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EVALUATION OF THE SYNERGISTIC HEPATOPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACTS OF *MORINGA OLEIFERA* LEAVES AND *MOMORDICA CHARANTIA* FRUITS AGAINST CCL_4-INDUCED HEPATOTOXICITY IN WISTAR ALBINO RATS

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Abstract:

Background: *Liver diseases are a leading cause of global morbidity and mortality, with chronic liver disease accounting for over 1.03 million deaths annually. Carbon tetrachloride (CCl_4)-induced hepatotoxicity is a well-validated experimental model of oxidative hepatic injury. Conventional hepatoprotective therapies have limited efficacy and significant adverse effects, creating demand for safe, plant-based alternatives.*

Objectives: *To evaluate the hepatoprotective potential of ethanolic extracts of Moringa oleifera leaves (MOLE) and Momordica charantia fruit (MCFE), individually and in combination, in CCl_4-induced hepatotoxicity in Wistar albino rats.*

Methods: *Thirty-six male Wistar albino rats were divided into six groups ($n = 6$). Hepatotoxicity was induced by CCl_4 (1 mL/kg i.p., twice weekly, 28 days). Test groups received MOLE (400 mg/kg), MCFE (250 mg/kg), or combination (MOLE 200 mg/kg + MCFE 125 mg/kg) orally once daily. Silymarin (100 mg/kg) served as the reference standard. Serum AST, ALT, ALP, total bilirubin, total protein, and albumin were estimated at study termination.*

Results: *CCl_4 significantly elevated serum AST (196.8±9.2 U/L), ALT (180.4±8.1 U/L), ALP (242.4±11.0 U/L), and total bilirubin (2.14±0.11 mg/dL) while reducing total protein (4.8±0.2 g/dL) and albumin (2.6±0.1 g/dL) vs. normal controls ($p < 0.001$). All treatment groups showed significant reversal ($p < 0.05$). The combination group reduced AST to 84.3±5.2 U/L and ALT to 76.5±4.8 U/L, statistically comparable to silymarin ($p > 0.05$).*

Conclusion: *MOLE and MCFE individually and synergistically demonstrated significant hepatoprotective activity attributable to their complementary phytoconstituent profiles including flavonoids, isothiocyanates, charantin, and momordicin. The combination formulation warrants further mechanistic, toxicological, and clinical evaluation.*

Keywords: *Moringa oleifera; Momordica charantia; hepatoprotective; CCl_4-induced hepatotoxicity; silymarin; oxidative stress; flavonoids; Wistar albino rats*

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1. INTRODUCTION:

The liver is the largest glandular organ of the human body, weighing approximately 1200–1500 g in an adult [1]. It occupies the right hypochondrium and epigastric region of the abdominal cavity and is composed of hepatocytes — the primary parenchymal cells — arranged in thin plates separated by sinusoidal spaces. In adults, hepatocytes constitute approximately 78% of the total liver volume, with non-parenchymal cells (Kupffer cells, hepatic stellate cells, sinusoidal endothelial cells) accounting for the remainder [2]. The outer surface is enclosed within a collagenous Glisson capsule overlaid by mesothelial cells.

The liver performs indispensable physiological functions including intermediary metabolism of carbohydrates, lipids, and proteins; biotransformation and detoxification of endogenous and exogenous substances; synthesis of plasma proteins (albumin, clotting factors, acute-phase proteins); bile production and secretion; and immunological surveillance via Kupffer cells [1,3]. Its central role in xenobiotic metabolism renders it acutely susceptible to drug- and chemical-induced injury [4].

Liver disease encompasses a wide clinical spectrum — from acute hepatitis and drug-induced liver injury (DILI) to non-alcoholic fatty liver disease (NAFLD), cirrhosis, and hepatocellular carcinoma (HCC). Globally, chronic liver disease and cirrhosis account for more than 1.03 million deaths annually [5]. According to the Global Burden of Disease 2019 study, liver cirrhosis and other chronic liver diseases affected an estimated 1.5 billion individuals worldwide [6]. In India, liver disorders account for approximately 2% of all hospital admissions and up to 20% of gastroenterology referrals [7,8].

Despite availability of several synthetic hepatoprotective agents and antiviral therapies, their clinical utility is constrained by high cost, significant adverse effects, and variable efficacy [9]. This has directed research interest towards medicinal plants as sources of safe, affordable, and multi-targeted hepatoprotective agents.

