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Amelioration of toxin-induced hepatic fibrosis and acute necrosis *via* modulation of oxidative stress and pro-inflammatory cytokines by *Belosynapsis vivipara* extract and a novel zinc-quercetin complex

Hanumantharayappa Bylappa* & Mukund Handral

PESU Institute of Pharmacy, PES University, Hosur Road Campus, Bangalore- 560 100, Karnataka, India

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Liver fibrosis and drug-induced liver injury represent significant global health challenges with limited therapeutic options. This study evaluated the hepatoprotective, antioxidant, and anti-inflammatory potential of the ethanolic leaf extract of *Belosynapsis vivipara* (EBL) and a novel synthesized Zinc-Quercetin (ZQ) complex CCl_4-induced chronic fibrosis and paracetamol (APAP)-induced acute hepatotoxicity in Wistar rats. EBL (500 mg/kg) and ZQ (60 mg/kg) evaluated alongside standard Silymarin and pure Quercetin treatments. In the CCl_4 model, treatment with ZQ and EBL significantly ($P< 0.0001$) attenuated the elevation of serum marker enzymes (ALT, AST, ALP), total bilirubin, and CRP. Specifically, the ZQ complex drastically reduced ALT levels to 76.4 ± 1.3 U/L, outperforming pure quercetin (96.4 ± 1.3 U/L) and showcasing marked recovery compared to the toxic control (187.6 ± 2.9 U/L). Both treatments better restored hepatic antioxidant status, significantly increasing SOD and GSH levels while suppressing lipid peroxidation (MDA). In the APAP model, ZQ and EBL effectively prevented hepatic necrosis and preserved hepatic synthetic functions by restoring total protein and albumin levels. Furthermore, the ZQ complex potently downregulated severe inflammatory surges, reducing TNF-α from 145.8 ± 6.2 pg/mL to 62.5 ± 3.1 pg/mL, alongside significant reductions in IL-6. Histopathological analysis confirmed the striking preservation of hepatic architecture and the prevention of steatohepatitis and cellular ballooning in the treated groups. The study demonstrates that the ZQ complex exhibits superior hepatoprotective, antioxidant, and anti-inflammatory efficacy at a substantially lower dose indicate that zinc-complexation is a highly effective structural strategy for overcoming the bioavailability limitations of therapeutic flavonoids.

Keywords: Antioxidant, *Belosynapsis vivipara*, Carbon tetrachloride, Hepatoprotection, Paracetamol, Zinc-Quercetin

Liver disease represents a significant global health crisis, responsible for approximately 2 million deaths annually[1]. Persistent liver injury, regardless of the cause, triggers fibrosis, a pathological wound-healing response driven by the activation of hepatic stellate cells (HSCs) and their excessive deposition of extracellular matrix (ECM)[2]. While fibrosis was once considered irreversible, recent evidence suggests it is a dynamic process with a critical therapeutic window for pharmacological intervention.

Evaluating novel hepatoprotective agents requires well-characterized preclinical models. The carbon tetrachloride (CCl_4) model induces chronic injury via CYP2E1-mediated bioactivation, generating highly reactive trichloromethyl free radicals that cause severe lipid peroxidation and centrilobular necrosis[3]. Conversely, the Paracetamol (APAP) model mimics acute drug-induced liver injury. In APAP overdose, detoxification pathways are saturated, leading to the severe depletion of hepatic glutathione (GSH) and the accumulation of the toxic metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI), which rapidly triggers fatal mitochondrial dysfunction and sterile inflammation[4]. Using both models allows for a robust assessment of therapies against injury driven by external free radicals (CCl_4) and by the failure of internal detoxification (APAP).

*Correspondence:
E-mail: findraya@gmail.com ; Mobile No: +91-9964859932

Abbreviations: ALP, Alkaline Phosphatase; ALT, Alanine Aminotransferase; ANOVA, Analysis of Variance; APAP, N-acetyl-p-aminophenol (Paracetamol); AST, Aspartate Aminotransferase; CCl_4, Carbon Tetrachloride; CPCSEA, Committee for the Purpose of Control and Supervision of Experiments on Animals; DILI, Drug-Induced Liver Injury; EBVL, Ethanolic Extract of *Belosynapsis vivipara* Leaves; GSH, Reduced Glutathione; H&E, Hematoxylin and Eosin; IAEC, Institutional Animal Ethics Committee; IIHR, Indian Institute of Horticultural Research; IL-6, Interleukin-6; MDA, Malondialdehyde; OECD, Organisation for Economic Co-operation and Development; SEM, Standard Error of the Mean; SOD, Superoxide Dismutase; TNF-α, Tumor Necrosis Factor-alpha; ZQ, Zinc-Quercetin

Current management for end-stage liver disease relies heavily on liver transplantation, a procedure severely limited by donor shortages. This therapeutic gap drives the urgent search for novel pharmacotherapies from natural sources. *Belosynapsis vivipara* (Dalzell) C.E.C. Fisch., a rare herb from the Western Ghats, has demonstrated antioxidant and anti-inflammatory properties[5]. Notably, phytochemical analyses reveal that *B. vivipara* contains both the flavonoid quercetin and remarkably high endogenous concentrations of zinc.

