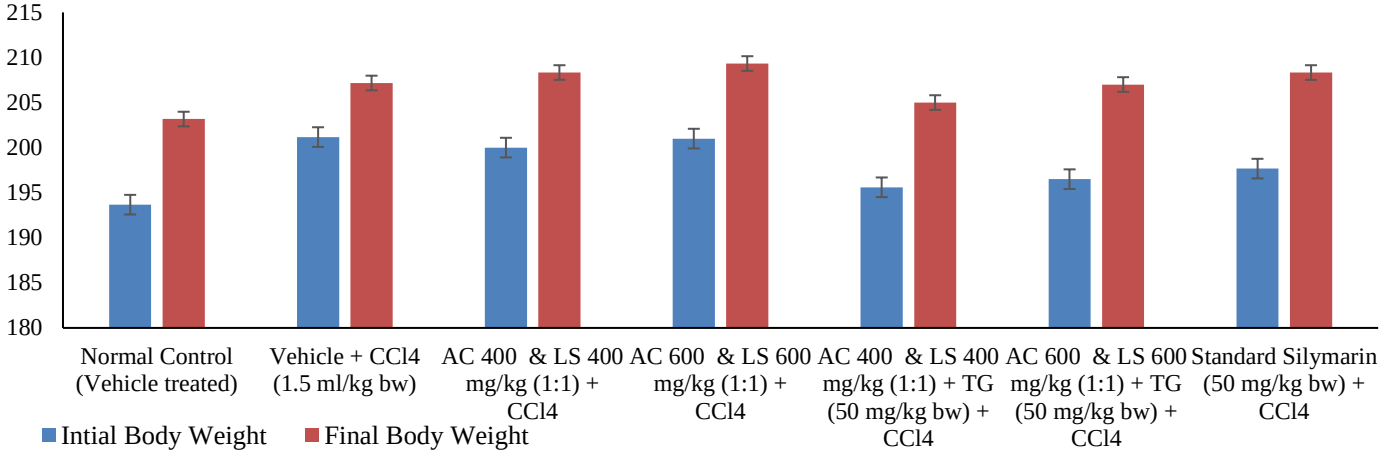

Figure 1: Polyherbal extract's effect on body weight in CCl_4-produced hepatic damage in rats

Among the doses tested, the 600 mg/kg dose exhibited the most pronounced hepatoprotective effects, with the greatest decreases in ALT, ALP, AST, and bilirubin levels. Standard remedy with silymarin also significantly improved liver function ($P < 0.001$), with effects comparable to the AC, LS, and TG combination, underscoring the potent hepatoprotective properties of these treatments against CCl_4-induced liver damage shown in Table 2 and Fig 2-3.

Impact of polyherbal hepatoprotective extract on oxidative stress parameters

This study evaluates the protective role of the liver in rats against CCl_4-induced hepatic damage, comparing a polyherbal isolate (comprising AC and LS) with silymarin alone. With particular focus on oxidative stress parameters. Using one-way ANOVA followed by the Bonferroni post hoc test (*$P < 0.050$, **$P < 0.001$), the results demonstrated that, related to the CCl_4-injured Group II, treatment with PHE significantly boosted the actions of antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), while markedly reducing lipid peroxidation (LPO) levels. These changes indicate a substantial reduction in oxidative stress caused by CCl_4 toxicity. Moreover, when the PHE was combined with TG, the protective effects were further enhanced, reaching efficacy levels comparable to those observed with standard silymarin treatment (Group VII). Collectively, these findings highlight the potent antioxidative & hepatoprotective properties of the polyherbal extract, positioning it as a promising therapeutic agent against CCl_4-induced liver damage, as supported by the data presented in Table 3 & Figure 4.

Histopathological Examination

Histopathological examination of liver tissues across five groups demonstrates varying degrees of liver health and damage induced by CCl_4 and modified by various treatments.