Carbon tetrachloride (CCl_4) is one of the most extensively validated chemical models of experimental hepatotoxicity. Following hepatic bioactivation by cytochrome P450 2E1 (CYP2E1), CCl_4 is metabolised to the trichloromethyl radical (CCl_3•), which rapidly reacts with oxygen to generate the trichloromethylperoxy radical (CCl_3OO•) [10]. These radicals initiate lipid peroxidation of cellular membranes, disrupt intracellular calcium homeostasis, impair mitochondrial oxidative phosphorylation, and ultimately cause hepatocyte necrosis and centrilobular fibrosis [11]. The characteristic biochemical sequelae — elevation of serum ALT, AST, ALP, and bilirubin with concurrent decline in total protein and albumin — closely parallel those observed in human toxic hepatitis [12].

Moringa oleifera Lam. (family Moringaceae), the drumstick tree, is indigenous to the Indian subcontinent and naturalised across tropical Africa, Asia, and Latin America [13]. Its leaves contain high concentrations of quercetin, kaempferol, isothiocyanates, β-sitosterol, phenolic acids, vitamins A, C, and E, and essential minerals [14]. Preclinical studies have established potent antioxidant, anti-inflammatory, and hepatoprotective activities for leaf extracts [15,16].

Momordica charantia L. (family Cucurbitaceae), bitter gourd, is widely cultivated in Asia, Africa, and the Caribbean and is a cornerstone of Ayurvedic, Unani, and Chinese traditional medicine [17]. Its fruit is rich in charantin (a steroidal glycoside), momordicin I and II (cucurbitane-type triterpenoids), polypeptide-p, flavonoids, and saponins [18], which collectively confer antidiabetic, antioxidant, anti-inflammatory, and hepatoprotective properties [19,20].

While individual hepatoprotective activity has been reported for both plants, no systematic comparative and combination study documenting dose optimisation, pharmacodynamic synergy, and comprehensive serum biochemical profiling has been published. A combination approach is compelling because MOLE and MCFE act through complementary mechanisms: MOLE via CYP2E1 inhibition and flavonoid-mediated free-radical scavenging, while MCFE enhances endogenous antioxidant enzyme activity and suppresses inflammatory cytokines through charantin and triterpenoids. The synergistic use of two mechanistically divergent agents at reduced individual doses may achieve superior efficacy while reducing dose-dependent toxicity risk. The present investigation was therefore designed to evaluate and compare the hepatoprotective effects of MOLE and MCFE, alone and in combination, against CCl_4-induced hepatotoxicity in Wistar albino rats, with silymarin as the reference standard, and to correlate biochemical findings with body and liver weight indices.