Quercetin is a well-established hepatoprotective agent known to scavenge free radicals and modulate inflammatory pathways such as NF-κB. However, its clinical application is severely hampered by poor aqueous solubility, extensive first-pass metabolism, and overall low oral bioavailability[6]. Interestingly, quercetin is an efficient chelator of divalent metal ions and acts as a zinc ionophore, facilitating the transport of zinc—an essential catalytic cofactor for antioxidant enzymes like Superoxide Dismutase (SOD)—across cell membranes[7,8].

This study is based on the rationale that the co-localization of zinc and quercetin in *B. vivipara* represents a natural, synergistic defense mechanism. We therefore investigated the crude ethanolic extract of *B. vivipara* alongside a synthetically prepared Zinc-Quercetin (ZQ) complex, hypothesizing that targeted metal complexation overcomes quercetin's bioavailability limitations, thereby amplifying its therapeutic efficacy against acute and chronic hepatic insults. While the antioxidant properties of pure quercetin are well-documented, its clinical utility is heavily restricted by poor bioavailability. This study adds significant novelty by providing the first *in vivo* hepatoprotective evaluation of the *B. vivipara* extract and conducting a direct comparative efficacy analysis between the crude extract, pure quercetin, and the synthesized Zinc-Quercetin complex at defined doses. By directly contrasting these interventions, this research elucidates whether zinc-complexation serves as a superior structural strategy to enhance the biological efficacy of therapeutic flavonoids in established liver injury models.

Materials and Methods

Plant material and chemicals

Leaves of *B. vivipara* were collected from Wayanad, Kerala, India. Botanical authentication was performed by Dr. Rajshekharan at the Indian Institute of Horticultural Research, Bengaluru. A voucher specimen (Herbarium No: PCOG/B.VIVIPARA-32/Leaves/MCP/2024) was deposited at Mallige College of Pharmacy. Hepatotoxicity was induced using Carbon Tetrachloride (CCl_4) and Paracetamol (APAP). Silymarin was used as the standard positive control.

Preparation of extract and synthesis

Cleaned, shade-dried leaves (500 g) were extracted with 80% ethanol utilizing a Soxhlet apparatus. The crude extract (EVBL) was concentrated using a rotary evaporator at 45°C and stored at 4°C. The Zinc-Quercetin (ZQ) complex was synthesized by reacting isolated quercetin with zinc sulphate. The ZQ complex has undergone complete physicochemical characterization, including UV-vis, FT-IR, XRD, DSC, and SEM analyses. However, the primary focus of this study is to evaluate the complex's biological activity, the detailed structural data are the subject of a separate article and are not duplicated here.

Experimental Animals

Healthy Wistar albino rats (120-160 g) were maintained under standard laboratory conditions (27 ± 2°C, 12 h light/dark cycle). The study protocol was approved by the Institutional Animal Ethics Committee (Registration number: 1432/PO/Re/S/11/ CPCSEA; Protocol No: MCP176/2025-26).

Acute oral toxicity

An acute toxicity study was conducted according to OECD 425 guidelines using female Wistar rats[9]. The maximum upper limit dose of 5000 mg/kg of EVBL was administered orally. Animals were observed for mortality and behavioral changes for 14 days.

Hepatoprotective study protocols CCl_4-induced chronic hepatotoxicity (14 Days)

Rats were randomly divided into six groups of six animals each (n=6). Group 1 (Vehicle) received 0.5% CMC vehicle orally (p.o.) daily and olive oil (1.5 ml/kg, i.p.) twice weekly. Group 2 (CCl4) was administered a 1:1 mixture of CCl_4 and olive oil (1.5 ml/kg, i.p.) twice a week (on the 1st, 4th, 8th, and 12th days) for 14 days. Group 3 (Silymarin) received the CCl_4 and olive oil induction (1.5 ml/kg, i.p., twice a week) concurrently with Silymarin (100 mg/kg, p.o.) daily. Group 4 (Quercetin) received the CCl_4 induction along with the Quercetin (60 mg/kg, p.o.) daily. Group 5 (Zn-Quercetin) received the CCl_4 induction along with the ZQ complex (60 mg/kg, p.o.)

daily. Finally, Group 6 (EBVL) received the CCl_4 induction concurrently with the EBVL extract (500 mg/kg, p.o.) daily.

Paracetamol-Induced Acute Hepatotoxicity (7 Days)

Rats were randomly divided into Six groups of six animals each (n=6). Group 1 (Vehicle) received a 0.5% CMC vehicle orally (p.o.) daily. Group 2 (APAP) was administered APAP (500 mg/kg, p.o.) daily. Group 3 (Silymarin) received the APAP induction concurrently with Silymarin (100 mg/kg, p.o.) daily. Group 4 (Quercetin) received the CCl_4 induction along with the Quercetin (60 mg/kg, p.o.) daily. Group 5 (Zn-Quercetin) received the APAP induction along with the ZQ complex (60 mg/kg, p.o.) daily. Finally, Group 6 (EBVL) received the APAP induction concurrently with the EBVL extract (500 mg/kg, p.o.) daily.

Dose Justification

The therapeutic dose of 60 mg/kg for the pure Quercetin and ZQ complex was meticulously selected based on established preclinical literature, which identifies 50–100 mg/kg as the optimal sub-maximal hepatoprotective range in rodent toxicity models[10,11]. Utilizing this moderate dose prevents receptor saturation and provides a highly precise baseline to comparatively evaluate whether zinc complexation successfully enhances the bioavailability of the flavonoid structure[6].