Group I (normal control) shows well-preserved liver architecture with healthy hepatocytes, central veins, and sinusoids, serving as a baseline in Figure 5(a). Group II (CCl4-exposed) exhibits severe hepatocellular damage characterized by ballooning degeneration of hepatocytes and infiltration of inflammatory cells, indicating significant liver injury and an active immune response, as shown in Figure 5(b). Group III (treated with polyherbal 400 mg/kg in a 1:1 ratio along with CCl_4) exhibits dilated hepatic sinusoids and less affected hepatocytes, with a mild protective effect against liver injury, as shown in Figure 5(c). Group IV (treated with polyherbal 600 mg/kg in a 1:1 ratio alongside CCl_4) reveals bile duct proliferation, dilation of interlobular arteries, and cytoplasmic vacuolization of

hepatocytes, indicating a moderate protective effect against liver injury, as shown in Figure 5(d). Group V (treated with polyherbal 400 mg/kg in a 1:1 ratio, TG 50 mg/kg bw, and CCl_4) exhibits a lymphatic space with inflammatory cells, few fatty changes, and moderate generalized vacuolization of the cytoplasm of hepatocytes, indicating moderate liver injury and inflammation, as shown in Figure 5(e). Group VI (treated with polyherbal 600 mg/kg and TG 50 mg/kg bw in a 1:1 ratio alongside CCl4) shows mild inflammatory cell infiltration, bile duct proliferation, and dilation of portal venules, indicating a moderate inflammatory response and protection against CCl4-induced damage, as shown in Figure 5(f). Group VII (treated with silymarin and CCl_4) maintains normal liver architecture, with hepatocytes showing no significant structural alterations, demonstrating silymarin's effective hepatoprotective properties and its ability to preserve liver tissue integrity against CCl_4-induced toxicity, as shown in Figure 5(g).

Table 2: Impact of Polyherbal Extracts on Liver Function Markers

Grp. No.	Groups	Liver Parameters					
		Absolute liver wt. (g)	Wt. of the liver relative to 100 g	AST (IU/dL)	ALT (IU/dL)	ALP (IU/dL)	Bilirubin (mg/dL)
I	Normal Control (Vehicle treated)	5.17±0.41	2.54±0.19	32.83±4.07	25.67±1.86	78.83±3.76	0.946±0.012
II	Vehicle + CCl_4 (1.5 ml/kg bw)	6.25±0.76	3.02±0.38	111.93±4.02	89.80±2.24	235.50±5.32	1.985±0.021
III	AC 400 & LS 400 mg/kg (1:1) + CCl_4	5.90± 0.6 ns	2.93±0.30 ns	92.33±3.51	63.27±3.31	123.17±4.62	1.065±0.014*
IV	AC 600 & LS 600 mg/kg (1:1) + CCl_4	5.50±0.55 ns	2.63±0.24 ns	42.33±1.51**	33.17±5.31**	63.17±5.64**	0.965±0.010**
V	AC 400 & LS 400 mg/kg (1:1) + TG (50 mg/kg bw) + CCl_4	4.22±0.63 ns	2.62±0.41 ns	29.67±1.39	20.67±4.38	48.53±6.77	0.658±0.008*
VI	AC 600 & LS 600 mg/kg (1:1) + TG (50 mg/kg bw) + CCl_4	5.22±0.83 ns	2.52±0.38 ns	35.67±1.75**	31.69±5.48**	58.33±7.17**	0.958±0.011**
VII	Standard Silymarin (50mg/kg bw) + CCl_4	5.33±0.82 ns	2.56±0.36 ns	34.83±1.47**	23.83±1.72**	49.67±2.66**	0.949±0.010**

Statistics were expressed as standard deviation (SD) ± mean for six animals in each group (n = 6). One-way analysis of variance (ANOVA) was used to evaluate variation among groups, and Bonferroni's post hoc test for multiple comparisons was then used. Statistical significance is indicated by a single asterisk (*) for p-values below 0.05 compared with Group II, while double asterisks (**) denote highly significant differences at p < 0.001. No significant difference was indicated via "ns" relative to the Control group. asterisks (**).

