Chinese Journal of Health Management
Volume 20 Issue 6, Year of Publication 2026, Page 1689-1709 | DoI-10.564220/1674-0815.2026.167

ISSN: 1674-0815

Chinese Journal of Health Management

Chinese Medical Association

Evaluation of Hepatoprotective Activity of Clidemia hirta in Carbon tetrachloride (CCl_4)-Induced Hepatotoxicity in Wistar Rats

Nishita Yadav [1], Dr. Swati Jogdand [2], Dr. Swati Deshmukh [3]
CAYMET's Siddhant college of Pharmacy, Sudumbare, Pune, Maharashtra, India.

Article Information
Received: 25-05-2026
Revised: 09-06-2026
Accepted: 23-06-2026
Published: 10-07-2026

Keywords
Clidemia hirta, Hepatoprotective activity, Carbon tetrachloride, Liver biomarkers, Oxidative stress.

ABSTRACT:
The present study evaluated the hepatoprotective activity of *Clidemia hirta* against carbon tetrachloride (CCl_4)-induced hepatotoxicity in Wistar rats. Preliminary phytochemical screening revealed the presence of flavonoids, phenolics, tannins and terpenoids, while the methanolic extract (MERH) showed significant antioxidant activity in various in vitro assays. Acute oral toxicity studies confirmed the safety of the extract. Hepatotoxicity induced by CCl_4 significantly elevated serum hepatic biomarkers, lipid peroxidation and inflammatory cytokines while reducing antioxidant enzymes and neurochemical parameters. Treatment with MERH at different doses significantly restored altered biochemical, antioxidant and neurochemical markers in a dose-dependent manner. Histopathological studies also demonstrated improvement in hepatic architecture. The study concludes that *Clidemia hirta* possesses significant hepatoprotective, antioxidant and anti-inflammatory activities and may serve as a promising natural therapeutic agent for liver disorders.

1. INTRODUCTION:
The liver is one of the most important organs of the human body and plays a vital role in metabolism, detoxification, storage of nutrients, secretion of bile, and maintenance of homeostasis [1]. It is continuously exposed to various toxic substances including alcohol, environmental pollutants, drugs, and chemicals, making it highly susceptible to injury. Liver disorders such as hepatitis, cirrhosis, fatty liver disease, and hepatocellular damage have become major global health problems [2]. Hepatotoxicity caused by chemicals and drugs is one of the leading causes of liver dysfunction and may result in severe complications if left untreated.

Carbon tetrachloride (CCl_4) is a well-known hepatotoxic agent commonly used in experimental studies to induce liver injury in animals [3]. The toxicity of CCl_4 is mainly associated with the generation of reactive oxygen species (ROS) and free radicals, which initiate lipid peroxidation, oxidative stress, inflammation, and necrosis of hepatic cells [3,4]. Oxidative stress also affects antioxidant defense systems such as superoxide dismutase, catalase, and glutathione, resulting in cellular damage and impaired liver function. In addition to hepatic injury, chronic liver diseases may also produce neurological and cognitive disturbances due to accumulation of toxic metabolites such

as ammonia.

Modern medicines used for the treatment of liver disorders often show limited efficacy and may produce adverse effects during long-term therapy. Therefore, there is increasing interest in medicinal plants and herbal formulations because of their therapeutic effectiveness, affordability, and comparatively fewer side effects [5,6]. Medicinal plants are rich sources of phytochemicals such as flavonoids, phenolics, tannins, alkaloids, and terpenoids, which possess antioxidant, anti-inflammatory, and hepatoprotective activities.

Natural antioxidants play an important role in protecting hepatic cells against free radical-mediated damage [4]. Herbal medicines with strong antioxidant potential may reduce oxidative stress and improve liver function by restoring endogenous antioxidant enzymes. In recent years, several medicinal plants have been investigated scientifically for their hepatoprotective effects against experimentally induced liver toxicity.

Among these medicinal plants, *Clidemia hirta* has attracted considerable attention due to its diverse pharmacological activities. The plant has been traditionally used for the treatment of various ailments and is reported to possess antioxidant, antimicrobial, anti-inflammatory, antidiabetic, and hepatoprotective properties [7]. The presence of bioactive constituents such as flavonoids and phenolic compounds may contribute to its therapeutic effects.

2. MATERIALS AND METHODS:

Materials:

Leaves of *Clidemia hirta* were used as plant material. Petroleum ether, chloroform, ethyl acetate and methanol were used for extraction. Folin–Ciocalteu reagent, gallic acid, quercetin, aluminium chloride and DPPH were used for phytochemical and antioxidant studies. Carbon tetrachloride (CCl_4), silymarin and carboxymethyl cellulose (CMC) were used for hepatoprotective activity. Wistar rats were used as experimental animals. ELISA kits for TNF-α, IL-1β and IL-6 were used for inflammatory marker estimation. Formalin, hematoxylin and eosin were used for histopathological studies. Soxhlet apparatus, rotary evaporator, UV–Visible spectrophotometer, HPLC system, centrifuge, microscope and microtome were used during the study.

IAEC Approval and Animal Housing

Wistar rats weighing 200–250 g were used for the study. Animals were housed in polyacrylic cages under standard laboratory conditions with free access to food and water ad libitum. The animals were maintained at room temperature of 22±2°C, relative humidity of 55–60% and 12 h light/dark cycle. All experiments were carried out according to CPCSEA guidelines under Registration No. 816/PO/Re/S/04/CPCSEA.

Methods:

Phytochemical Test:

The leaves of *Clidemia hirta* were shade dried, powdered and successively extracted with petroleum ether, chloroform, ethyl acetate, methanol and water using Soxhlet apparatus. The extracts were concentrated and lyophilized [8].

The extracts were subjected to preliminary phytochemical screening for alkaloids, saponins, sterols, carbohydrates, tannins, proteins, flavonoids and triterpenoids using standard qualitative tests [9].

Total phenolic content was estimated by Folin–Ciocalteu method using gallic acid as standard [10,11]. Total flavonoid content was determined by aluminium chloride colorimetric method using quercetin as standard [12].

In Vitro Antioxidant Activity of all Extracts:

DPPH radical scavenging activity was performed using 0.1 mM DPPH solution in methanol. Different concentrations of extracts (50–500 µg/ml) were mixed with DPPH solution and incubated for 30 min at room temperature. Absorbance was measured at 517 nm and percentage inhibition was calculated. Ascorbic acid was used as standard [13].

Reducing power assay was carried out by mixing different concentrations of extract (50–500 µg/ml) with phosphate buffer and potassium ferricyanide followed by incubation at 50°C for 20 min. After addition of trichloroacetic acid and centrifugation, the supernatant was mixed with distilled water and ferric chloride.

Absorbance was measured at 700 nm. Ascorbic acid was used as standard [13,14].

Metal chelating activity was determined by adding extract (50–500 µg/ml) to ferrous chloride solution followed by ferrozine reagent. The mixture was incubated for 10 min and absorbance was measured at 562 nm. Percentage inhibition was calculated using standard formula. EDTA was used as standard chelating agent [15].

Hydrogen peroxide scavenging activity was estimated using H_2O_2 solution prepared in phosphate buffer (pH 7.4). Different concentrations of extracts (50–500 µg/ml) were added and absorbance was measured at 230 nm. Percentage scavenging activity was calculated [15].