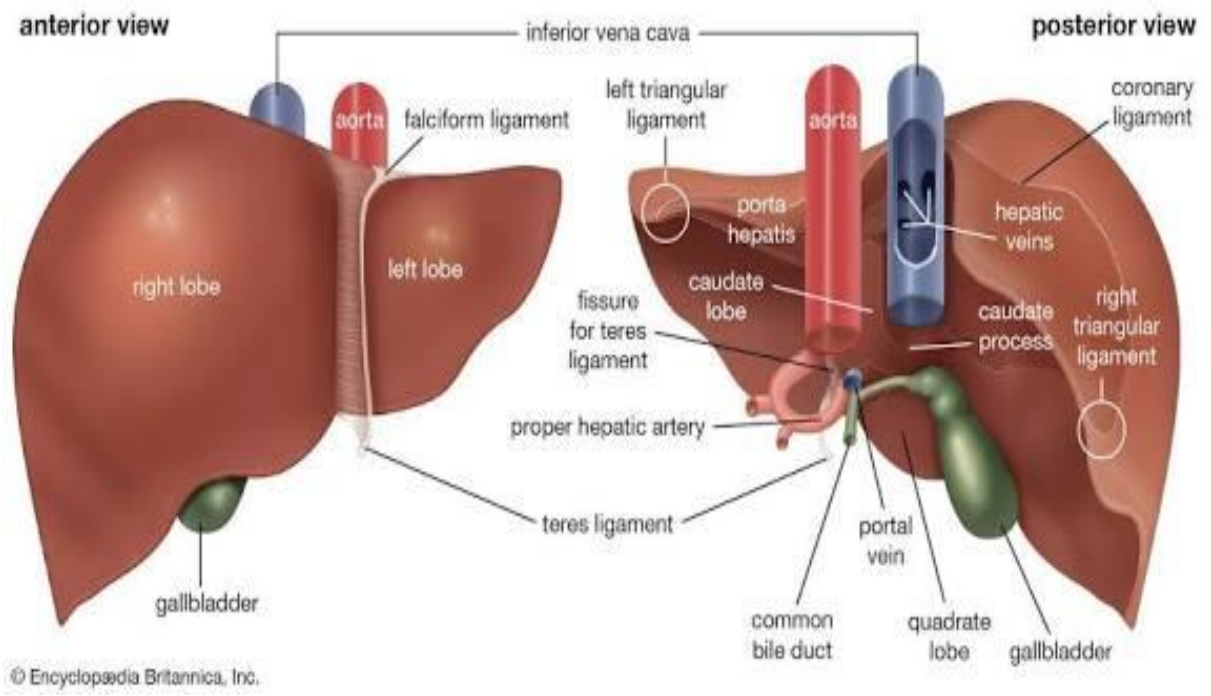

Figure 1. Gross anatomical views of the human liver: anterior (left) and posterior (right) surfaces.

2. PLANT PROFILES

2.1 Moringa oleifera Lam. (Family: Moringaceae)

Moringa oleifera is a fast-growing, drought-tolerant, deciduous tree typically attaining 5–12 m height under cultivation. The trunk is straight with soft, corky, whitish-grey bark. Leaves are tripinnate, feathery, and light green, measuring 30–60 cm. Flowers are small, fragrant, creamy-white to pale yellow, borne in axillary drooping panicles. The fruit (drumstick) is an elongated, three-angled, pendulous capsule measuring 20–45 cm, containing 8–12 round dark-brown seeds with three papery wings. It is native to north-western India and widely naturalised in tropical and subtropical regions of Asia, Africa, and the Americas [13,27].

Phytochemistry: Leaves of *M. oleifera* contain flavonoids (quercetin-3-glucoside, kaempferol-3-glucoside, rhamnetin), phenolic acids (chlorogenic acid, caffeic acid, ferulic acid), isothiocyanates, β-sitosterol, zeatin and zeatin riboside, vitamins A (as β-carotene), C, E, and B-complex vitamins, and macro- and micro-minerals [14,15]. Alkaloids, terpenoids, tannins, and saponins are also present [22].

Traditional uses: Antidiabetic, anti-inflammatory, antimicrobial, antioxidant, hepatoprotective, antihypertensive, antianemic, analgesic, antipyretic, and nutritional supplement in malnutrition [27].

2.2 Momordica charantia L. (Family: Cucurbitaceae)

Momordica charantia is an annual, monoecious, climbing vine with slender, angular, grooved stems that attach by simple tendrils, growing to 2–5 m. Leaves are alternate, palmately lobed (5–7 lobes), dark green, 4–12 cm diameter. Flowers are yellow, unisexual, and axillary. The fruit is an elongated to fusiform, warty or ridged berry, 10–30 cm long, green when immature, turning yellow-orange upon ripening. Ripe fruits dehisce into three loculicidal valves revealing bright red-arillate seeds. The plant is native to tropical Asia and widely cultivated throughout the tropics [17,28].

Phytochemistry: The fruit of *M. charantia* is notable for charantin (a steroidal glycoside), momordicin I and II (cucurbitane-type triterpenoids), polypeptide-p (plant insulin analogue), momordin, momorcharins (lectins), flavonoids (myricetin, quercetin, catechin, epicatechin, caffeic acid), vitamin C and E, and β-carotene [17,18,19]. Saponins, alkaloids, and glycosides are also present in significant quantities [23].

Traditional uses: Hypoglycaemic, anthelmintic, carminative, laxative, antimalarial, antiviral, appetiser, digestive, hepatoprotective, anti-inflammatory, and immunostimulant [17,20].

Figure 2. Moringa oleifera Lam. (drumstick tree).

Figure 3. Momordica charantia L. (bitter gourd).