Biochemical and Histopathological Estimation

Blood was collected via carotid bleeding, and serum was isolated. Serum ALT, AST, and ALP were estimated using the Reitman and Frankel method[12]. Bilirubin, total protein, and albumin were quantified using standard colorimetric kits[13,14]. Hepatic reduced glutathione (GSH) was determined using the Ellman method[15]. Lipid peroxidation (MDA) was measured per Ohkawa et al., and SOD activity was assayed via the Marklund method[16,17]. Pro-inflammatory cytokines (TNF-α and IL-6) were quantified using specific rat ELISA kits. Dissected livers were fixed in 10% formalin, embedded in paraffin, sectioned (4-5 µm), and stained with hematoxylin and eosin (H&E).

Statistical Analysis

Data are expressed as Mean ± SEM. Prior to analysis, the data were assessed for normal distribution using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. Statistical differences were analyzed using one-way ANOVA followed by Tukey's post-hoc test (GraphPad Prism). A P value < 0.05 was considered significant.

Results

Acute Oral Toxicity

In the OECD 425 acute toxicity study, EVBL administered at 5000 mg/kg resulted in zero mortality or morbidity over 14 days. Consequently, 1/10th of this limit dose (500 mg/kg) was established as the safe therapeutic dose for the extract.

Effect on CCl_4-Induced Hepatotoxicity

Administration of CCl_4 induced severe hepatocellular injury, significantly elevating serum marker enzymes compared to normal controls, including ALT (187.6±2.9 U/L), AST (248.6±3.5 U/L), and ALP (391.1±4.2 U/L). CCl_4 toxicity also caused a marked increase in total bilirubin (2.96±0.04 mg/dL) and the inflammatory marker CRP (8.4±0.4 mg/L). Treatment with the ZQ complex (60 mg/kg) drastically attenuated these elevations, demonstrating efficacy that outperformed pure quercetin and was comparable to the standard Silymarin. Specifically, ZQ reduced ALT to 76.4±1.3 U/L, AST to 106.4±1.6 U/L, ALP to 177.7±2.9 U/L, and total bilirubin to 0.96±0.02 mg/dL alongside a significant reduction in CRP to 3.1±0.2 mg/L. The analysis of results is presented in (Table 1 and Fig. 1).

Table 1 — Effect of *Belosynapsis vivipara* extract and Zn-Quercetin complex on serum and tissue biochemical parameters in CCl_4-induced hepatotoxicity in rats.

Sr. No	Biochemical Parameters	Normal	CCl4 Control	Silymarin	Quercetin	Zn-Quercetin	BV Extract
1	ALT (U/L)	43.6 ± 1.2	187.6 ± 2.9	70.3 ± 1.3	96.4 ± 1.3	76.4 ± 1.3	86.6 ± 1.3
2	AST (U/L)	56.2 ± 1.4	248.6 ± 3.5	96.6 ± 1.4	130.1 ± 1.7	106.4 ± 1.6	117.8 ± 1.8
3	ALP (U/L)	113.6 ± 1.6	391.1 ± 4.2	167.6 ± 2.9	217.6 ± 2.9	177.7 ± 2.9	198.5 ± 3.4
4	T. Bilirubin (mg/dL)	0.47 ± 0.01	2.96 ± 0.04	0.88 ± 0.02	1.37 ± 0.03	0.96 ± 0.02	1.15 ± 0.02
5	MDA (nmol/mg prot)	1.25 ± 0.08	6.85 ± 0.25	2.15 ± 0.15	3.45 ± 0.25	2.35 ± 0.18	2.85 ± 0.22
6	GSH (µmol/g tissue)	5.82 ± 0.15	1.95 ± 0.12	4.65 ± 0.18	3.25 ± 0.22	4.42 ± 0.21	3.95 ± 0.25
7	SOD (U/mg prot)	12.45 ± 0.32	4.15 ± 0.28	9.85 ± 0.35	6.85 ± 0.35	9.25 ± 0.41	8.15 ± 0.38
8	CRP (mg/L)	1.5 ± 0.1	8.4 ± 0.4	2.8 ± 0.2	4.5 ± 0.3	3.1 ± 0.2	3.8 ± 0.3

