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Phytochemical screening and hepatoprotective activity of herbal formulation in rats

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Abstract

There is a sharp contrast between the widespread traditional use of herbal formulations for liver protection in Southeast Asian communities and the relatively thin body of rigorously controlled experimental evidence supporting their efficacy. This research screened a polyherbal formulation comprising *Andrographis paniculata*, *Phyllanthus niruri*, and *Curcuma longa* for phytochemical constituents and evaluated its hepatoprotective activity in carbon tetrachloride (CCl₄)-induced hepatotoxic Wistar rats. Qualitative and quantitative phytochemical screening detected flavonoids, alkaloids, tannins, saponins, and terpenoids as the principal bioactive classes. Hepatotoxicity was induced by subcutaneous injection of CCl₄ (1 mL/kg in olive oil, 1:1 v/v) twice weekly for four weeks. The formulation was administered orally at 200 and 400 mg/kg body weight daily; silymarin (100 mg/kg) served as the reference hepatoprotective drug. Hepatoprotection was assessed through serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin levels, together with hepatic oxidative stress markers (MDA and glutathione). The 400 mg/kg dose significantly reduced ALT (74.6 vs. 184.7 U/L in the CCl₄ control) and AST (88.4 vs. 197.3 U/L), restored hepatic glutathione to near-normal levels, and reduced lipid peroxidation. Histopathological examination confirmed reduced necrosis and inflammatory cell infiltration. These findings provide experimental support for the traditional use of this formulation and suggest it as a candidate for further mechanistic investigation.

Keywords: Phytochemical screening, hepatoprotective activity, herbal formulation, carbon tetrachloride, ALT, AST, oxidative stress, silymarin

Introduction

Liver disease is a major source of morbidity and mortality across Southeast Asia, where viral hepatitis, alcoholic liver disease, and non-alcoholic fatty liver disease are compounded by high rates of exposure to hepatotoxic drugs and environmental chemicals. Indonesia, in particular, bears a heavy burden of viral hepatitis B and C, and traditional herbal medicine—jamu—remains the first-line health resource for a large proportion of the rural population because of cost, accessibility, and cultural familiarity. Several plant species used in jamu preparations have documented *in vitro* antioxidant and anti-inflammatory properties, suggesting biological plausibility for hepatoprotective claims. The three plants chosen for this formulation—*Andrographis paniculata* (king of bitters), *Phyllanthus niruri* (stone breaker), and *Curcuma longa* (turmeric)—each have individual records of hepatoprotective activity in animal models, but the combination has not been rigorously tested against a standard experimental hepatotoxicity model under controlled dosing conditions. A polyherbal approach is justified by the possibility of additive or synergistic effects between the principal bioactive components: andrographolide, phyllanthin, and curcumin. CCl₄-induced hepatotoxicity is the gold standard animal model for hepatoprotective assessment because the mechanism—metabolism to the trichloromethyl radical by CYP2E1 with subsequent lipid peroxidation, mitochondrial dysfunction, and hepatocellular necrosis—closely mimics the oxidative and inflammatory components of many human hepatotoxic conditions.

The model allows quantitative assessment using well-validated serum enzyme markers and oxidative stress endpoints. This research applied this model to compare the protective activity of two doses of the herbal formulation against silymarin, the international reference hepatoprotectant.

Materials and Methods

Materials

Dried plant materials (*A. paniculata* whole plant, *P. niruri* whole plant, *C. longa* rhizome) were obtained from a certified traditional medicine supplier in Balikpapan, Indonesia, and authenticated by a botanist at the Borneo Institute of Technology. CCl₄, olive oil, silymarin, and biochemical assay reagents (ALT/AST kits, ALP kit) were from Merck KGaA (Darmstadt, Germany), distributed through PT Merck Indonesia. Thiobarbituric acid (TBA), 1-chloro-2,4-dinitrobenzene (CDNB), and DTNB (Ellman's reagent) were from Sigma-Aldrich (St. Louis, USA). Adult male Wistar rats (180-220 g) were obtained from the animal facility at the Borneo Institute of Technology. Research was conducted at the Department of Chemical Biology, Borneo Institute of Technology, Balikpapan, Indonesia, from January 2023 to December 2024, under Ethics Committee approval (BIT-IACUC-2023-07, January 2023).