Nitric oxide scavenging activity was evaluated using sodium nitroprusside and Griess reagent method. Different concentrations of extracts (50–500 µg/ml) were incubated with sodium nitroprusside followed by addition of sulfanilic acid and naphthylethylenediamine dihydrochloride. Absorbance was measured at 540 nm and percentage inhibition was calculated. Ascorbic acid was used as standard [13,14].

Experimental Design
Acute oral toxicity studies were carried out according to OECD guideline 423 for determination of safety profile and LD_{50} estimation.

For hepatoprotective activity, 36 rats were divided into six groups containing six animals each. Group I received vehicle (1% CMC), Group II received CCl_4, Group III received CCl_4 along with silymarin (25 mg/kg), while Groups IV, V and VI received CCl_4 along with MERH at doses of 100, 200 and 400 mg/kg respectively for four weeks [16,17].

At the end of the treatment period, animals were fasted overnight and blood samples were collected through retro-orbital plexus puncture. Serum was separated by centrifugation at 3000 rpm for 15 min for biochemical analysis. Liver and brain tissues were isolated for biochemical and histopathological studies.

Behavioral Parameter – Novel Object Recognition Test [17]
Novel Object Recognition (NOR) test was performed to evaluate recognition memory in experimental animals. Rats were habituated individually to an open field arena for 5 min, 24 h before testing. During the acquisition phase, two identical objects were placed symmetrically in the chamber and animals were allowed to explore for 5 min. After 24 h, one familiar object was replaced with a novel object and exploratory behavior was observed for another 5 min.

Exploration was defined as sniffing, rearing or touching the object with the nose from a distance of less than 2 cm. Recognition memory was assessed by calculating the preference index using the formula:

Preference Index = (Time spent near novel object − Time spent near familiar object) / (Time spent near novel object + Time spent near familiar object)

Measurement of Serum Biochemical Parameters
Serum biochemical parameters including AST, ALT, ALP, total protein, albumin, direct bilirubin and total bilirubin were estimated using a clinical chemistry autoanalyzer [18].

SGPT/ALT activity was estimated by IFCC method based on conversion of L-alanine and α-ketoglutarate to pyruvate and L-glutamate in the presence of GPT enzyme. The decrease in absorbance of NADH at 340 nm was measured and was proportional to ALT activity [18].

SGOT/AST activity was determined by IFCC method in which L-aspartate and α-ketoglutarate were converted into oxaloacetate and L-glutamate. The decrease in absorbance of NADH at 340 nm was measured and was proportional to AST activity.

ALP activity was estimated by IFCC method using p-nitrophenyl phosphate (pNPP) as substrate. Formation of yellow coloured p-nitrophenol was measured at 405 nm and was proportional to ALP activity.

Albumin estimation was carried out by Bromocresol Green (BCG) end point assay method. Formation of green coloured albumin-BCG complex was measured at 630 nm and was proportional to albumin concentration [24]. Total protein was estimated by Modified Biuret end point assay method based on formation of Cu-protein complex in alkaline medium. Absorbance was measured at 578 nm and was proportional to total protein concentration [20].

Estimation of Ammonia:
Ammonia estimation was carried out using Ammonia Quantitation Kit based on enzyme-coupled reaction method. Formation of bluish green coloured product was measured colorimetrically at 660–670 nm using a microplate reader. The intensity of colour produced was directly proportional to ammonia concentration in the sample [16].

Assessment of Antioxidant Enzymes in Liver Tissue:
Liver tissues were excised, washed with ice-cold saline and homogenized in 0.15 M Tris-HCl buffer (pH 7.4). The homogenate was used for estimation of lipid peroxidation (LPO), reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activities using standard procedures.

Lipid Peroxidation (LPO):
Lipid peroxidation was estimated by measuring malondialdehyde (MDA) levels using thiobarbituric acid reactive substances (TBARS) method. Absorbance was measured at 532 nm and results were expressed as nmoles TBARS/mg protein.

Estimation of Reduced Glutathione (GSH) [19]
Reduced glutathione level was estimated using Ellman's reagent method. Absorbance was measured at 412 nm and values were compared with standard GSH curve.

of Superoxide Dismutase (SOD) [20]
SOD activity was determined by Kakkar et al. method using sodium pyrophosphate buffer, phenazine methosulphate, nitroblue tetrazolium and NADH. Absorbance was measured at 560 nm and results were expressed as units/mg protein.

Estimation of Catalase (CAT) [21]
Catalase activity was estimated by Aebi's method based on decomposition of hydrogen peroxide. Changes in absorbance were measured at 240 nm and activity was expressed as units/mg protein.
Neurochemical Estimation in Brain Tissue
Brain tissues were isolated, homogenized in ice-cold phosphate buffer (pH 7.4) and centrifuged. The supernatant obtained was used for estimation of GABA, glutamate, AChE and pro-inflammatory markers [24].

Estimation of GABA and Glutamate [22]
GABA and glutamate levels were estimated by HPLC using electrochemical detector according to Donzanti and Yamamoto method with slight modifications. Brain samples were derivatized using OPA/β-ME reagent and analyzed using reverse phase C18 column. Concentrations were calculated from standard calibration curve.

Estimation of Acetylcholinesterase (AChE) Activity
AChE activity was estimated by photometric method based on formation of yellow coloured complex produced from thiocholine and dithiobisnitrobenzoate ion. Absorbance was measured at 412 nm and enzyme activity was calculated accordingly.

Neuroinflammatory Markers
TNF-α, IL-1β and IL-6 levels were estimated using ELISA kits according to manufacturer's instructions. Absorbance was measured at 450 nm using ELISA reader and concentrations were calculated from standard calibration curves [23].

Histopathology [24]
Liver tissues were isolated and fixed in 10% formalin followed by dehydration with graded isopropyl alcohol. Tissues were embedded in paraffin wax, sectioned using microtome and stained with hematoxylin and eosin. Histopathological changes were observed under microscope.

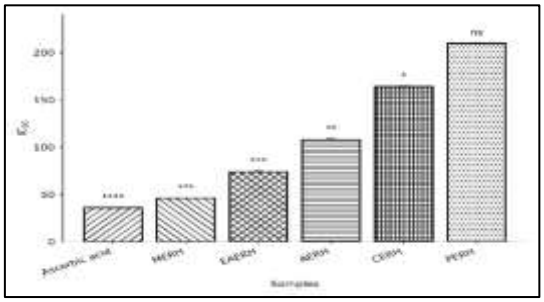
Statistical Analysis
Results were expressed as mean ± SEM. Behavioral and biochemical parameters were analyzed using one-way ANOVA followed by Bonferroni's multiple comparison test. Neuroinflammatory parameters were analyzed using two-way ANOVA followed by Bonferroni's test using GraphPad Prism software. IC_{50} values were calculated by linear regression analysis.

3. RESULTS

Preliminary Phytochemical Screening
Phytochemical screening of *Clidemia hirta* extracts revealed the presence of saponins, sterols, carbohydrates, tannins, flavonoids, fatty acids, terpenoids and glycosides in different solvent extracts. Methanolic extract (MERH) showed the presence of maximum phytoconstituents, while alkaloids were absent in all extracts.