Table 1. Comparative taxonomic classification and vernacular names of the study plants

Taxonomic Rank	***Moringa oleifera* Lam.**	***Momordica charantia* L.**
Kingdom	Plantae	Plantae
Division	Magnoliophyta	Magnoliophyta
Class	Magnoliopsida	Magnoliopsida
Order	Brassicales	Cucurbitales
Family	Moringaceae	Cucurbitaceae
Genus	Moringa	Momordica
Species	M. oleifera Lam.	M. charantia L.
Common Name	Drumstick tree / Miracle tree	Bitter gourd / Bitter melon
Vernacular (Telugu)	Munaga	Pavakkai
Vernacular (Hindi)	Sahjan	Karela

3. MATERIALS AND METHODS:

3.1 Chemicals and Reagents

Carbon tetrachloride (AR grade), olive oil, silymarin (≥98% purity; Sigma-Aldrich, St. Louis, MO, USA), and 95% ethanol were used. Carboxymethylcellulose sodium (CMC) and thiopental sodium were procured from S.D. Fine Chemicals, Mumbai. All biochemical assay kits were obtained from Erba Mannheim (Transasia Bio-Medicals Ltd., Mumbai) and used as per manufacturer instructions.

3.2 Plant Material Collection and Authentication

Fresh mature leaves of *Moringa oleifera* Lam. and unripe green fruits of *Momordica charantia* L. were collected from cultivated gardens in Narasaraopet, Palnadu District, Andhra Pradesh, India, in August 2025 (09:00–11:00 h). Plant materials were washed under running tap water then distilled water to remove surface contaminants. Botanical identity was authenticated by a qualified taxonomist at the Department of Botany, Acharya Nagarjuna University (ANU), Guntur. Voucher specimens were deposited in the ANU Herbarium (Voucher No. ANU/BOT/2025/MO-118 for *M. oleifera* leaf; ANU/BOT/2025/MC-119 for *M. charantia* fruit).

3.3 Preparation of Ethanolic Extracts

Collected plant materials were shade-dried at 25±2°C for 10–14 days until constant weight was obtained, coarsely powdered, and passed through mesh No. 40 sieve. Each powder (50 g) was packed in a Whatman No. 1 filter paper thimble and subjected to continuous Soxhlet extraction (Figure 4) using 300 mL of 95% v/v ethanol at 60–70°C for 6–8 hours until the recycling solvent ran colourless [21]. Filtrates were concentrated using a rotary vacuum evaporator (Buchi R-100) at 45°C. Semisolid residues designated *Moringa oleifera* Leaf Ethanolic Extract (MOLE; yield: 14.8% w/w) and *Momordica charantia* Fruit Ethanolic Extract (MCFE; yield: 11.3% w/w) were stored at 4°C in amber vials. Working suspensions were prepared fresh daily in 0.5% w/v CMC.

3.4 Preliminary Phytochemical Screening

Standard qualitative phytochemical analyses were performed to detect alkaloids (Dragendroff's and Mayer's tests), flavonoids (alkaline reagent test), terpenoids (Salkowski test), tannins (ferric chloride test), phenolics (lead acetate test), glycosides (Keller–Killiani test), saponins (foam test), and starch (iodine test), following the methods of Harborne [22] and Trease and Evans [23]. Results are presented in Table 4.

3.5 Experimental Animals and Housing

Thirty-six healthy male Wistar albino rats weighing 150–200 g (8–10 weeks) were procured from Sri Venkateswara Enterprises, Hyderabad (CPCSEA Reg. No. [verify with supplier]). Animals were housed in groups of four per well-ventilated polypropylene cage under standard laboratory conditions: ambient temperature 22±2°C, relative humidity 50±10%, 12 h light/dark cycle. Standard rodent pellet diet and filtered drinking water were provided ad libitum. All animals were acclimatised for 7 days. All protocols were approved by the IAEC of Narasaraopeta Institute of Pharmaceutical Sciences under CPCSEA guidelines (IAEC Approval No. NIPS/IAEC/2025/PHM/07).

3.6 Experimental Design and Treatment Protocol

After acclimatisation, animals were randomly allocated to six groups (n = 6) by stratified randomisation based on body weight. Hepatotoxicity was induced in Groups II–VI by intraperitoneal (i.p.) injection of CCl_4 (1 mL/kg body weight, dissolved in olive oil 1:1 v/v) twice weekly for 28 consecutive days [24,25]. All oral treatments were administered once daily by gastric gavage. Group I received 0.5% CMC orally and olive oil i.p. Full details are provided in Table 2.

3.7 Sample Collection and Biochemical Estimations

At the end of the 28-day study period, all animals were fasted overnight (12 h) with unrestricted access to water. Animals were anaesthetised with thiopental sodium (50 mg/kg, i.p.) and blood was collected by cardiac puncture using a sterile syringe. Blood was allowed to clot at room temperature for 30 minutes, then centrifuged at 3000 rpm for 15 min at 4°C. Serum was stored at −20°C until analysis. Hepatic injury markers (Table 3) were estimated spectrophotometrically on a semi-automated biochemistry analyser (Erba Chem-7, Transasia Bio-Medicals) using validated commercial reagent kits.

