

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 properties⁵. 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- κ B. However, its clinical application is severely hampered by poor aqueous solubility, extensive first-pass metabolism, and overall low oral bioavailability⁶. 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 (CCl₄) 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-hour 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 rats⁹. 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 CCl₄-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 CCl₄ 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 CCl₄ 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 CCl₄ induction along with the Quercetin (60 mg/kg, p.o.) daily. Group 5 (Zn-Quercetin) received the CCl₄ induction along with the ZQ complex (60 mg/kg, p.o.)

daily. Finally, Group 6 (EBVL) received the CCl₄ 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 CCl₄ 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 structure⁶.

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 method¹². Bilirubin, total protein, and albumin were quantified using standard colorimetric kits^{13,14}. Hepatic reduced glutathione (GSH) was determined using the Ellman method¹⁵. 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 μ m), and stained with hematoxylin and eosin (H&E).

Statistical Analysis

Data are expressed as Mean $\pm$ 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, EBVL 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 CCl₄-Induced Hepatotoxicity

Administration of CCl₄ induced severe hepatocellular injury, significantly elevating serum marker enzymes compared to normal controls, including ALT (187.6 $\pm$ 2.9 U/L), AST (248.6 $\pm$ 3.5 U/L), and ALP (391.1 $\pm$ 4.2 U/L). CCl₄ toxicity also caused a marked increase in total bilirubin (2.96 $\pm$ 0.04 mg/dL) and the inflammatory marker CRP (8.4 $\pm$ 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 $\pm$ 1.3 U/L, AST to 106.4 $\pm$ 1.6 U/L, ALP to 177.7 $\pm$ 2.9 U/L, and total bilirubin to 0.96 $\pm$ 0.02 mg/dL alongside a significant reduction in CRP to 3.1 $\pm$ 0.2 mg/L. The analysis of results is presented in Table 1 and Fig. 1. The extract EBVL

Table 1 — Effect of *Belosynapsis vivipara* extract and Zn-Quercetin complex on serum and tissue biochemical parameters in CCl₄-induced hepatotoxicity in rats.

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

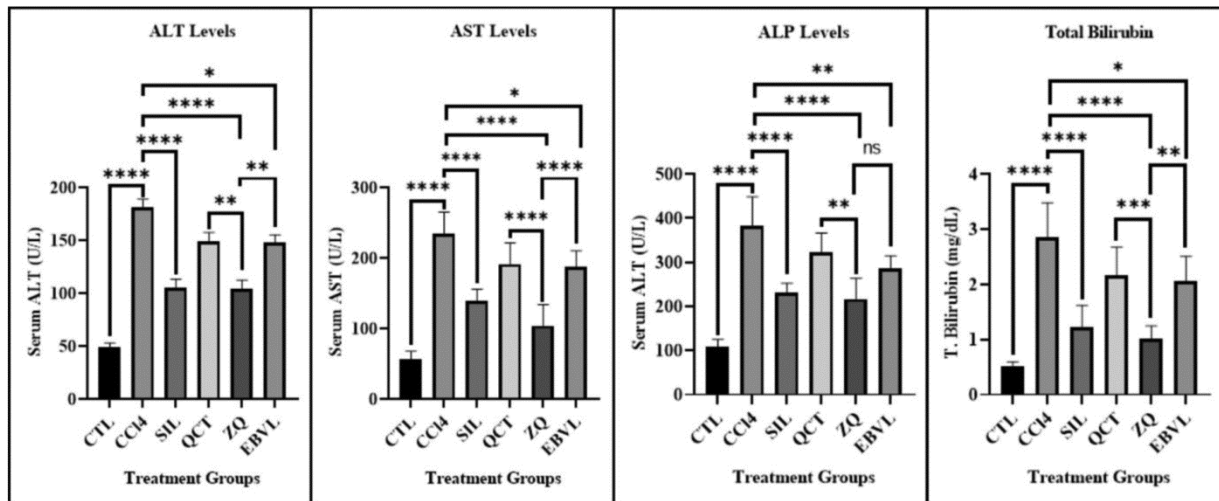


Fig. 1 — Effect of different treatments on serum liver function markers in CCl₄-induced hepatotoxicity over 14 days. Data are expressed as Mean $\pm$ SEM (n=6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Asterisks denote statistical significance as indicated by the brackets: * $P < 0.05$, $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; ns = non-significant.