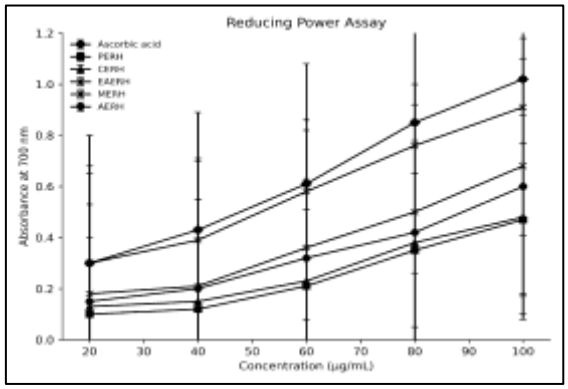
Determination of Total Phenolic Content, Total Flavonoid Content and Percentage Yield
Among all the extracts, methanolic extract showed the highest total phenolic content (121.7±5.947 mg GAE/g extract), total flavonoid content (165.9±3.99 mg QE/g extract) and percentage yield (13.33%). Ethyl acetate extract also exhibited considerable phenolic (82.21±1.542 mg GAE/g extract) and flavonoid content (88.42±1.949 mg QE/g extract). Aqueous extract showed moderate phenolic and flavonoid content with 12.65% yield. Petroleum ether and chloroform extracts showed comparatively lower phenolic and flavonoid contents.

***In Vitro* Antioxidant Activity**
DPPH Assay



Graph 1: DPPH free radical scavenging activity of different extracts of *Clidemia hirta**. Data are expressed as mean ± SEM (n = 3). Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test. # $p < 0.05$ compared with Ascorbic acid; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with MERH*

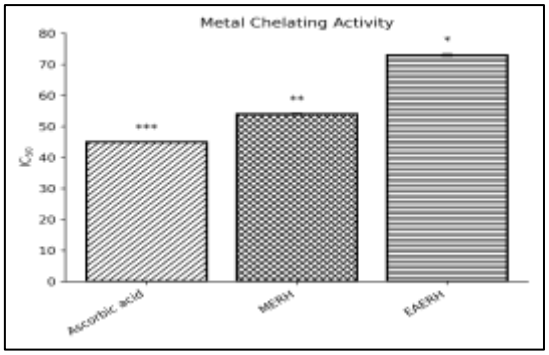
Reducing Power Assay



Graph 2: *Reducing power activity of different extracts of* Clidemia hirta*. Data are expressed as mean ± SEM (n = 3). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Significance levels were considered at* p *< 0.05, $p < 0.01$, and $p < 0.001$.

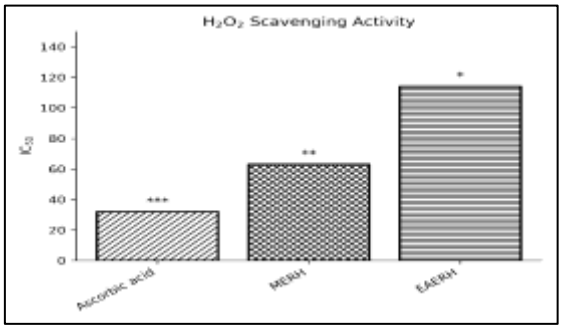
Metal chelating activity

Chinese Journal of Health Management
Volume 20 Issue 6, Year of Publication 2026, Page 1689-1709 | DoI-10.564220/1674-0815.2026.167



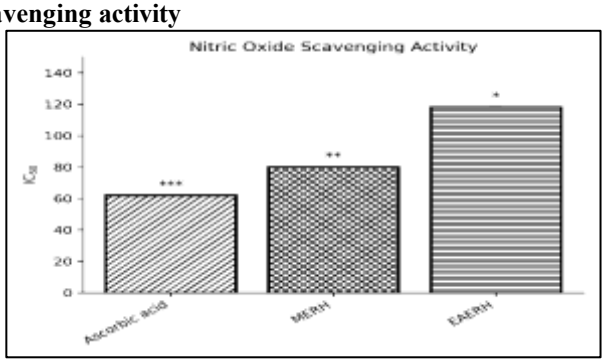
Graph 3: Metal chelating activity of different extracts of Clidemia hirta. *Data are expressed as mean ± SEM (n = 3). Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test. # p < 0.05 compared with Ascorbic acid; * p < 0.05, ** p < 0.01, and *** p < 0.001 compared with MERH.

H_2O_2 activity



Graph 4: *Hydrogen peroxide scavenging activity of different extracts of Clidemia hirta*. Data are expressed as mean ± SEM (n = 3). Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test. # *p* < 0.05 compared with Ascorbic acid; * *p* < 0.05, ** *p* < 0.01, and *** *p* < 0.001 compared with MERH.

Nitric oxide radical (NO˙) scavenging activity



Graph 5: *Nitric oxide scavenging activity of different extracts of Clidemia hirta*. Data are expressed as mean ± SEM (n = 3). Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test. # *p* < 0.05 compared with Ascorbic acid; * *p* < 0.05, ** *p* < 0.01, and *** *p* < 0.001 compared with MERH.

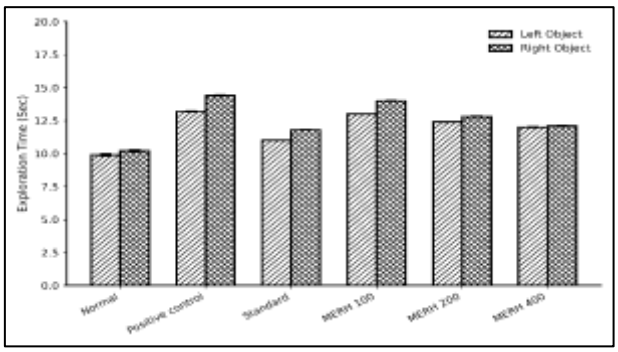
Acute Oral Toxicity Studies of MERH:

Acute oral toxicity study of MERH was carried out according to OECD guideline 423. Animals were observed continuously for the first 4 h and periodically up to 24 h after administration of the extract for any behavioral, neurological and autonomic changes. Further observations were continued daily for 14 days.

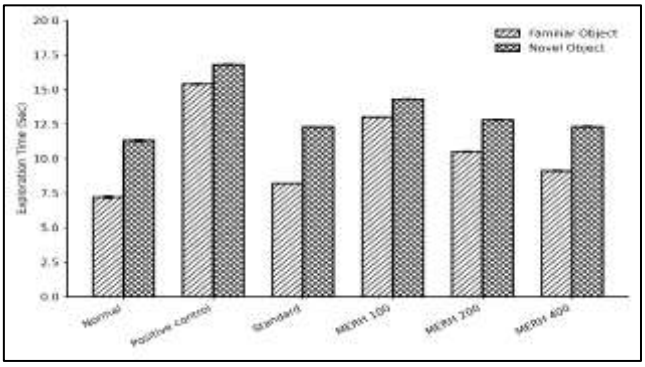
Parameters such as alertness, spontaneous motor activity, respiration, body tone, limb tone, reactivity to sound

and touch, urination, lacrimation, salivation, diarrhea and pupil size remained normal throughout the observation period. No signs of ataxia, body tremors, convulsions, writhing, sedation, coma, cyanosis or piloerection were observed in any experimental animal. No mortality or toxic symptoms were detected during the study period, indicating the safety of MERH at the administered dose.