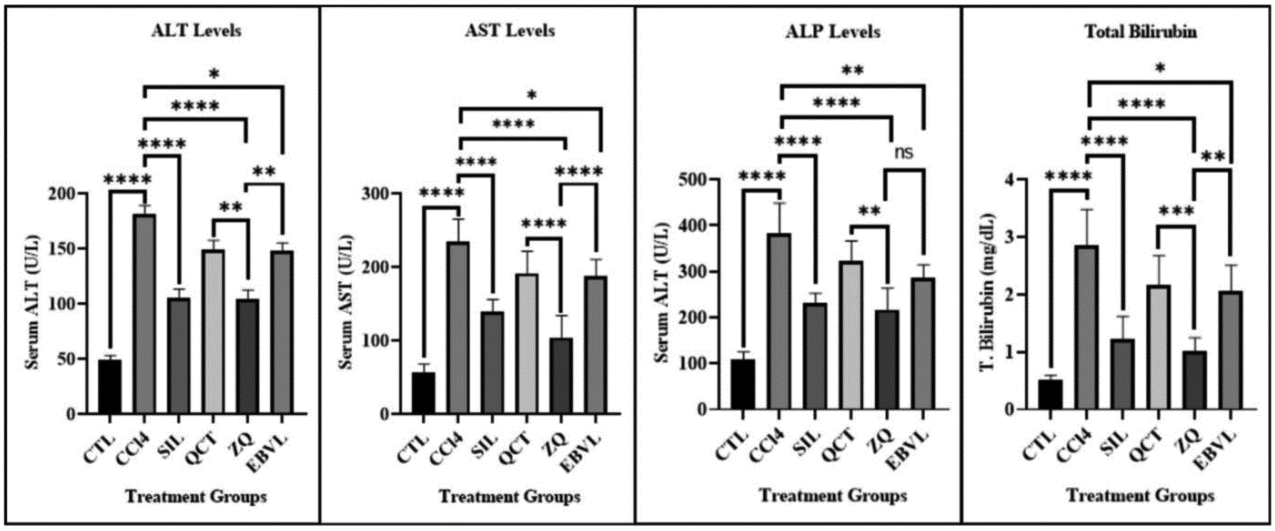


Fig. 1 — Effect of different treatments on serum liver function markers in CCl_4-induced hepatotoxicity over 14 days. Data are expressed as Mean ± SEM (n=6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Asterisks denote statistical significance as indicated by the brackets: **P*< 0.05, *P*< 0.01, ****P*< 0.001, *****P*< 0.0001; ns = non-significant

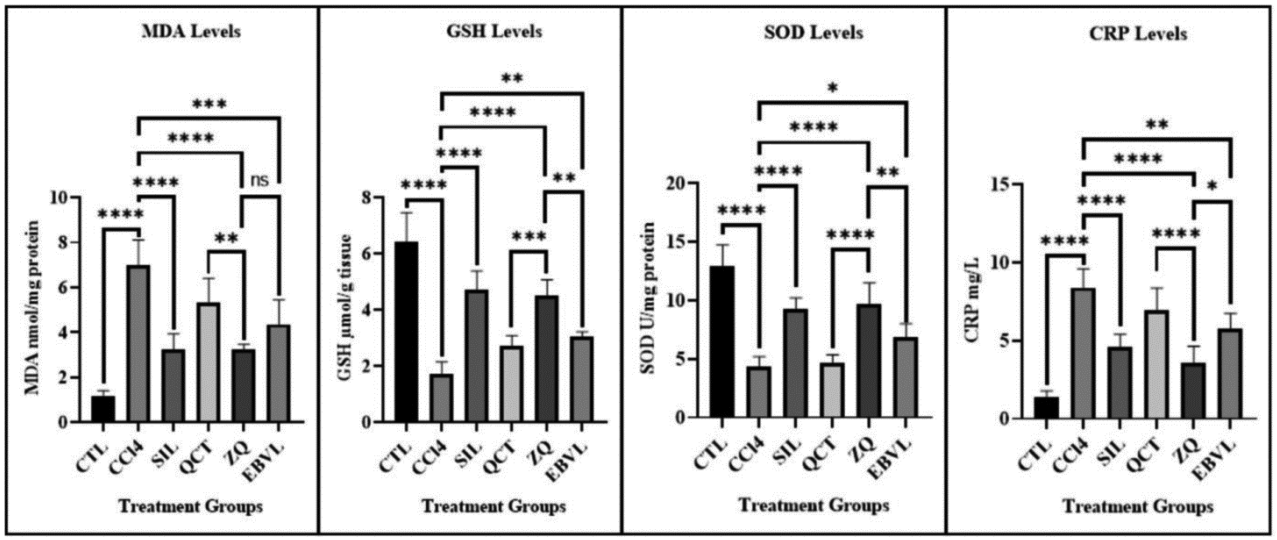


Fig. 2 — Effect of different treatments on hepatic antioxidant status and inflammation markers in CCl_4-induced hepatotoxicity over 14 days. Data are expressed as Mean ± SEM (n=6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Asterisks denote statistical significance as indicated by the brackets: **P*< 0.05, *P*< 0.01, ****P*< 0.001, *****P*< 0.0001; ns = non-significant

The extract EBVL (500 mg/kg) also showed significant protection across all markers, albeit slightly less potent than the synthesized ZQ complex.

Furthermore, CCl_4 triggered profound oxidative stress, evidenced by severely depleted tissue GSH (1.95±0.12 µmol/g) and SOD (4.15±0.28 U/mg prot), alongside heavily elevated lipid peroxidation (MDA: 6.85±0.25 nmol/mg prot). The ZQ complex robustly restored hepatic GSH to 4.42±0.21 µmol/g and SOD to 9.25±0.41 U/mg prot, while effectively suppressing lipid peroxidation by lowering MDA levels to 2.35±0.18 nmol/mg prot as presented in (Table 1 and Fig. 2). Histopathological sections of the ZQ-treated and EBVL-treated groups showed striking preservation of normal cellular architecture, effectively preventing the centrilobular necrosis and