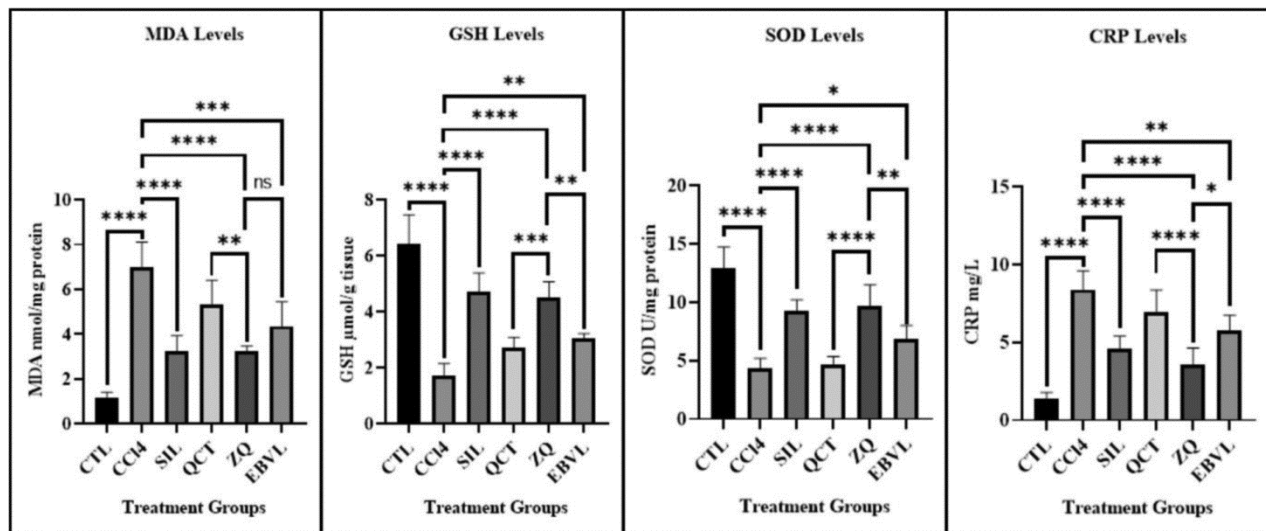


Fig. 2 — Effect of different treatments on hepatic antioxidant status and inflammation markers in CCl₄-induced hepatotoxicity over 14 days. Data are expressed as Mean $\pm$ SEM (n=6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Asterisks denote statistical significance as indicated by the brackets: * $P < 0.05$, $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$; ns = non-significant.

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

Furthermore, CCl₄ triggered profound oxidative stress, evidenced by severely depleted tissue GSH ($1.95 \pm 0.12 \mu\text{mol/g}$) and SOD ($4.15 \pm 0.28 \text{ U/mg prot}$), alongside heavily elevated lipid peroxidation (MDA: $6.85 \pm 0.25 \text{ nmol/mg prot}$). The ZQ complex robustly restored hepatic GSH to $4.42 \pm 0.21 \mu\text{mol/g}$ and SOD

to $9.25 \pm 0.41 \text{ U/mg prot}$, while effectively suppressing lipid peroxidation by lowering MDA levels to $2.35 \pm 0.18 \text{ nmol/mg prot}$ 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 steatosis observed in the toxic control as presented in Fig. 3.