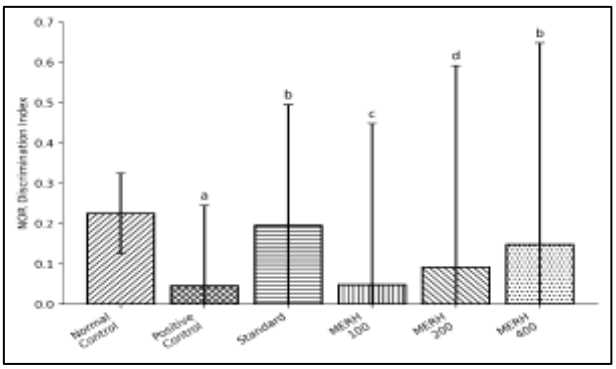
Effect of MERH Treatment in Ethanol Induced Hepatotoxicity.
Behavioral Parameter
Effect of MERH treatment on novel object recognition task in Ethanol induced hepatotoxicity



Graph 6: *Effect of MERH on recognition memory in ethanol-induced hepatotoxicity rats using the Novel Object Recognition (NOR) test. Data are expressed as mean ± SEM (n = 6).* Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparison test. ### $p < 0.001$ compared with the Normal Control group; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and ns = non-significant compared with the Positive Control group*



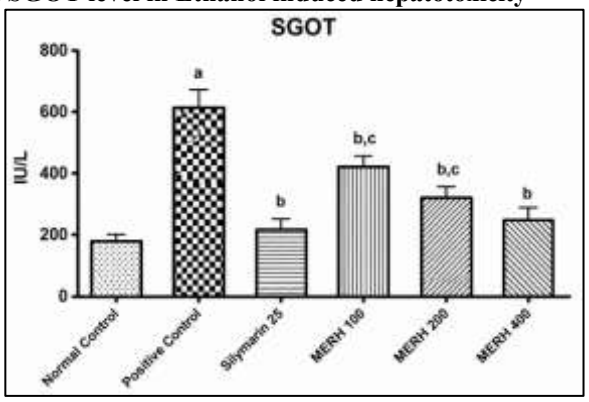
Graph 7: Effect of MERH on exploration time in retention trial using novel object recognition task.



Graph 8: Effect of MERH on NOR discrimination index in novel object recognition task in Ethanol induced hepatotoxicity. Values are expressed as mean ± SEM.[a]$p<0.001$ vs Normal Control, [b]$p<0.001$ vs Positive control, [c]$p<0.001$, [d]$p<0.01$ vs Standard. Data were statistically analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests.

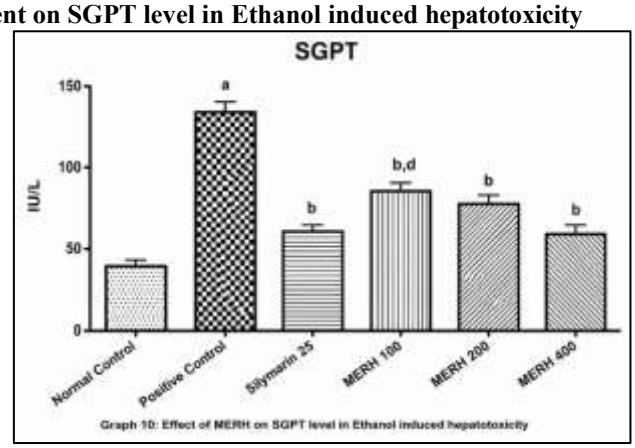
Effect of MERH treatment on serum biochemical biomarkers in Ethanol induced hepatotoxicity

Effect of MERH treatment on SGOT level in Ethanol induced hepatotoxicity



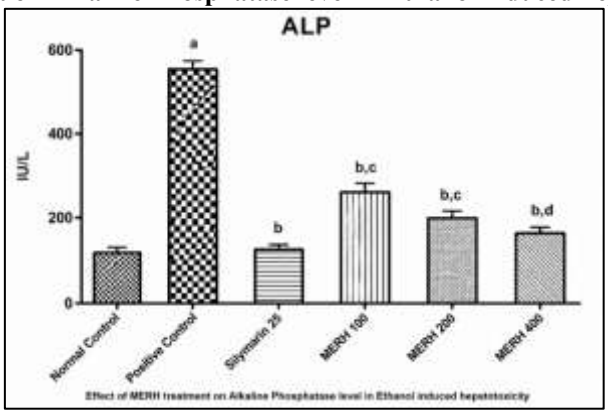
Graph 9: Effect of MERH on SGOT level in Ethanol induced hepatotoxicity. Values are expressed as mean ± SEM.[a]p<0.001 vs Normal Control, [b]p<0.001 vs Positive control, [c]p<0.001 vs Standard. Data were statistically analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests.

Effect of MERH treatment on SGPT level in Ethanol induced hepatotoxicity



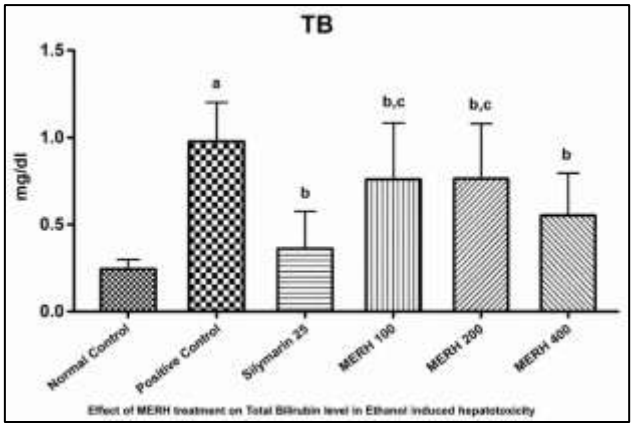
Graph 10: Effect of MERH on SGPT level in Ethanol induced hepatotoxicity. Values are expressed as mean ± SEM.[a]p<0.001 vs Normal Control, [b]p<0.001 vs Positive control, [d]p<0.05 vs Standard. Data were statistically analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests.

Effect of MERH treatment on Alkaline Phosphatase level in Ethanol induced hepatotoxicity



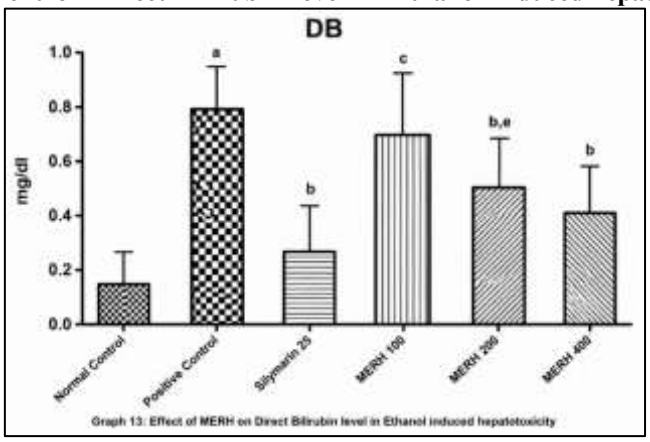
Graph 11: Effect of MERH on ALP level in Ethanol induced hepatotoxicity. Values are expressed as mean ± SEM.[a]p<0.001 vs Normal Control, [b]p<0.001 vs Positive control, [c]p<0.001, [d]p<0.05 vs Standard. Data were statistically analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests.

Effect of MERH treatment on Total Bilirubin level in Ethanol induced hepatotoxicity



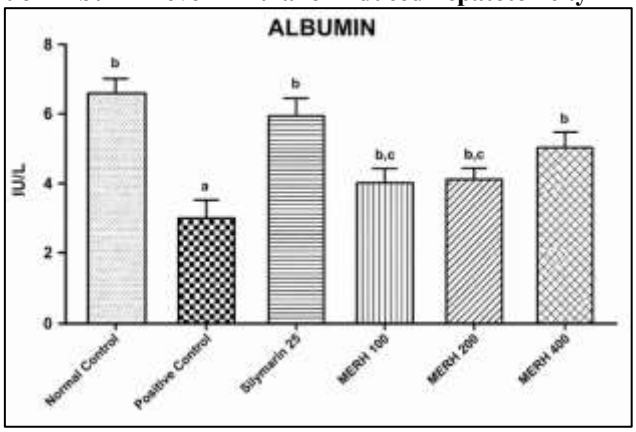
Graph 12: Effect of MERH on Total Bilirubin level in Ethanol induced hepatotoxicity. Values are expressed as mean ± SEM.[a]p<0.001 vs Normal Control, [b]p<0.001 vs Positive control, [c]p<0.001 vs Standard. Data were statistically analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests.