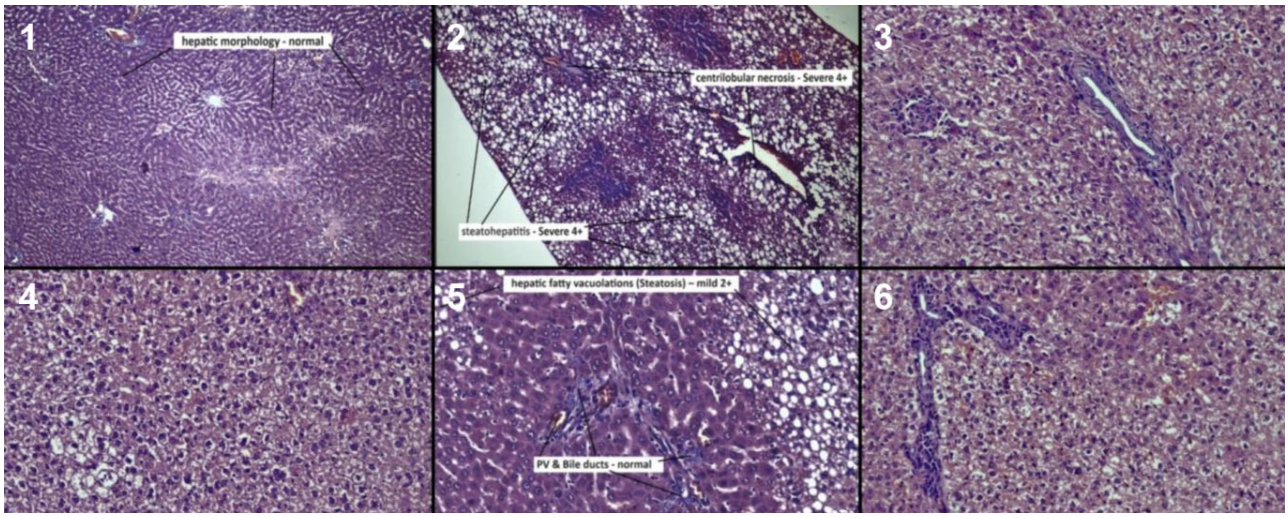


Fig. 3 — Histopathological examination of rat liver sections in CCl_4-induced hepatotoxicity (H&E stain). (1) Normal Control (CTL): Normal hepatic architecture. (2) CCl_4 Control: Severe (4+) centrilobular necrosis and massive steatohepatitis. (3) Silymarin (SIL, 100 mg/kg): Significant preservation and reduced necrosis. (4) Pure Quercetin (QCT, 60 mg/kg): Moderate recovery and reduced fat deposition. (5) Zn-Quercetin (ZQ, 60 mg/kg): Near-normal architecture with only mild (2+) steatosis. (6) *Belosynapsis vivipara* Extract (EVBL, 500 mg/kg): Cellular recovery and reduced fibrotic changes

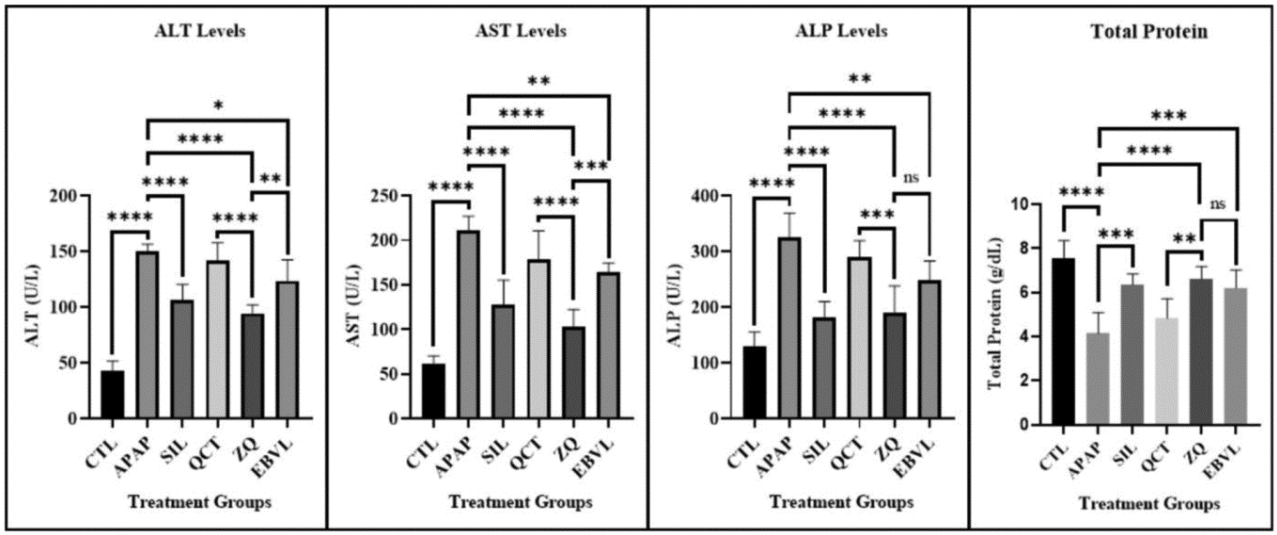


Fig. 4 — Effect of different treatments on serum liver function markers in Paracetamol (APAP)-induced hepatotoxicity over 7 days. Data are expressed as Mean ± SEM (n=6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Asterisks denote statistical significance as indicated by the brackets: *P< 0.05, P< 0.01, ***P< 0.001, ****P< 0.0001; ns = non-significant

steatosis observed in the toxic control as presented in (Fig. 3).