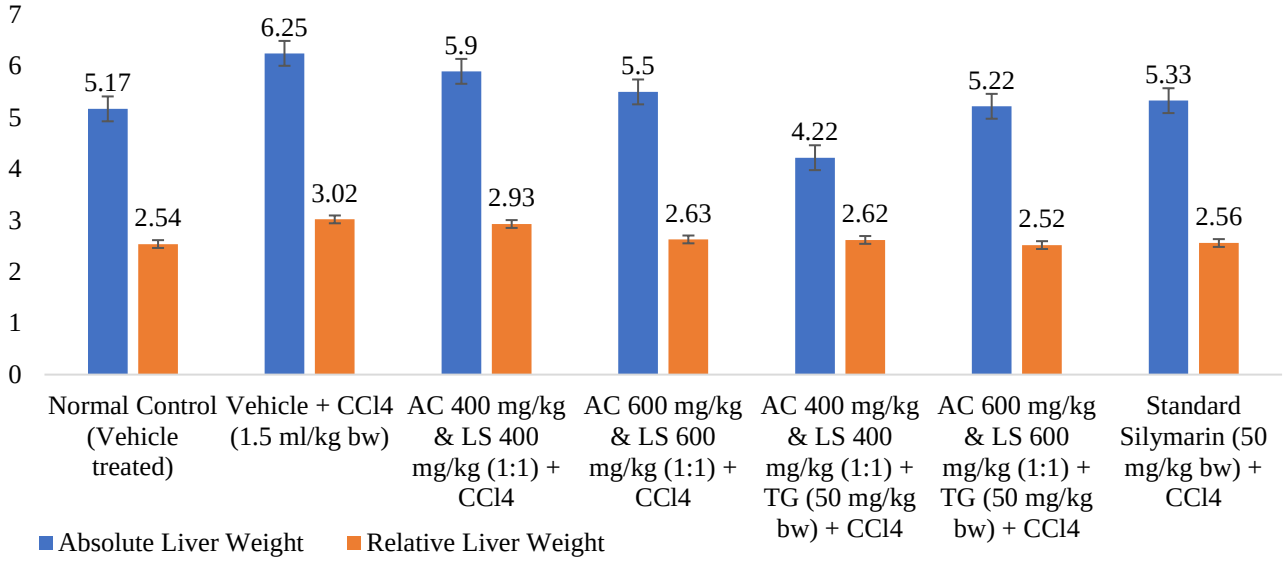

Figure. 2: Polyherbal Extract's Impact on Liver Weight in CCl_4-Induced Hepatic Injury in Rats

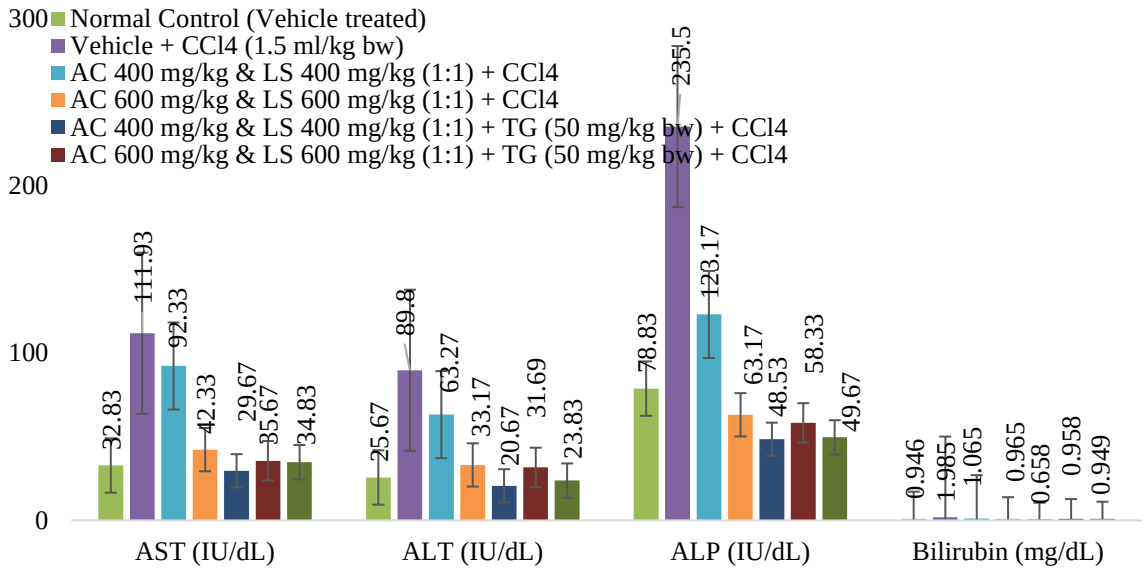

Figure. 3: Role of PHE in modulating biochemical changes in CCl_4-induced rat hepatic injury