Effect of MERH treatment on Direct Bilirubin level in Ethanol induced hepatotoxicity



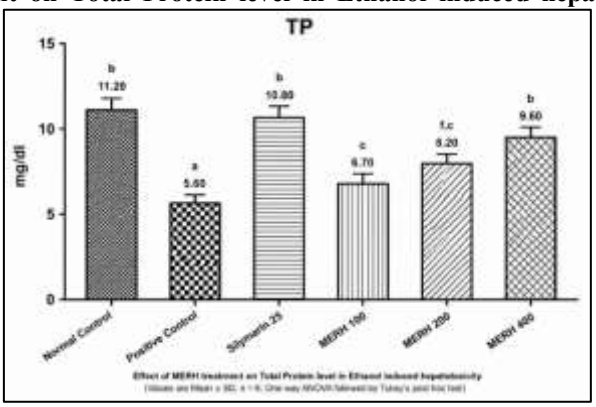
Graph 13: Effect of MERH on Direct Bilirubin level in Ethanol induced hepatotoxicity. Values are expressed as mean ± SEM.[a]p<0.001 vs Normal Control, [b]p<0.001 vs Positive control, [c]p<0.001, [e]p<0.01 vs Standard. Data were statistically analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests.

Effect of MERH treatment on Albumin level in Ethanol induced hepatotoxicity



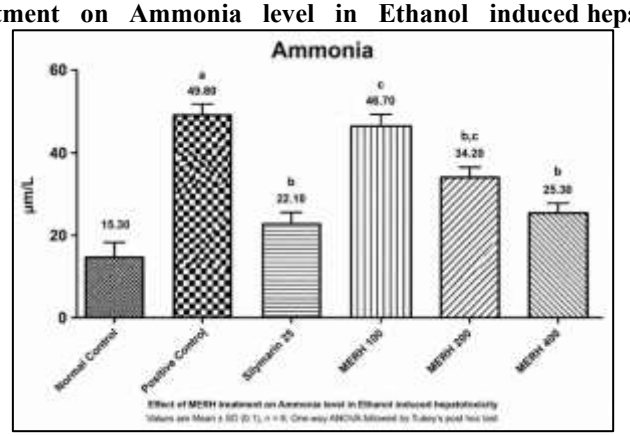
Graph 14: Effect of MERH on Albumin level in Ethanol induced hepatotoxicity. Values are expressed as mean ± SEM.[a]p<0.001 vs Normal Control, [b]p<0.001 vs Positive control, [c]p<0.001 vs Standard. Data were statistically analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests.

Effect of MERH treatment on Total Protein level in Ethanol induced hepatotoxicity.



Graph 15: Effect of MERH on Total Protein level in Ethanol induced hepatotoxicity. Values are expressed as mean ± SEM.[a]p<0.001 vs Normal Control, [b]p<0.001, [f]p<0.01 vs Positive control, [c]p<0.001 vs Standard. Data were statistically analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests.

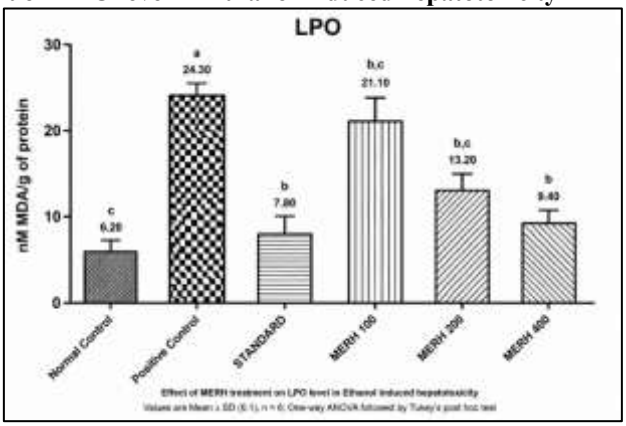
Effect of MERH treatment on Ammonia level in Ethanol induced hepatotoxicity



Graph 16: Effect of MERH on Ammonia level in Ethanol induced hepatotoxicity. Values are expressed as mean ± SEM.[a]p<0.001 vs Normal Control, [b]p<0.001 vs Positive control, [c]p<0.001 vs Standard. Data were statistically analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests**.**

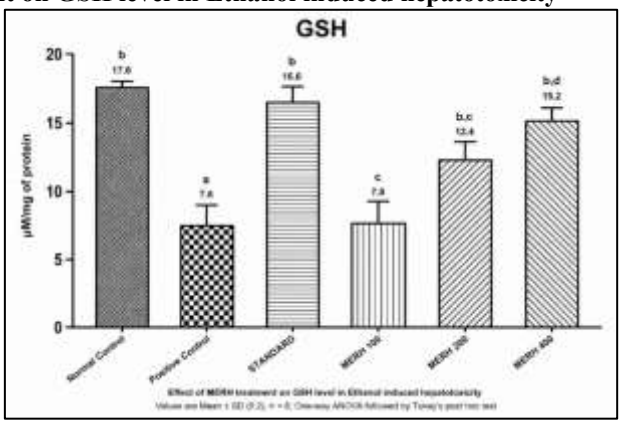
***In vivo* antioxidant activity in liver tissue**

Effect of MERH treatment on LPO level in Ethanol induced hepatotoxicity



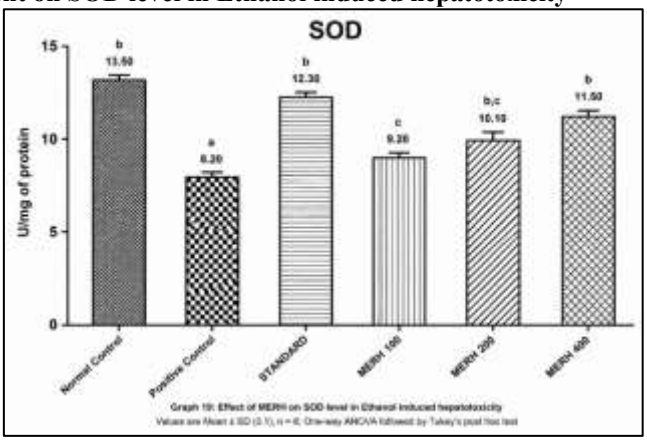
Graph 17: Effect of MERH on LPO level in Ethanol induced hepatotoxicity. Values are expressed as mean ± SEM.[a]p<0.001 vs Normal Control, [b]p<0.001 vs Positive control, [c]p<0.001 vs Standard. Data were statistically analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests.