Effect on APAP-induced hepatotoxicity

APAP overdose caused acute hepatic necrosis, severely elevating serum enzymes including ALT (157.7±2.9 U/L), AST (217.1±3.8 U/L), and ALP (321.5±4.0 U/L) compared to the normal group. The toxicity also severely impaired hepatic synthetic function, significantly reducing total protein to 4.65±0.08 g/dL and albumin to 2.42±0.06 g/dL. Pre-treatment with ZQ (60 mg/kg) significantly protected the liver, maintaining ALT at 70.3±1.3 U/L, AST at 96.6±1.4 U/L, and ALP at 168.2±2.9 U/L. ZQ also effectively restored total protein to 6.28±0.06 g/dL, closely mirroring the protective effects of Silymarin as presented in (Table 2 and Fig. 4). Furthermore, ZQ robustly restored serum albumin to

Table 2 — Effect of *Belosynapsis vivipara* extract and Zn-Quercetin complex on serum and tissue biochemical parameters in Paracetamol (APAP)-induced hepatotoxicity in rats

S. No	Biochemical Parameters	Normal	APAP Control	Silymarin	Quercetin	Zn-Quercetin	BV Extract
1	ALT (U/L)	47.1 ± 0.9	157.7 ± 2.9	63.6 ± 1.3	90.2 ± 1.4	70.3 ± 1.3	80.2 ± 1.4
2	AST (U/L)	63.7 ± 1.2	217.1 ± 3.8	89.9 ± 1.5	120.4 ± 1.7	96.6 ± 1.4	108.1 ± 1.9
3	ALP (U/L)	128.2 ± 1.9	321.5 ± 4.0	157.7 ± 2.9	208.4 ± 2.9	168.2 ± 2.9	188.3 ± 2.9
4	T. Protein (g/dL)	7.28 ± 0.06	4.65 ± 0.08	6.58 ± 0.06	5.58 ± 0.06	6.28 ± 0.06	5.88 ± 0.06
5	Albumin (g/dL)	4.18 ± 0.06	2.42 ± 0.06	3.68 ± 0.06	2.98 ± 0.06	3.48 ± 0.06	3.18 ± 0.06
6	TNF-α (pg/mL)	25.4 ± 1.5	145.8 ± 6.2	55.2 ± 2.8	88.5 ± 4.2	62.5 ± 3.1	78.4 ± 3.5
7	IL-6 (pg/mL)	18.2 ± 1.2	98.5 ± 5.4	38.4 ± 2.1	65.4 ± 3.5	45.2 ± 2.5	58.6 ± 2.9

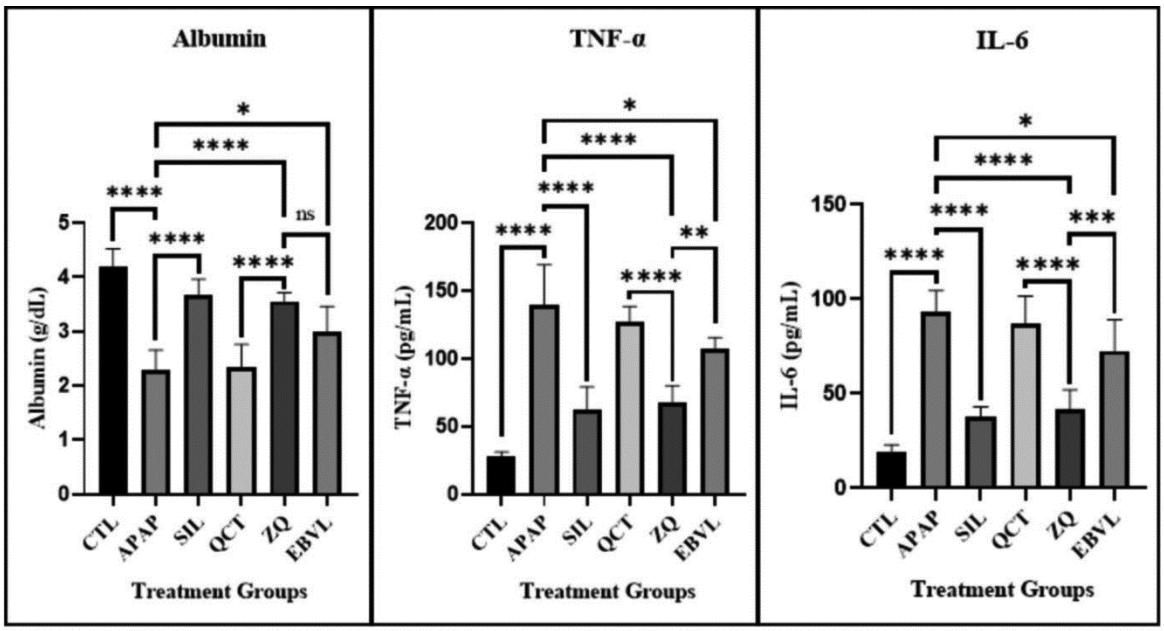


Fig. 5 — Effect of different treatments on serum albumin and pro-inflammatory cytokines (TNF-α and IL-6) in Paracetamol (APAP)-induced hepatotoxicity over 7 days. Data are expressed as Mean ± SEM (n=6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Asterisks denote statistical significance as indicated by the brackets: **P*< 0.05, *P*< 0.01, ****P*< 0.001, *****P*< 0.0001; ns = non-significant

3.48±0.06 g/dL. The extract EBVL (500 mg/kg) similarly exhibited substantial protective effects across these hepatic functional markers.