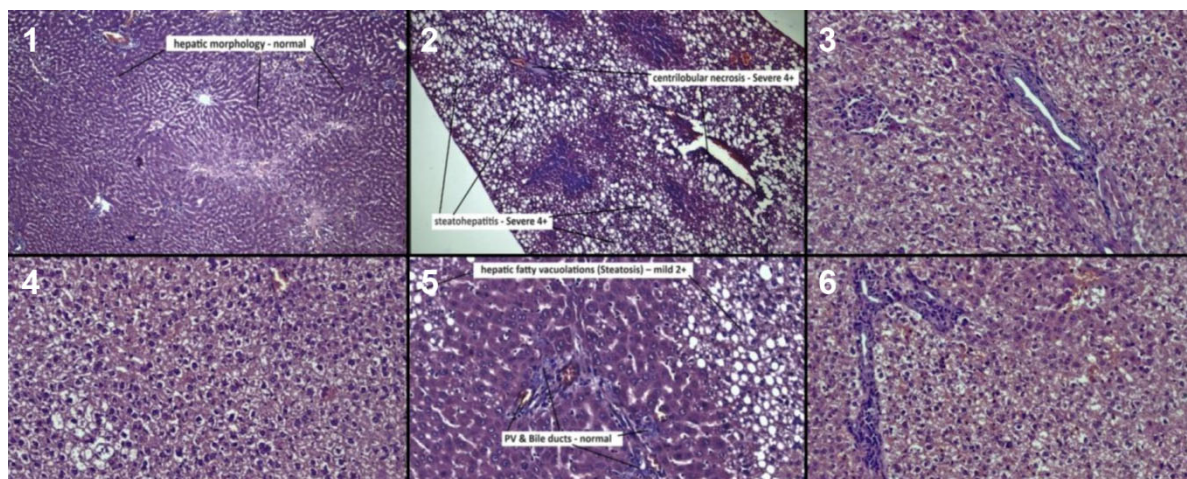


Fig. 3 — Histopathological examination of rat liver sections in CCl₄-induced hepatotoxicity (H&E stain). (1) Normal Control (CTL): Normal hepatic architecture. (2) CCl₄ Control: Severe (4+) centrilobular necrosis and massive steatohepatitis. (3) Silymarin (SIL, 100 mg/kg): Significant preservation and 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.

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

Sr. 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

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 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

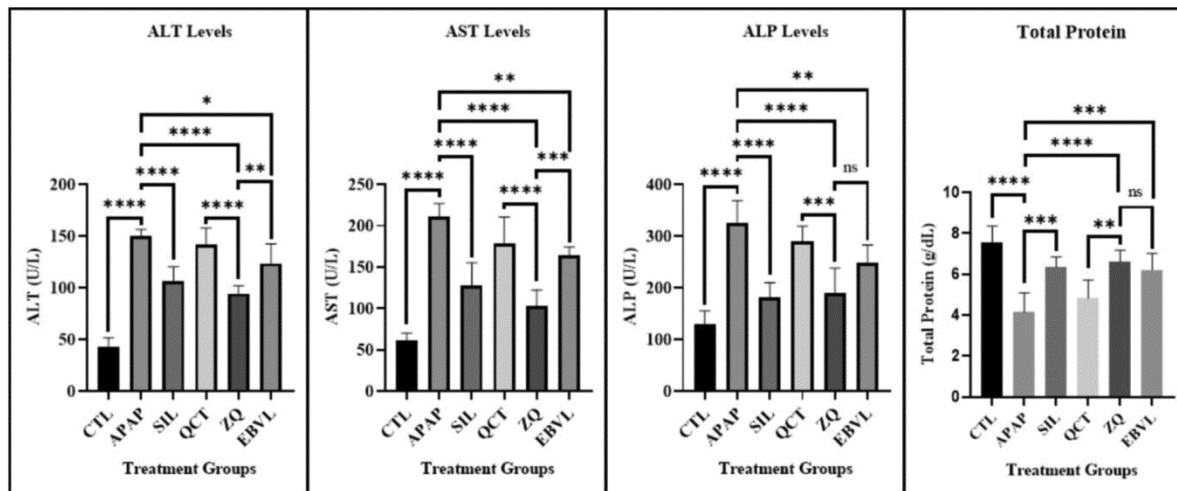


Fig. 4 — Effect of different treatments on serum liver function markers in Paracetamol (APAP)-induced hepatotoxicity over 7 days. Data are expressed as Mean $\pm$ SEM (n=6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Asterisks denote statistical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