3.8 Statistical Analysis

All data are expressed as mean±SEM (n = 6). Groups were compared by one-way ANOVA followed by Tukey's HSD post-hoc test using SPSS v26.0 (IBM Corp., Armonk, NY). A two-tailed $p < 0.05$ was considered statistically significant. Symbols: ***$p < 0.001$ vs. Group I (normal control); #$p < 0.05$ and ##$p < 0.01$ vs. Group II (hepatotoxic control) by Tukey's HSD.

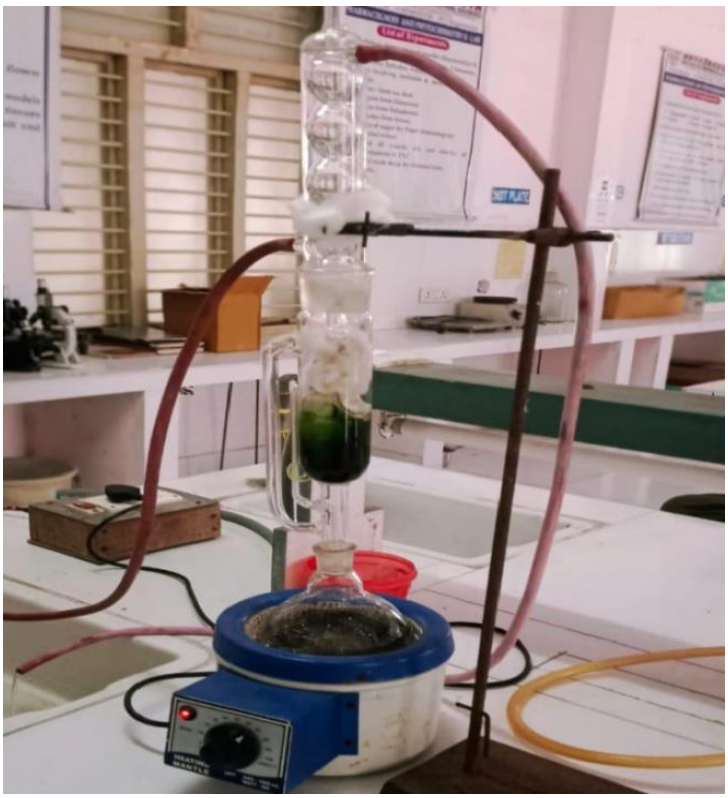

Figure 4. Soxhlet extraction apparatus used for preparation of MOLE and MCFE.

Table 2. Experimental group design and treatment schedule

Group	Designation	Treatment	Dose	Route	Duration
I	Normal Control	0.5% CMC (p.o.) + olive oil (i.p.)	—	p.o./i.p.	28 days
II	Hepatotoxic Control	CCl_4 in olive oil 1:1 v/v, i.p., twice weekly	1 mL/kg	i.p.	28 days
III	Standard (Silymarin)	Silymarin (p.o.) + CCl_4 (i.p.)	100 mg/kg/day	p.o.+i.p.	28 days
IV	Test I – MOLE	MOLE (p.o.) + CCl_4 (i.p.)	400 mg/kg/day	p.o.+i.p.	28 days
V	Test II – MCFE	MCFE (p.o.) + CCl_4 (i.p.)	250 mg/kg/day	p.o.+i.p.	28 days
VI	Test III – Combination	MOLE + MCFE (p.o.) + CCl_4 (i.p.)	MOLE 200 + MCFE 125 mg/kg/day	p.o.+i.p.	28 days

MOLE = M. oleifera Leaf Ethanolic Extract; MCFE = M. charantia Fruit Ethanolic Extract; CMC = carboxymethylcellulose; p.o. = per os (oral); i.p. = intraperitoneal.