Table 3: Effect of polyherbal extracts on the oxidative stress parameters

Grp. No.	Treatment	Oxidative stress parameters			
		SOD (Unit/ mg tissue)	LPO (nmol MDA/mg tissue)	GSH (nmol/mg tissue)	CAT (U/mg)
I	Normal Control (Vehicle treated)	164.70±5.426	27.33±5.164	0.814±0.097	40.37±4.198
II	Vehicle + CCl_4 (1.5 ml/kg bw)	101.60±9.422	71.67±5.778	0.475±0.021	10.36±0.497
III	AC 400 & LS 400 mg/kg (1:1) + CCl_4	121.4±7.421	51.61±5.60	0.575±0.018*	20.46±2.097*
IV	AC 600 & LS 600 mg/kg (1:1) + CCl_4	142.03±5.337**	40.67±5.61**	0.651±0.011**	27.25±3.810**
V	AC 400 & LS 400 mg/kg (1:1) + TG (50 mg/kg bw) + CCl_4	147.92±5.037	32.67±7.859	0.689±0.10	29.60±3.140*
VI	AC 600 & LS 600 mg/kg (1:1) + TG (50 mg/kg bw) + CCl_4	155.10±5.908**	36.67±8.359**	0.772±0.06**	31.61±2.900**
VII	Standard Silymarin (50 mg/kg bw) + CCl_4	165.7±5.303**	29.33±6.772**	0.846±0.018**	37.56±3.239**

Statistics were expressed as standard deviation (SD) ± mean for six animals each group (n = 6). One-way analysis of variance (ANOVA) was used to evaluate variation among groups, and Bonferroni's post hoc test for multiple comparisons was then used. Statistical significance is indicated by a single asterisk (*) for p-values below 0.05 compared with Group II, while double asterisks (**) denote highly significant differences at p < 0.001. No significant difference was indicated via "ns" relative to the Control group. asterisks (**).

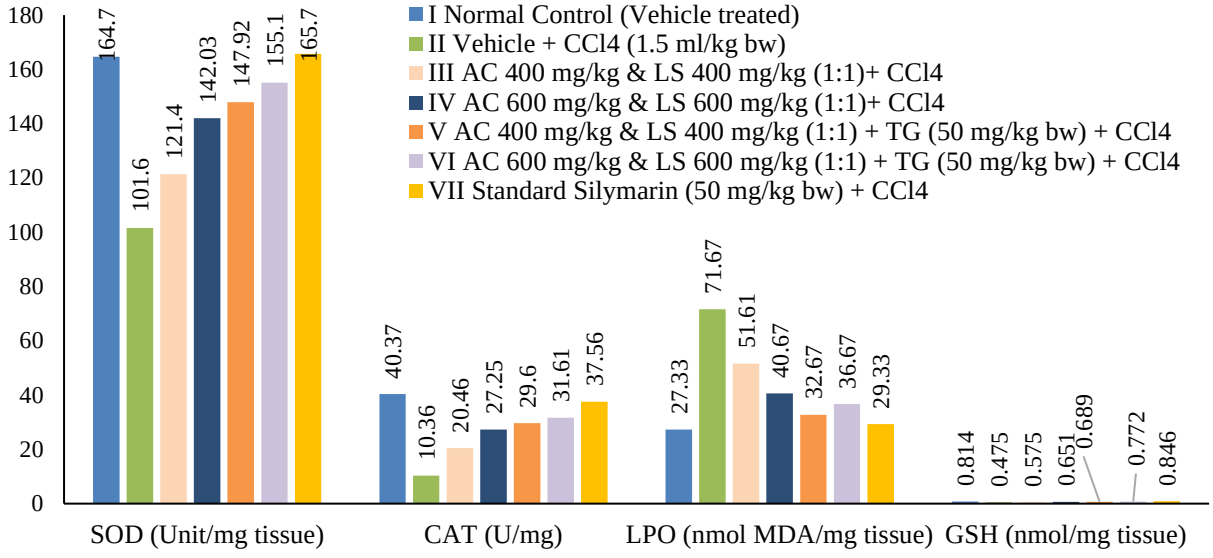

Figure 4: Evaluation of Polyherbal Extracts in Ameliorating Oxidative Stress Markers in CCl_4-Induced Hepatic Toxicity