Effect of MERH treatment on GSH level in Ethanol induced hepatotoxicity



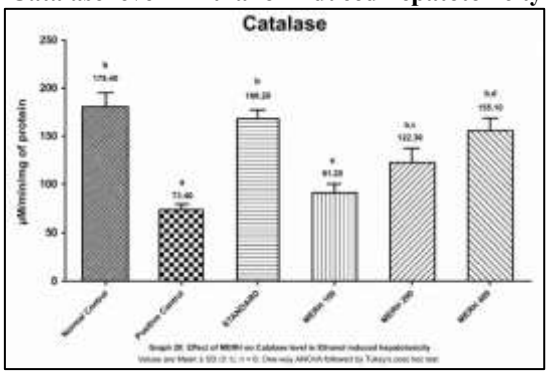
Graph 18: Effect of MERH on GSH level in Ethanol induced hepatotoxicity. Values are expressed as mean ± SEM.[a]p<0.001 vs Normal Control, [b]p<0.001 vs Positive control, [c]p<0.001, [d]p<0.05 vs Standard. Data were statistically analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests.

Effect of MERH treatment on SOD level in Ethanol induced hepatotoxicity



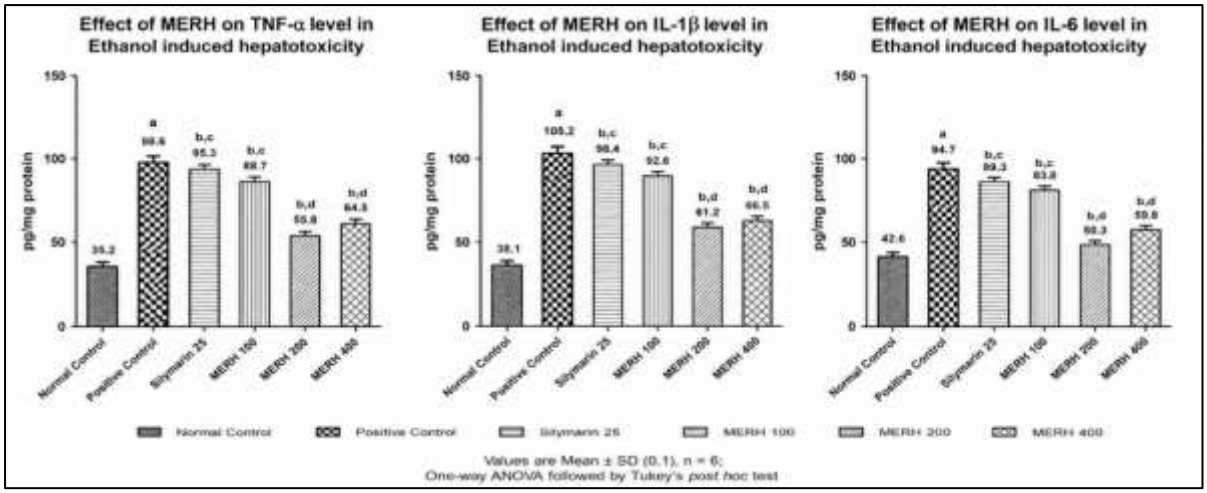
Graph 19: Effect of MERH on SOD level in Ethanol induced hepatotoxicity. Values are expressed as mean ± SEM.[a]p<0.001 vs Normal Control, [b]p<0.001 vs Positive control, [c]p<0.001 vs Standard. Data were statistically analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests.

Effect of MERH treatment on Catalase level in Ethanol induced hepatotoxicity



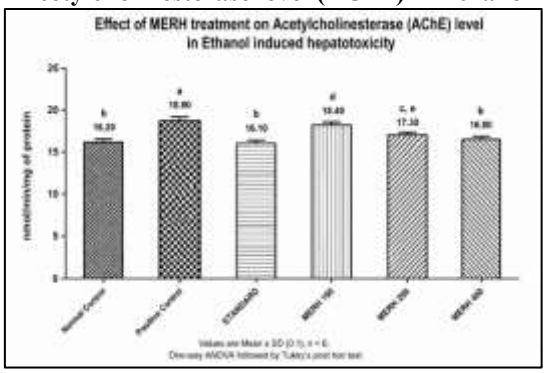
Graph 20: Effect of MERH on Catalase level in Ethanol induced hepatotoxicity. Values are expressed as mean ± SEM. [a]p<0.001 vs Normal Control, [b]p<0.001, [c]p<0.01 vs. Positive control, [c]p<0.001, [d]p<0.05 vs Standard. Data were statistically analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests.

Effect of MERH treatment on pro-inflammatory cytokines levels (TNF-α, IL-1β, IL-6) in Ethanol induced hepatotoxicity



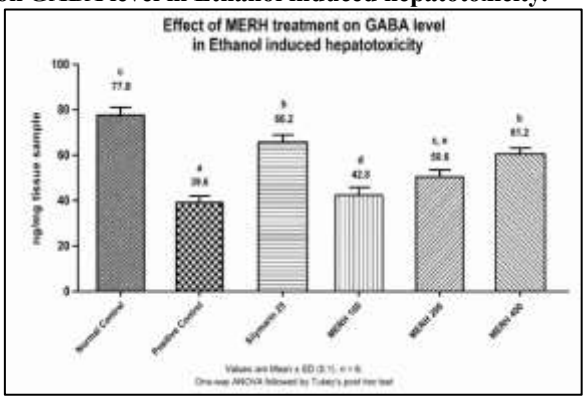
Graph 21: Effect of MERH on TNF-α, IL-1β and IL-6 levels in Ethanol induced hepatotoxicity. Values are expressed as mean ± SEM.[a]p<0.001 vs Normal Control, [b]p<0.001 vs Positive control, [c]p<0.001, [d]p<0.05 vs Standard. Data were statistically analyzed by two-way ANOVA followed by Bonferroni's multiple comparison tests.

Effect of MERH treatment on AChE, GABA and Glutamate level in ethanol induced hepatotoxicity
Effect of MERH treatment on Acetylcholinesterase level (AChE) in Ethanol induced hepatotoxicity



Graph 22: Effect of MERH on AChE level in Ethanol induced hepatotoxicity. Values are expressed as mean ± SEM.[a]p<0.001 vs Normal Control, [b]p<0.001, [c]p<0.05 vs Positive control, [d]p<0.001, [e]p<0.01 vs Standard. Data were statistically analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests.

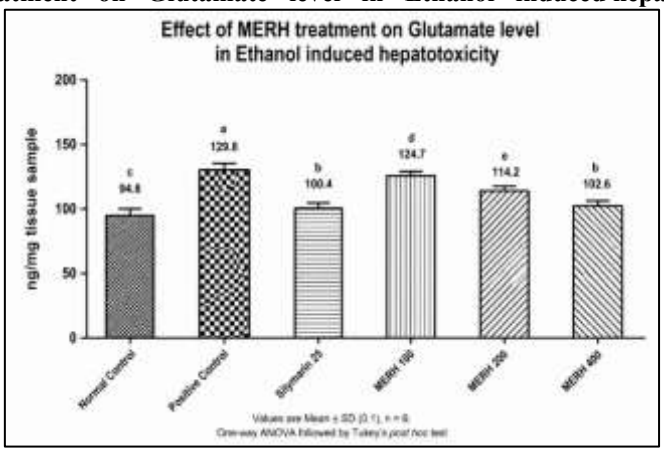
Effect of MERH treatment on GABA level in Ethanol induced hepatotoxicity.



Graph 23: Effect of MERH on GABA level in Ethanol induced hepatotoxicity. Values are expressed as mean ± SEM.[a]p<0.001 vs Normal Control, [b]p<0.001, [c]p<0.05 vs Positive control, [d]p<0.001, [e]p<0.01 vs Standard. Data were statistically analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests.

Chinese Journal of Health Management
Volume 20 Issue 6, Year of Publication 2026, Page 1689-1709 | DoI-10.564220/1674-0815.2026.167

Effect of MERH treatment on Glutamate level in Ethanol induced hepatotoxicity.