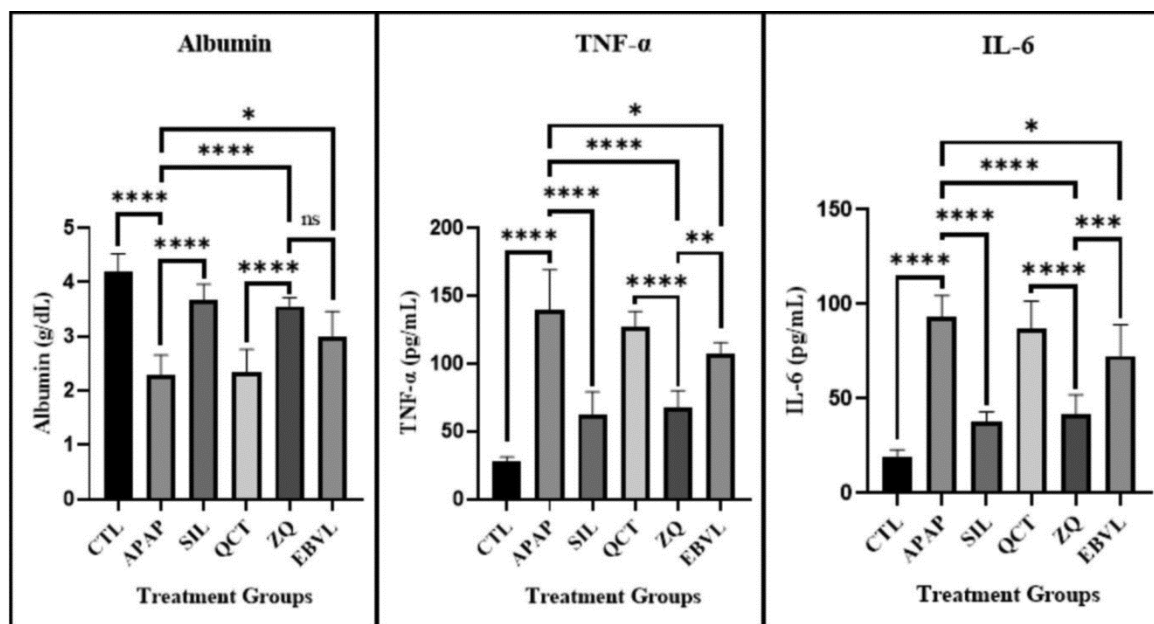


Fig. 5 — Effect of different treatments on serum albumin and pro-inflammatory cytokines (TNF- α and IL-6) in Paracetamol (APAP)-induced hepatotoxicity over 7 days. Data are expressed as Mean $\pm$ SEM (n=6). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison 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.

trichloromethyl radicals via the CYP2E1 enzyme³. These free radicals aggressively attack lipid membranes, initiating a chain reaction of lipid peroxidation that destroys cellular integrity. The massive elevation of MDA alongside the depletion of endogenous SOD and GSH in our CCl₄ 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 mechanisms⁴. At high doses, the accumulation of NAPQI rapidly depletes hepatic glutathione, leaving mitochondria

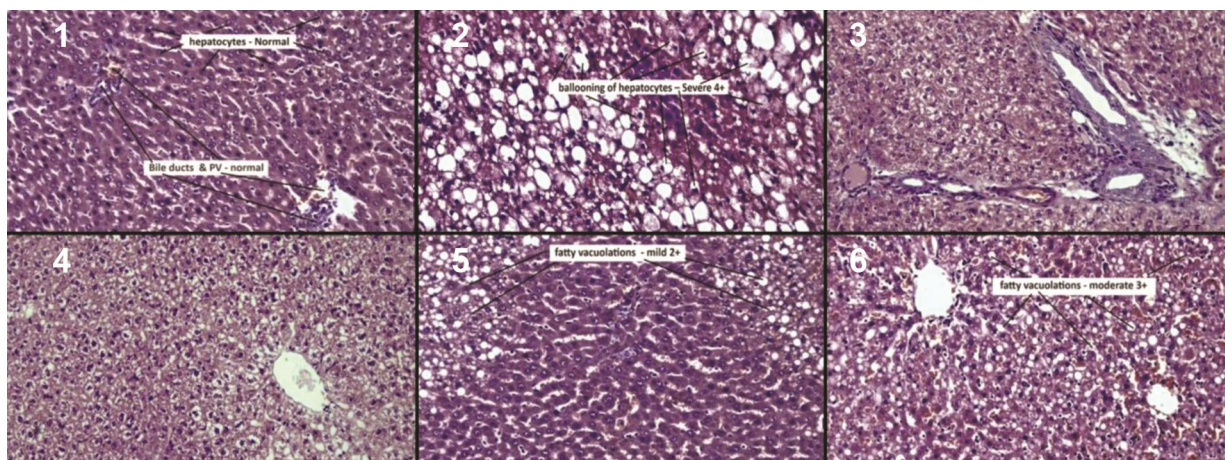


Fig. 6 — Histopathological examination of rat liver sections in APAP-induced hepatotoxicity (H&E stain). (1) Normal Control (CTL): Normal hepatic architecture. (2) APAP Control: Severe (4+) ballooning degeneration and necrosis. (3) Silymarin (SIL, 100 mg/kg): Marked protection against necrosis. (4) Pure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

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 suggests that the complex dynamically boosts the endogenous nucleophilic defense system, safely neutralizing NAPQI before irreversible mitochondrial permeability occurs. Furthermore, the profound suppression of $\text{TNF-}\alpha$ 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* utility⁶. 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 membrane⁸. 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 *Belosynopsis 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 ($\text{TNF-}\alpha$, 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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Ethical Approval: 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).

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