Table 3. Serum biochemical parameters evaluated for hepatoprotective activity assessment

S.No	Parameter	Method / Principle	Unit	Normal Range (Rat)
1	ALT (SGPT)	Kinetic UV (IFCC); NADH oxidation at 340 nm	U/L	17–45 U/L
2	AST (SGOT)	Kinetic UV (IFCC); NADH oxidation at 340 nm	U/L	45–80 U/L
3	ALP	p-Nitrophenyl phosphate colorimetric; 405 nm	U/L	50–120 U/L
4	Total Bilirubin	Diazotisation – Jendrassik & Grof; 546 nm	mg/dL	0.20–0.60 mg/dL
5	Total Protein	Biuret method; 540 nm	g/dL	6.0–8.5 g/dL
6	Serum Albumin	Bromocresol green (BCG) dye-binding; 630 nm	g/dL	3.5–5.0 g/dL

4. RESULTS:

4.1 Percentage Yield and Phytochemical Screening

Soxhlet extraction of dried powders yielded semisolid ethanolic extracts of 14.8% w/w (MOLE) and 11.3% w/w (MCFE), calculated on dry weight basis. Preliminary qualitative phytochemical screening (Table 4) confirmed the presence of alkaloids, flavonoids, terpenoids, tannins, phenolics, glycosides, saponins, and starch in both extracts. MOLE showed higher flavonoid and phenolic content while MCFE showed higher saponin and glycoside content. These phytoconstituent classes are well-established contributors to antioxidant, membrane-stabilising, and anti-inflammatory activities relevant to hepatoprotection [29,30].

4.2 Effect on Body Weight and Relative Liver Weight

CCl_4-treated animals (Group II) exhibited a significant decrease in percentage body weight gain (6.9±1.2%) compared to normal controls (32.8±1.8%; $p < 0.001$), consistent with systemic toxicity. Concurrently, relative liver weight was significantly elevated in Group II (5.84±0.14 g/100 g body weight) vs. Group I (3.12±0.08 g/100 g), reflecting hepatic oedema and inflammatory infiltration. Treatment with MOLE, MCFE, and their combination progressively restored body weight gain and reduced relative liver weight in a dose-dependent manner ($p < 0.05$ vs. Group II). Complete data are presented in Table 5.

4.3 Effect on Serum Biochemical Parameters

CCl_4 administration (Group II) caused a significant ($p < 0.001$) elevation in serum AST (196.8±9.2 U/L), ALT (180.4±8.1 U/L), ALP (242.4±11.0 U/L), and total bilirubin (2.14±0.11 mg/dL) compared to normal control Group I. Concurrently, serum total protein (4.8±0.2 g/dL) and albumin (2.6±0.1 g/dL) were significantly reduced ($p < 0.001$), confirming extensive hepatocellular injury and impaired synthetic function (Table 6).

Oral treatment with MOLE at 400 mg/kg/day (Group IV) produced significant reductions in all elevated hepatic enzymes and bilirubin ($p < 0.05$ vs. Group II), along with partial restoration of total protein and albumin. MCFE at 250 mg/kg/day (Group V) demonstrated comparable hepatoprotection. The combination group (Group VI: MOLE 200 mg/kg + MCFE 125 mg/kg/day) showed the most pronounced normalisation of all biochemical parameters ($p < 0.01$ vs. Group II), with

AST reduced to 84.3±5.2 U/L and ALT to 76.5±4.8 U/L, values statistically comparable to silymarin (AST: 71.4±4.0 U/L; ALT: 64.2±3.7 U/L; $p > 0.05$, combination vs. silymarin).

Table 4. Preliminary phytochemical screening of ethanolic extracts of MOLE and MCFE

Phytoconstituent	MOLE (M. oleifera)	MCFE (M. charantia)
Alkaloids	+++	++
Flavonoids	+++	+++
Terpenoids	++	++
Tannins	++	+
Phenolics	+++	++
Glycosides	+	+++
Saponins	+	+++
Starch	+	+

(+) = Trace/weak positive; (++) = Moderate positive; (+++) = Strong positive. All tests performed in triplicate. MOLE = M. oleifera Leaf Ethanolic Extract; MCFE = M. charantia Fruit Ethanolic Extract.