Graph 24: Effect of MERH on Glutamate level in Ethanol induced rats. Values are expressed as mean ± SEM.[a]$p<0.001$ vs Normal Control, [b]$p<0.001$ vs Positive control, [d]$p<0.001$, [e]$p<0.01$ vs Standard. Data were statistically analyzed by one-way ANOVA followed by Bonferroni's multiple comparison tests.

Histopathology Examination

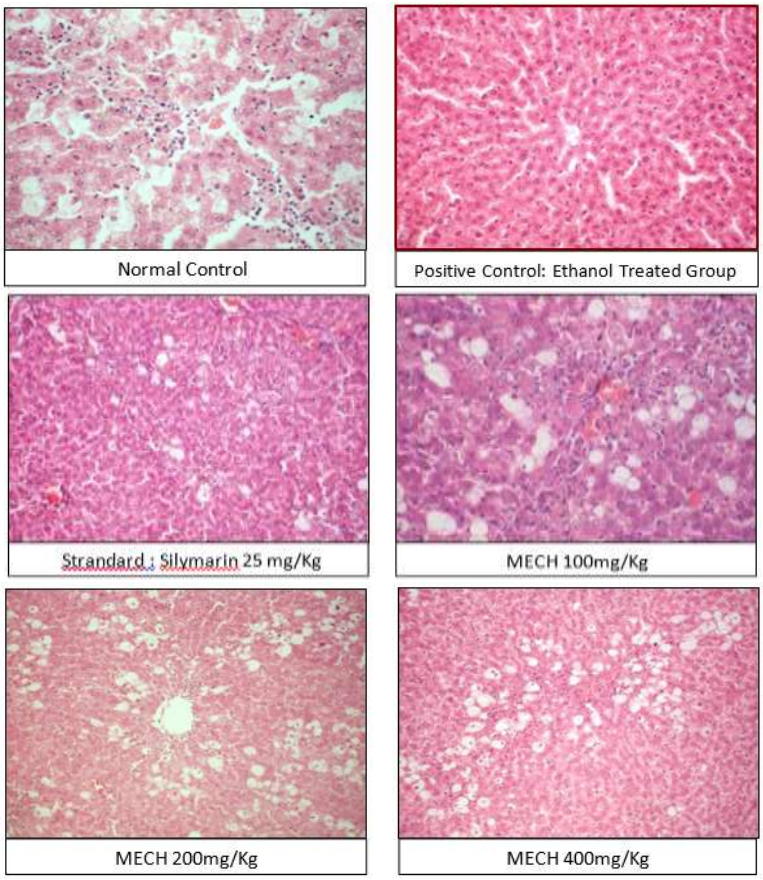
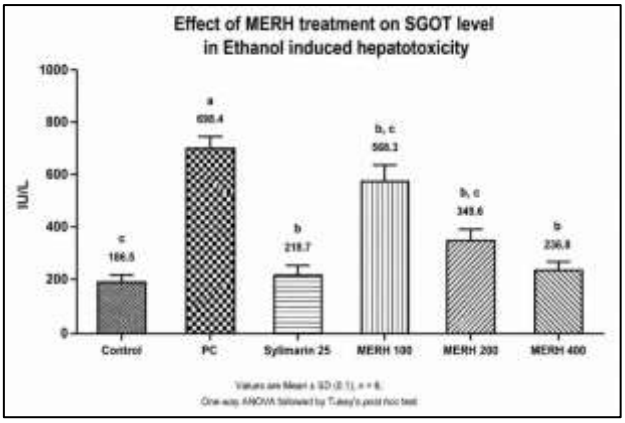


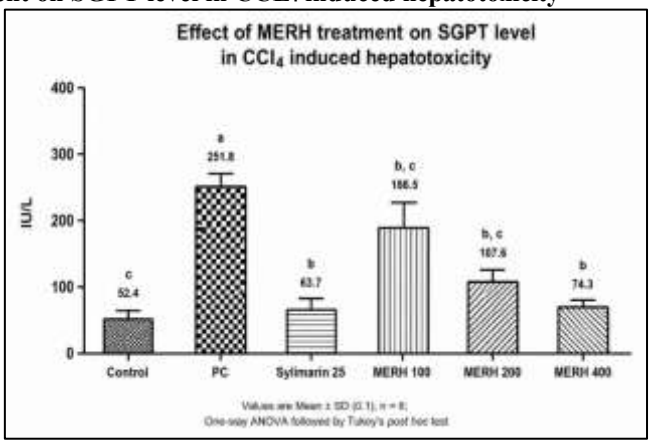
Figure 1: Effect of Ethanol, Silymarin and MERH on liver gross and histology in Ethanol - induced hepatotoxicity, (HEx 400).

Effect of CCL_4, Silymarin, MERH on serum hepatic biomarker in CCL_4 -induced hepatotoxicity in rats
Effect of MERH treatment on SGOT level in CCL_4 induced hepatotoxicity



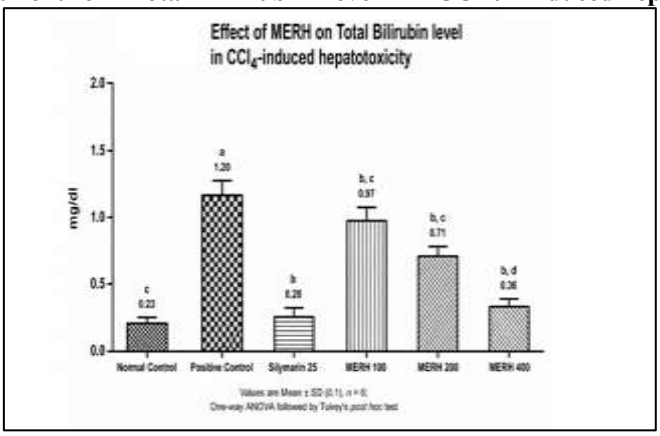
Graph 25: Effect of MERH treatment on SGOT level in ethanol-induced hepatotoxicity. Values are expressed as mean ± SD (n = 6). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test.

Effect of MERH treatment on SGPT level in CCL_4 induced hepatotoxicity



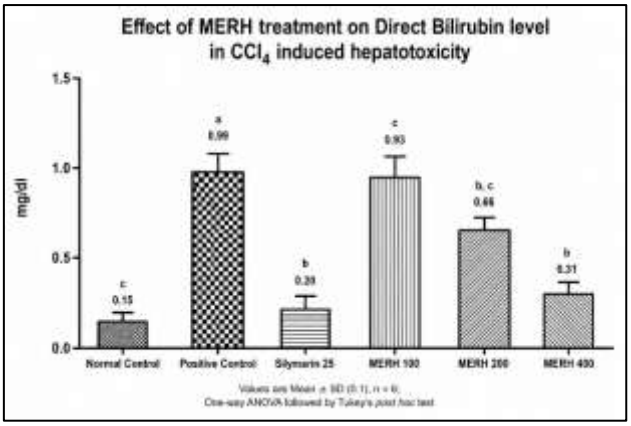
Graph 26: Effect of MERH treatment on SGPT level in CCl_4-induced hepatotoxicity. Values are expressed as mean ± SD (n = 6). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test.