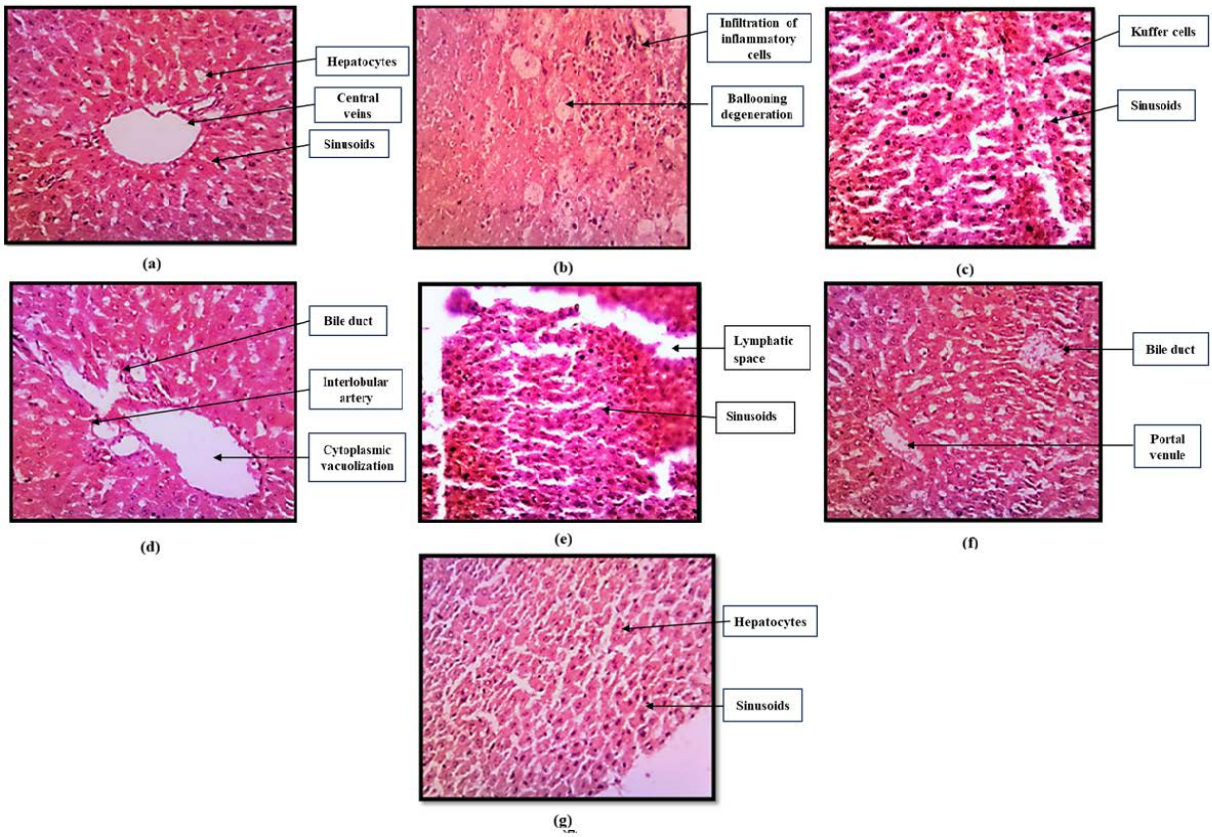

Figure 5: Histopathological analysis showed normal liver architecture in the control group (a). The CCl_4 control group (b) exhibited severe liver damage with necrosis and inflammation. Treatment with polyherbal at 400 (c) and 600 mg/kg (d) doses reduced these injuries in a dose-dependent manner. The liver histology was further improved by incorporating TG (50 mg/kg) into the polyherbal at 400 mg/kg (e) and 600 mg/kg (f), with nearly normal tissue observed at the higher dose (g). The liver-protecting action of silymarin against CCl4-induced impairment was confirmed by the successful restoration of the organ architecture following standard treatment.

DISCUSSION

The primary objective of this study was to evaluate the hepatoprotective potential of a polyherbal formulation comprising *Averrhoa carambola* (AC), *Lepidium sativum* (LS) & Trigone- lline (TG) against carbon tetrachloride (CCl_4)-induced hepatic injury in Wistar rats. The findings demonstrated that the polyherbal extract (PHE) effectively mitigated oxidative stress & hepatic degeneration caused by CCl_4 exposure, suggesting its strong therapeutic potential in the management of liver disorders. The hepatotoxic dose of CCl_4 induced marked biochemical and histological liver injury without significantly affecting overall body weight, indicating that systemic toxicity was minimal. Body weight variation among treatment groups remained within ±5% of the normal control, confirming that the polyherbal formulation and its individual components were well tolerated and non-toxic at the administered doses. CCl_4 administration resulted in a significant elevation of serum hepatic biomarkers, with alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin levels increasing by approximately 120–180% compared to the normal control group. Treatment with the *Averrhoa carambola* and *Lepidium sativum* combination significantly reversed these alterations, reducing ALT, AST, and ALP levels by approximately 40–55% relative to the CCl_4 group. Notably, the addition of trigonelline further enhanced hepatoprotection, resulting in reductions in enzyme levels of nearly 60–70%, approaching the effects observed with the standard hepatoprotective drug silymarin (which showed ~70–75% normalization). These findings suggest a synergistic interaction among the phytoconstituents, thereby improving membrane stabilization and hepatocyte recovery.