Table 5. Effect of MOLE and MCFE on body weight gain and relative liver weight in CCl_4-induced hepatotoxic rats (Mean±SEM, n = 6)

Parameter	Gp I Normal	Gp II CCl_4	Gp III Silymarin	Gp IV MOLE	Gp V MCFE	Gp VI Combo
Initial body wt. (g)	174.3±4.2	176.1±3.8	175.4±4.5	173.8±3.6	175.2±4.1	174.6±4.4
Final body wt. (g)	231.6±5.4	188.4±4.6***	224.2±5.1##	208.6±4.8#	212.4±5.2#	220.8±4.9##
% Weight gain	32.8±1.8	6.9±1.2***	27.8±1.6##	19.9±1.4#	21.2±1.5#	26.6±1.7##
Relative liver wt. (g/100 g body wt.)	3.12±0.08	5.84±0.14***	3.46±0.09##	4.28±0.11#	4.02±0.10#	3.72±0.09##

*Values are Mean±SEM (n = 6). ***p < 0.001 vs. Group I (normal control); #p < 0.05, ##p < 0.01 vs. Group II (hepatotoxic control); one-way ANOVA, Tukey's HSD test.*

Table 6. Effect of MOLE and MCFE on serum biochemical parameters in CCl_4-induced hepatotoxic rats (Mean±SEM, n = 6)

Parameter (Unit)	Gp I Normal	Gp II CCl_4 Control	Gp III Silymarin 100 mg/kg	Gp IV MOLE 400 mg/kg	Gp V MCFE 250 mg/kg	Gp VI Combo 200+125 mg/kg	Normal Range
AST (SGOT) U/L	44.2±3.1	196.8±9.2***	71.4±4.0##	112.6±6.8#	98.4±5.9#	84.3±5.2##	45–80
ALT (SGPT) U/L	37.8±2.6	180.4±8.1***	64.2±3.7##	102.8±6.2#	89.6±5.4#	76.5±4.8##	17–45
T. Bilirubin mg/dL	0.50±0.04	2.14±0.11***	0.86±0.05##	1.38±0.09#	1.18±0.07#	1.02±0.06##	0.1–1.2
ALP U/L	66.8±4.4	242.4±11.0***	96.2±5.6##	162.4±8.2#	138.6±7.1#	118.8±6.4##	50–120
Total Protein g/dL	7.4±0.2	4.8±0.2***	6.9±0.1##	6.1±0.2#	6.3±0.2#	6.6±0.2##	6.0–8.3
Serum Albumin g/dL	4.2±0.1	2.6±0.1***	3.9±0.1##	3.4±0.1#	3.5±0.1#	3.7±0.1##	3.5–5.0

*Values are Mean±SEM (n = 6). ***p < 0.001 vs. Group I (normal control) by one-way ANOVA; #p < 0.05, ##p < 0.01 vs. Group II (hepatotoxic control) by Tukey's HSD post-hoc test.*

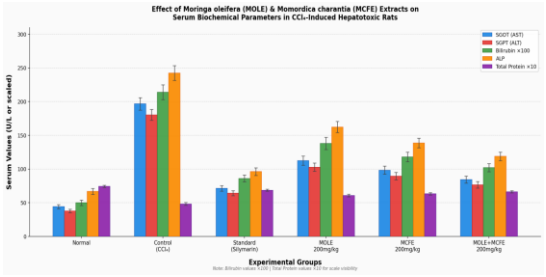

Figure 5. Combined serum biochemical parameters across all six experimental groups (Mean±SEM).

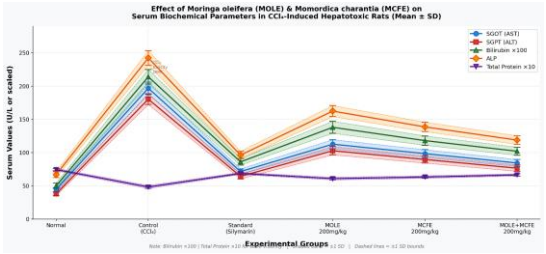

Figure 6. Comparative effect on serum biochemical parameters across all experimental groups.

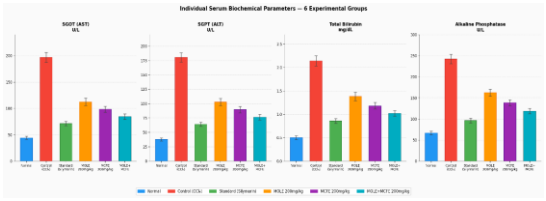

Figure 7. Effect on serum AST (SGOT) levels across all experimental groups.

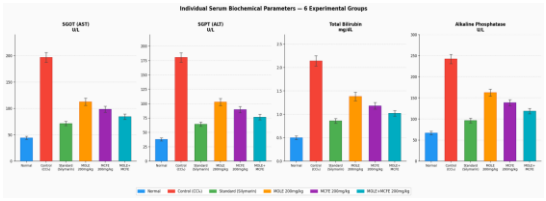

Figure 8. Effect on serum ALT (SGPT) levels across all experimental groups.