APAP toxicity also initiated a severe inflammatory cascade. The ZQ complex potently downregulated serum pro-inflammatory cytokines, reducing TNF-α to 62.5±3.1 pg/mL and IL-6 to 45.2±2.5 pg/mL. This is a drastic reduction compared to the massive surges of 145.8±6.2 pg/mL (TNF-α) and 98.5±5.4 pg/mL (IL-6) observed in the untreated APAP control group as presented in (Table 2 and Fig. 5). Microscopic analysis corroborated these findings; ZQ and EBVL treatments effectively prevented ballooning degeneration and restricted inflammatory cell infiltration, promoting active hepatocyte regeneration, as presented in (Fig. 6).

Discussion

The current study comprehensively validates the hepatoprotective efficacy of the Belosynapsis vivipara ethanolic extract (EBVL) and explores the pharmacological advantage of synthesizing a Zinc-Quercetin (ZQ) coordination complex. Both interventions demonstrated marked protection against chemically induced liver injury, rivalling the clinical standard Silymarin.

Carbon tetrachloride initiates fibrosis through the continuous generation of highly reactive trichloromethyl radicals via the CYP2E1 enzyme[3]. These free radicals aggressively attack lipid membranes, initiating a chain reaction of lipid peroxidation that destroys cellular integrity. The

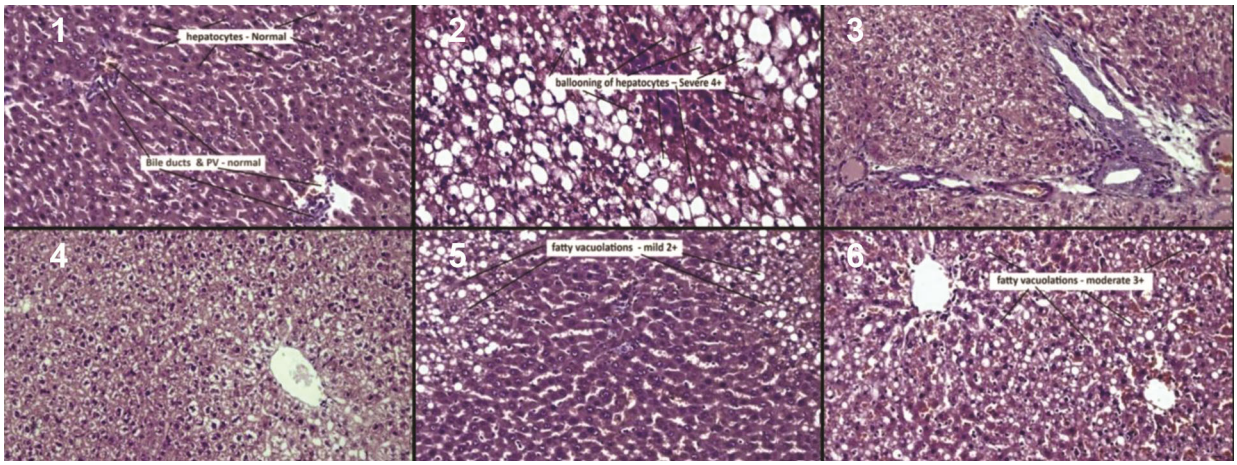


Fig. 6 — Histopathological examination of rat liver sections in APAP-induced hepatotoxicity (H&E stain). (1) Normal Control (CTL): Normal hepatic architecture. (2) APAP Control: Severe (4+) ballooning degeneration and necrosis. (3) Silymarin (SIL, 100 mg/kg): Marked protection against necrosis. (4) Pure Quercetin (QCT, 60 mg/kg): Partial tissue recovery. (5) Zn-Quercetin (ZQ, 60 mg/kg): Excellently preserved architecture with mild (2+) fatty vacuolations. (6) *Belosynapsis vivipara* Extract (EVBL, 500 mg/kg): Significant structural recovery with moderate (3+) fatty vacuolations

massive elevation of MDA alongside the depletion of endogenous SOD and GSH in our CCl_4 control group perfectly illustrates this oxidative demolition. Treatment with the ZQ complex significantly suppressed MDA formation and replenished SOD and GSH. This indicates that the ZQ complex acts as a formidable radical scavenger and membrane stabilizer, halting the fibrotic cascade at its oxidative root.

Conversely, Paracetamol (APAP) toxicity is driven by the exhaustion of cellular defense mechanisms[4]. At high doses, the accumulation of NAPQI rapidly depletes hepatic glutathione, leaving mitochondria vulnerable to oxidative stress, which culminates in massive centrilobular necrosis and sterile inflammation. The robust restoration of GSH observed in the ZQ-treated rats is a pivotal finding; it Suggest that the complex dynamically boosts the endogenous nucleophilic defense system, safely neutralizing NAPQI before irreversible mitochondrial permeability occurs. Furthermore, the profound suppression of TNF-α and IL-6 underscores the complex's ability to interrupt the secondary inflammatory response that typically exacerbates APAP-induced necrosis.