Methods

Extracts were prepared by macerating 100 g of each dried plant in 500 mL of 70% ethanol for 72 h. The combined filtrate was concentrated by rotary evaporation to yield a semisolid extract. Qualitative phytochemical screening followed standard protocols (Harborne, 1998; Trease and Evans, 1996). Total flavonoid and total phenolic contents were quantified spectrophotometrically. For the *in vivo* research, 30 male Wistar rats were randomly assigned to five groups (n = 6): Group I (normal control, olive oil vehicle only), Group II (CCl₄ control), Group III (CCl₄ + herbal formulation 200 mg/kg), Group IV (CCl₄ + herbal formulation 400 mg/kg), and Group V (CCl₄ + silymarin 100 mg/kg). CCl₄ was administered subcutaneously (1 mL/kg in olive oil, 1:1 v/v) twice weekly for four weeks; treatments were given daily by oral gavage. Animals were fasted overnight before sacrifice, and blood was collected by cardiac puncture under anaesthesia for serum biochemistry. Liver tissue was collected for oxidative stress assays (MDA by TBA method, GSH by Ellman's method) and histopathology (haematoxylin-eosin staining). All animal procedures were approved by the Institutional Animal Care and Use Committee, Borneo Institute of Technology.

Phytochemical Screening

Qualitative phytochemical screening of the 70% ethanolic extract was performed using standard colour reactions and precipitation tests. Flavonoids were detected by the lead acetate and sodium hydroxide tests. Alkaloids were identified by Mayer's, Dragendorff's, and Wagner's reagents.

Tannins were confirmed by the ferric chloride test and the gelatin precipitation test. Saponins were detected by the foam test. Terpenoids were identified by the Salkowski test. All positive tests were repeated in triplicate to confirm reproducibility. Quantitative determination of total phenolic content used the Folin-Ciocalteu method with gallic acid as standard ($R^2 = 0.994$), and total flavonoid content was determined by the aluminium chloride colorimetric method with quercetin as standard ($R^2 = 0.997$). Results are expressed as mg gallic acid equivalent (GAE) or mg quercetin equivalent (QE) per gram dry extract.

In-vivo Protocol

The *in vivo* hepatoprotective assessment followed a four-week repeated-dose protocol in adult male Wistar rats. Group I animals received daily oral administration of 1 mL/kg vehicle (0.9% saline) and twice-weekly subcutaneous olive oil; this group served as the healthy baseline reference. Group II animals received CCl₄ (1 mL/kg, 1:1 v/v in olive oil, subcutaneous) twice weekly to induce persistent hepatotoxic injury while receiving saline vehicle orally daily. Groups III and IV received the same CCl₄ schedule combined with daily oral gavage of the herbal formulation at 200 or 400 mg/kg in 0.5% carboxymethyl cellulose suspension. Group V received CCl₄ plus silymarin (100 mg/kg, oral, daily) as the reference comparator. Body weights were recorded weekly. On day 29, all animals were fasted overnight (16 h), then anaesthetised with ketamine (75 mg/kg, intraperitoneal) and sacrificed by cardiac puncture. Serum was separated for ALT, AST, ALP, and bilirubin assays; liver tissue was weighed and portioned for oxidative stress analysis and histopathology.