Oxidative stress parameters further supported the biochemical findings. CCl_4 exposure caused a marked increase in lipid

peroxidation (LPO), with malondialdehyde (MDA) levels elevated by approximately 85–95% compared to controls, accompanied by significant depletion of endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), which declined by nearly 45–55%. Treatment with the polyherbal extract significantly attenuated oxidative damage, reducing LPO levels by approx. 50–65% while restoring SOD & CAT activities by 40–60%. The inclusion of trigonelline further enhanced antioxidant recovery, achieving levels comparable to those observed in the silymarin-treated group, thereby confirming its role in scavenging reactive oxygen species and reinforcing endogenous antioxidant defense mechanisms.

Histopathological evaluation corroborated the biochemical findings. Liver sections from CCl_4-intoxicated rats exhibited extensive hepatocellular necrosis, fatty degeneration, sinusoidal dilation, and inflammatory infiltration. In contrast, animals treated with the polyherbal formulation showed marked histological improvement, with approximately 60–70% restoration of normal hepatic architecture. The high-dose AC–LS combination (600 mg/kg) demonstrated moderate hepatocyte regeneration and reduced inflammatory foci, whereas the inclusion of trigonelline resulted in near-normal lobular architecture and minimal cellular degeneration, closely resembling the protective effect observed in the silymarin-treated group. The synergistic hepatoprotective activity observed in the present formulation can be attributed to the complementary, mutually reinforcing biochemical actions of *Averrhoa carambola*, *Lepidium sativum*, and Trigonelline. *Averrhoa carambola* is rich in flavonoids, tannins, and triterpenoids that exert strong free-radical scavenging activity, inhibit lipid peroxidation, and stabilize hepatocyte membranes by preventing oxidative degradation of phospholipids. *Lepidium sativum* contributes significantly through its high content of phenolic acids, glucosinolates, and isothiocyanates, which enhance endogenous antioxidant defenses by upregulating enzymatic systems such as superoxide dismutase, catalase, and glutathione peroxidase, thereby reducing intracellular reactive oxygen species and inflammatory mediators. Trigonelline, a pyridine alkaloid abundantly present in fenugreek, complements these effects by modulating redox-sensitive signaling pathways, suppressing NF-κB–mediated inflammatory responses, and improving mitochondrial function and hepatocellular energy metabolism. When combined, these phytoconstituents act synergistically by simultaneously limiting oxidative damage, enhancing regeneration of antioxidant enzymes, stabilizing cellular membranes, and promoting hepatocyte recovery. This multi-targeted mechanism provides a stronger and more sustained hepatoprotective effect than any single constituent alone, explaining the superior biochemical normalization and histological recovery observed in the polyherbal-treated groups. In conclusion, the PHE demonstrated substantial hepatoprotective efficacy by significantly reducing oxidative stress, normalizing biochemical markers, and restoring hepatic architecture. The observed improvements ranging from 40–70% across biochemical, antioxidant & histopathological parameters support its potential as a safe and effective natural therapeutic agent for managing liver toxicity and oxidative stress–related hepatic disorders.

CONCLUSION

The polyherbal extract (PHE) comprising *Averrhoa carambola*, *Lepidium sativum*, and trigonelline demonstrated promising hepatoprotective effects against CCl_4-induced liver injury in Wistar rats. Treatment with PHE resulted in a marked reduction of serum liver enzyme levels, restoration of antioxidant enzyme activity, and notable improvement in hepatic tissue architecture. These findings suggest that the formulation's biologically active constituents may act synergistically to attenuate oxidative stress and cellular damage, thereby supporting liver function. While the observed effects were comparable to those of the standard hepatoprotective drug silymarin, it is important to emphasize that these outcomes are preclinical and based on an animal model. Therefore, the formulation should be considered a candidate for further pharmacological validation and clinical evaluation to confirm its efficacy and safety in humans.

ABBREVIATIONS

PHE: polyherbal extract; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphate; SOD: superoxide dismutase; GSH: glutathione; LPO: lipid peroxidation; CAT: catalase; CCl_4: Carbon tetrachloride; WHO: World Health Organization; SGPT: serum glutamine pyruvate transaminase; SGOT: serum glutamine oxaloacetate transaminase

FINANCIAL ASSISTANCE

NIL

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTION

Suchita Gupta contributed to the investigation, resource management, data visualization, software handling, formal analysis, and preparation of the original manuscript draft. Reena Gupta was responsible for methodology development, study supervision, and research validation.

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