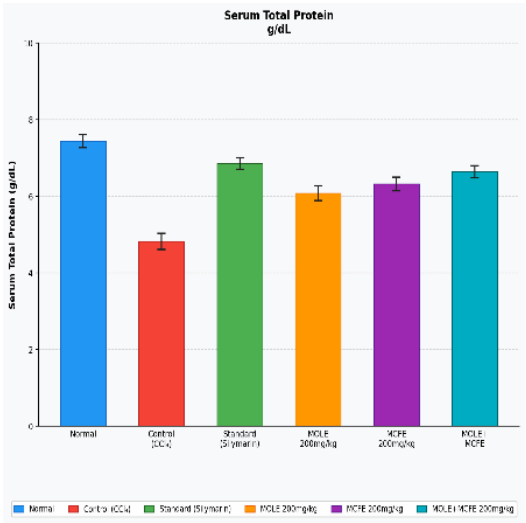

Figure 9. Effect on serum ALP and total bilirubin levels across all experimental groups.

5. DISCUSSION:

CCl_4-induced hepatotoxicity in Group II produced significant elevations in serum AST (196.8±9.2 U/L), ALT (180.4±8.1 U/L), ALP (242.4±11.0 U/L), and total bilirubin (2.14±0.11 mg/dL), with concurrent reductions in total protein and albumin ($p < 0.001$ vs. normal), confirming extensive hepatocellular injury and impaired synthetic function [12]. Treatment with MOLE (400 mg/kg) significantly restored all parameters ($p < 0.05$ vs. Group II), attributable to quercetin and kaempferol-mediated NF-κB inhibition and CYP2E1 inhibitory activity of isothiocyanates that limits CCl_4 bioactivation [14,15,29]. MCFE (250 mg/kg) demonstrated comparable efficacy via charantin and Momordicine-mediated enhancement of SOD and catalase activities and suppression of TNF-α and IL-6 [19,20].

The combination of MOLE + MCFE at half the individual doses (200 + 125 mg/kg) produced hepatoprotective activity statistically comparable to silymarin across all six biochemical endpoints ($p > 0.05$, combination vs. silymarin; $p < 0.01$ vs. hepatotoxic control), demonstrating pharmacodynamic synergism through complementary mechanisms: CYP2E1 inhibition and flavonoid-mediated radical scavenging (MOLE) combined with antioxidant enzyme upregulation and triterpenoid anti-inflammatory activity (MCFE) [29,30]. This is clinically relevant as equivalent efficacy at reduced individual doses lowers the risk of dose-dependent adverse effects.

Limitations of the present study include the absence of oxidative stress markers (MDA, GSH, SOD, CAT) and histopathological data, which will be reported separately. Future studies should evaluate dose-response relationships, sub-chronic toxicity, pharmacokinetic profiles, and molecular targets

(Nrf2/HO-1, NF-κB, TGF-β pathways) to fully establish the mechanistic basis of the observed synergistic hepatoprotection.

6. CONCLUSION:

Ethanolic extracts of *Moringa oleifera* leaf (MOLE) and *Momordica charantia* fruit (MCFE) individually and synergistically demonstrated significant hepatoprotective activity against CCl_4-induced hepatotoxicity in Wistar albino rats. Both extracts significantly reduced elevated serum liver enzymes (AST, ALT, ALP) and bilirubin, and restored total protein and albumin levels towards normal. The combination formulation (MOLE 200 mg/kg + MCFE 125 mg/kg) exhibited hepatoprotective activity statistically comparable to silymarin ($p > 0.05$ vs. silymarin; $p < 0.01$ vs. hepatotoxic control), indicating a pharmacodynamic synergism attributable to their complementary phytoconstituent profiles — including flavonoids, isothiocyanates, and phenolics (MOLE) and charantin, Momordicine, triterpenoids, and saponins (MCFE). These results provide a robust preclinical pharmacological basis for the traditional use of both plants in hepatic disorders and support further mechanistic investigation, toxicity profiling, and clinical evaluation towards the development of an evidence-based polyherbal hepatoprotective formulation.

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ETHICAL APPROVAL

All animals experiments were conducted in compliance with CPCSEA guidelines and were approved by the IAEC of Narasaraopeta institute of pharmaceutical sciences (Approval. No. NIPS/IAEC/2025/PHM/07)

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