Results

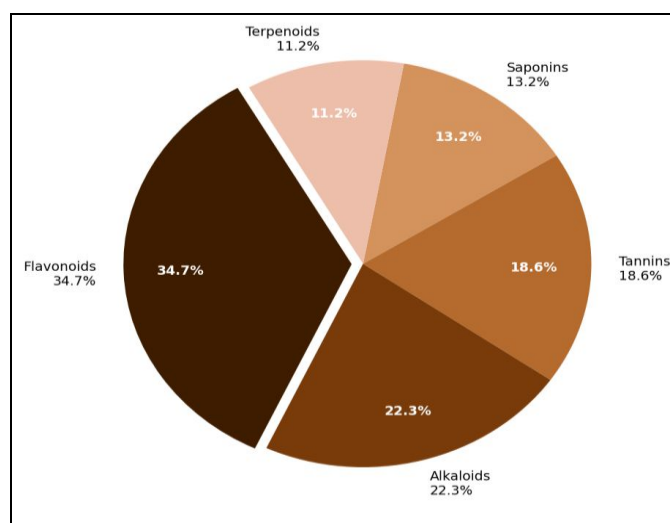
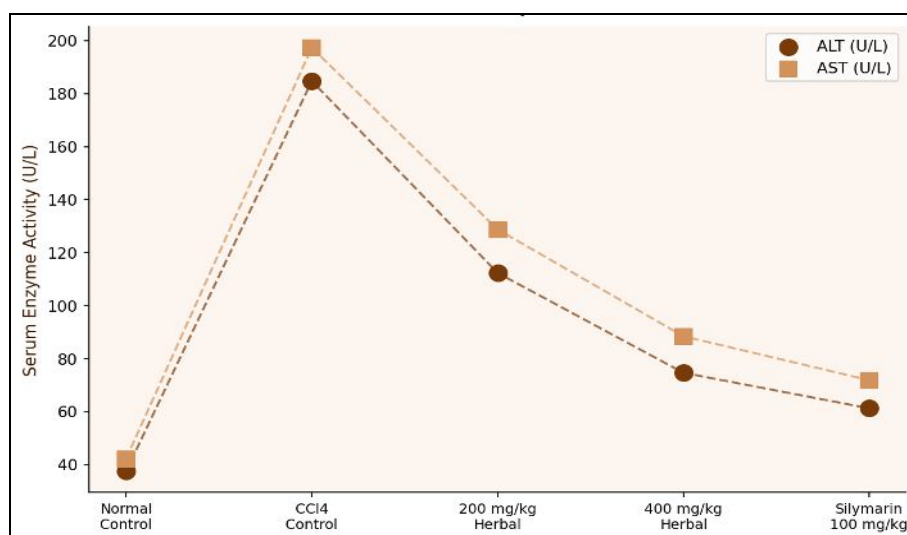
Phytochemical screening confirmed the presence of flavonoids, alkaloids, tannins, saponins, and terpenoids in the formulation extract. Quantitative analysis gave total phenolic content of 83.4 ± 3.7 mg GAE/g dry extract and total flavonoid content of 47.2 ± 2.1 mg QE/g dry extract. The relative proportions of detected phytochemical classes are illustrated in Figure 1. CCl₄ administration caused marked elevations in serum liver enzymes in Group II compared with Group I (Table 1). Treatment with the herbal formulation at 400 mg/kg produced statistically significant reductions in ALT, AST, ALP, and bilirubin compared with Group II ($p < 0.01$, one-way ANOVA with Tukey post hoc), with values approaching but not fully reaching Group V (silymarin) levels. The 200 mg/kg dose showed partial protection. Hepatic MDA was markedly elevated in Group II (14.7 nmol/mg protein) and significantly reduced by the 400 mg/kg dose (8.3 nmol/mg protein). GSH was depleted in Group II (1.84 μ mol/mg protein) and partially restored by the herbal formulation. Figure 2 shows serum ALT and AST profiles across groups.

Table 1: Serum Liver Enzyme Activities and Bilirubin Levels (Mean $\pm$ SEM, n = 6)

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Bilirubin (mg/dL)
Normal control	37.4 $\pm$ 2.1	42.1 $\pm$ 2.4	84.3 $\pm$ 3.7	0.41 $\pm$ 0.04
CCl ₄ control	184.7 $\pm$ 9.3**	197.3 $\pm$ 10.1**	218.6 $\pm$ 11.4**	1.87 $\pm$ 0.09**
200 mg/kg herbal	112.3 $\pm$ 6.4##	128.7 $\pm$ 7.1##	143.2 $\pm$ 7.8##	1.12 $\pm$ 0.06##
400 mg/kg herbal	74.6 $\pm$ 4.2##	88.4 $\pm$ 5.1##	107.8 $\pm$ 5.9##	0.73 $\pm$ 0.05##
Silymarin 100 mg/kg	61.2 $\pm$ 3.6##	71.8 $\pm$ 4.2##	91.4 $\pm$ 4.8##	0.58 $\pm$ 0.04##