The most significant translational finding of this study is the comparative superiority of the Zinc-Quercetin complex. While pure quercetin possesses antioxidant properties, its low lipophilicity and poor absorption restrict its *in vivo* utility[6]. By complexing quercetin with zinc, we observed outstanding therapeutic efficacy at a relatively low dose of 60 mg/kg—vastly outperforming the crude extract (required at 500 mg/kg) and pure uncomplexed quercetin. This synergistic enhancement likely occurs because the flavonoid acts as an ionophore, shuttling zinc across the hepatocellular membrane[8]. Once inside, zinc acts as a critical structural cofactor for SOD and induces metallothioneins, while the quercetin moiety directly neutralizes reactive oxygen species.

The marked efficacy of the crude *B. vivipara* extract scientifically validates its traditional medicinal use. The plant's innate phytochemical profile rich in both natural flavonoids and elemental zinc essentially functions as a naturally occurring complex, providing a dual mechanism of direct antioxidant protection and essential mineral supplementation.

Conclusion

This study provides robust preclinical evidence that the ethanolic leaf extract of *Belosynapsis vivipara* and a synthesized Zinc-Quercetin complex possess potent hepatoprotective properties against both chronic fibrotic injury and acute toxic necrosis. These protective effects are multidimensional, driven by the attenuation of lipid peroxidation, the regeneration of antioxidant reserves (GSH/SOD), and the potent suppression of pro-inflammatory cytokines (TNF-α, IL-6). Crucially, the ZQ complex achieved remarkable efficacy at lower doses, proving that structural metal-chelation is a highly effective pharmacological strategy for overcoming the bioavailability barriers of therapeutic flavonoids. These findings position Zinc-Quercetin formulations as highly promising candidates for clinical

development in the management of severe hepatic disorders.

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All animal experiments were strictly conducted in accordance with CPCSEA guidelines and approved by the Institutional Animal Ethics Committee (Registration number: 1432/PO/Re/S/11/CPCSEA; Protocol No: MCP176/2025-26).

Conflict of interest

Both the authors declare no conflict of interest.

References

1 Asrani SK, Devarbhavi H, Eaton J & Kamath PS, Burden of liver diseases in the world. *J Hepatol*, 70 (2019) 151.

2 Roehlen N, Crouchet E & Baumert TF, Liver Fibrosis: Mechanistic Concepts and Therapeutic Perspectives. *Cells*, 9 (2020) 875.

3 Weber LW, Boll M & Stampfl A, Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Crit Rev Toxicol*, 33 (2003) 105.

4 Hinson JA, Roberts DW & James LP, Mechanisms of acetaminophen-induced liver necrosis. *Handb Exp Pharmacol*, 196 (2010) 369.

5 Sasane NA, Gaikar PS, Gaikwad SL, Valvi AK & Wadhawa GC, Phytochemical analysis, anti-oxidant and anti-inflammatory of Belosynapsis vivipara leaves and roots. *Int J Bot Stud*, 6 (2021) 138.

6 Wang W, Sun C, Mao L, Ma P, Liu F, Yang J & Gao Y, The biological activities, chemical stability, metabolism and delivery systems of quercetin: A review. *Trends Food Sci Technol*, 56 (2016) 21.

7 Nakamura M, Urakawa D, He Z, Akagi I, Hou DX & Sakao K, Apoptosis induction in HepG2 and HCT116 cells by a novel quercetin-zinc (II) complex: Enhanced absorption of quercetin and zinc (II). *Int J Mol Sci*, 24 (2023) 17457.

8 Dabbagh-Bazarbachi H, Clergeaud G, Quesada IM, Ortiz M, O'Sullivan CK & Fernández-Larrea JB, Zinc ionophore activity of quercetin and epigallocatechin-gallate: from theory to practice. *J Agric Food Chem*, 62 (2014) 8085.

9 OECD. Test No. 425: Acute oral toxicity: Up-and-down procedure, in *OECD Guidelines for the Testing of Chemical. Section 4*, (OECD Publishing, Paris), 2008.

10 Domitrović R, Jakovac H, Tomac J & Sain I, Differential hepatoprotective mechanisms of rutin and quercetin in CCl4-intoxicated BALB/cN mice. *Acta Pharmacol Sin*, 33 (2012) 1260.

11 Yousef MI, Omar SAM, El-Guendi MI & Abdelmegid LA, Potential protective effects of quercetin and curcumin on paracetamol-induced histological changes, oxidative stress, impaired liver and kidney functions and haematotoxicity in rat. Food Chem Toxicol, 48 (2010) 3246.

12 Reitman S & Frankel S, A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol*, 28 (1957) 56.

13 Malloy HT & Evelyn KA, The determination of bilirubin with the photoelectric colorimeter. *J Biol Chem*, 119 (1937) 481.

14 Doumas BT, Watson WA & Biggs HG, Albumin standards and the measurement of serum albumin with bromcresol green. *Clin Chim Acta*, 31 (1971) 87.

15 Ellman GL, Tissue sulfhydryl groups. *Arch Biochem Biophys*, 82 (1959) 70.

16 Ohkawa H, Ohishi N & Yagi K, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem, 95 (1979) 351.

17 Marklund S & Marklund G, Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem*, 47 (1974) 469.