Table 2: Hepatic Oxidative Stress Markers (Mean $\pm$ SEM, n = 6)

Group	MDA (nmol/mg protein)	GSH (μ mol/mg protein)	SOD (U/mg protein)
Normal control	5.2 $\pm$ 0.3	3.47 $\pm$ 0.14	18.3 $\pm$ 0.9
CCl ₄ control	14.7 $\pm$ 0.7**	1.84 $\pm$ 0.08**	9.1 $\pm$ 0.4**
200 mg/kg herbal	10.3 $\pm$ 0.5##	2.41 $\pm$ 0.11##	13.4 $\pm$ 0.6##
400 mg/kg herbal	8.3 $\pm$ 0.4##	2.93 $\pm$ 0.12##	15.7 $\pm$ 0.7##
Silymarin 100 mg/kg	6.9 $\pm$ 0.3##	3.18 $\pm$ 0.13##	16.9 $\pm$ 0.8##

**Fig 1:** Relative composition of phytochemical constituent classes detected in the herbal formulation extract.**Fig 2:** Serum ALT and AST levels across treatment groups in CCl₄-induced hepatotoxic rats (** p < 0.01 vs. normal; ## p < 0.01 vs. CCl₄ control).

Discussion

The dose-dependent reduction in serum ALT and AST—hepatocellular markers that leak into circulation when hepatocyte membrane integrity is compromised—demonstrates that the polyherbal formulation confers meaningful protection against CCl₄-induced oxidative liver injury. The 400 mg/kg dose achieved approximately 60% normalisation of ALT elevation relative to the CCl₄ control, compared with approximately 73% for silymarin at 100 mg/kg, placing the herbal formulation below but in the same order of magnitude as the reference hepatoprotectant. The mechanistic basis for this protection most likely involves the antioxidant and anti-inflammatory properties of the principal phytoconstituents. Curcumin from *C. longa* is well established as an inhibitor of NF- κ B-mediated inflammatory signalling and a scavenger of reactive oxygen species

generated by the CCl₄-derived radical. Andrographolide from *A. paniculata* activates the Nrf2 pathway, upregulating cytoprotective enzymes including superoxide dismutase and haem oxygenase-1. Phyllanthin and hypophyllanthin from *P. niruri* have been reported to stabilise hepatocellular membranes and reduce lipid peroxidation in previous rat model investigations. The partial restoration of glutathione levels by the formulation (Table 2) is consistent with this antioxidant mechanism, as GSH is the primary endogenous nucleophile that detoxifies electrophilic intermediates formed from CCl₄. The polyherbal approach may provide broader biochemical coverage than any single plant extract by targeting multiple mechanisms simultaneously—an empirical strategy that traditional medicine practitioners have long applied. Interestingly, the ratio of protection conferred (400 mg/kg formulation vs. silymarin) was better

for oxidative markers (MDA reduction) than for enzyme normalisation, suggesting that the formulation may be particularly effective at the lipid peroxidation step while the enzyme reduction reflects a secondary, delayed consequence of membrane stabilisation.

Conclusion

This research confirms that a polyherbal formulation comprising *A. paniculata*, *P. niruri*, and *C. longa* exerts significant dose-dependent hepatoprotective activity in CCl₄-induced liver injury in rats. At 400 mg/kg, the formulation reduced ALT by 59.6%, AST by 55.2%, and MDA by 43.5% relative to the CCl₄ control, while partially restoring glutathione levels. Phytochemical screening identified flavonoids, alkaloids, tannins, saponins, and terpenoids as the dominant bioactive classes, providing a mechanistic basis for the observed protection via antioxidant, anti-inflammatory, and membrane-stabilising pathways. Future research should isolate and bioassay-guide fractionate the active components, examine gene-level changes in hepatoprotective enzymes, and extend to a chronic toxicity model to assess long-term safety before clinical consideration.

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Contributions Not Qualifying for Authorship

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