Effect of MERH treatment on Total Bilirubin level in CCL_4 induced hepatotoxicity

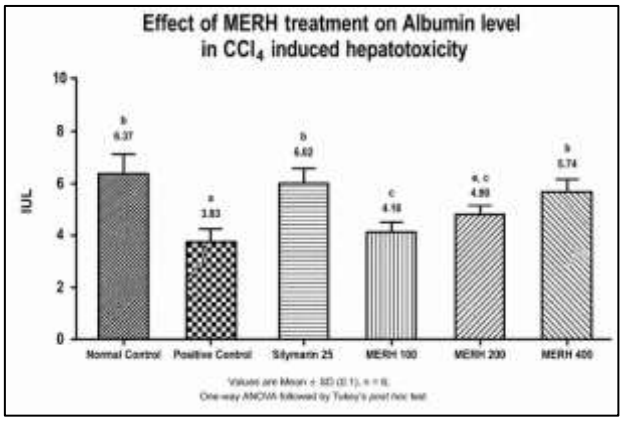


Graph 27: Effect of MERH treatment on total bilirubin level in CCl_4-induced hepatotoxicity. Values are expressed as mean ± SD (n = 6). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test.

Effect of MERH treatment on Direct Bilirubin level in CCL_4 induced hepatotoxicity.

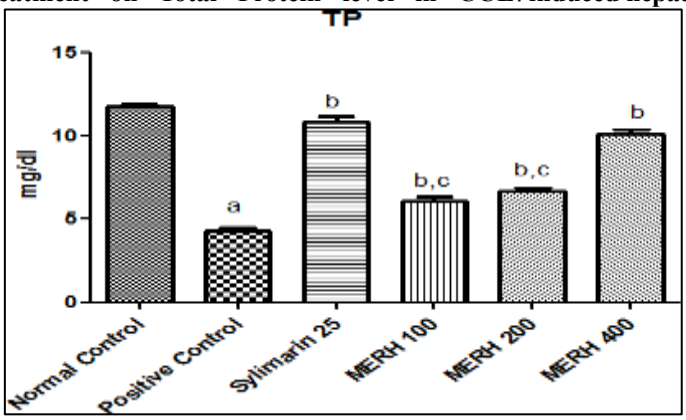


Graph 28: Effect of MERH treatment on direct bilirubin level in CCl_4-induced hepatotoxicity. Values are expressed as mean ± SD (n = 6). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test. Effect of MERH treatment on Albumin level in CCL_4 induced hepatotoxicity



Graph 29: Effect of MERH treatment on albumin level in CCl_4-induced hepatotoxicity. Values are expressed as mean ± SD (n = 6). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test.

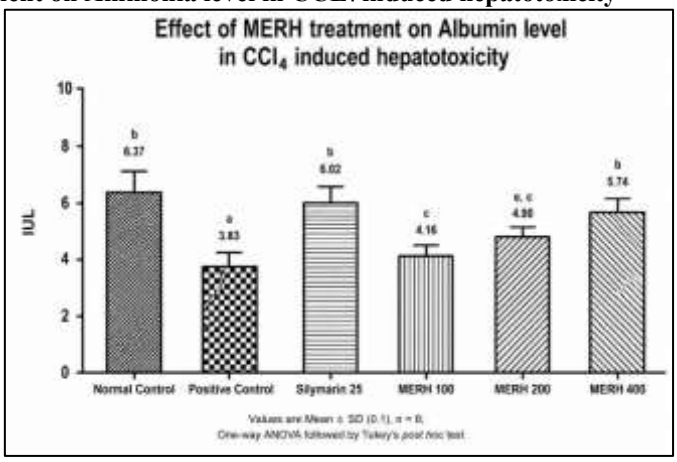
Effect of MERH treatment on Total Protein level in CCL_4 induced hepatotoxicity



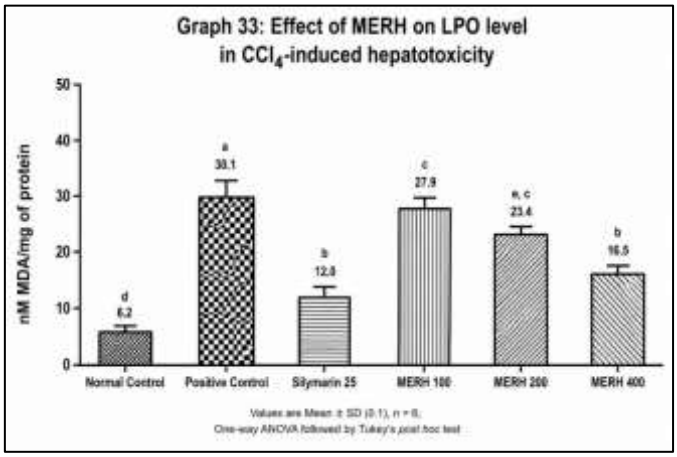
Graph 30: Effect of MERH treatment on total protein (TP) level in CCl_4-induced hepatotoxicity. Data are expressed as mean ± SEM (n = 6). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test.

Chinese Journal of Health Management
Volume 20 Issue 6, Year of Publication 2026, Page 1689-1709 | DoI-10.564220/1674-0815.2026.167

Effect of MERH treatment on Ammonia level in CCL_4 induced hepatotoxicity

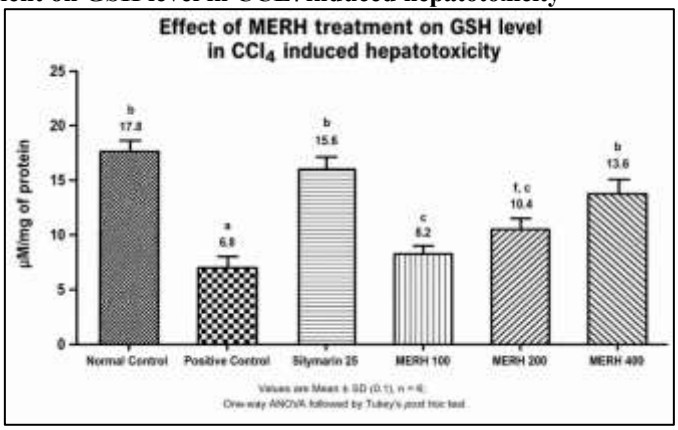


Graph 31: Effect of MERH treatment on albumin level in CCl_4-induced hepatotoxicity. Values are expressed as mean ± SD (n = 6). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test.Effect of MERH treatment on LPO level in CCL_4 induced hepatotoxicity



Graph 32: Effect of MERH treatment on lipid peroxidation (LPO) level in CCl_4-induced hepatotoxicity. Values are expressed as mean ± SD (n = 6). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test.

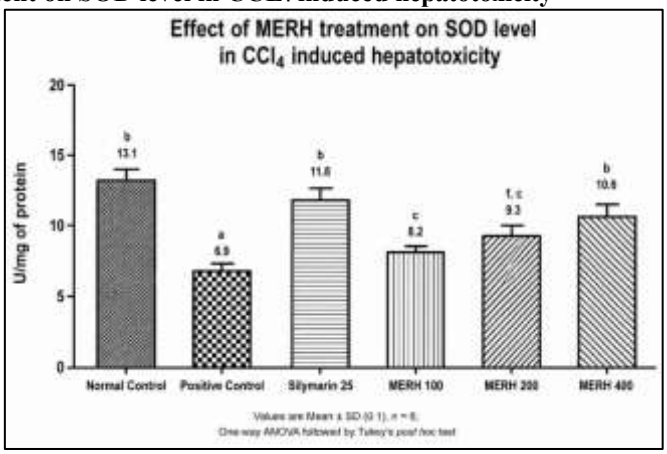
Effect of MERH treatment on GSH level in CCL_4 induced hepatotoxicity



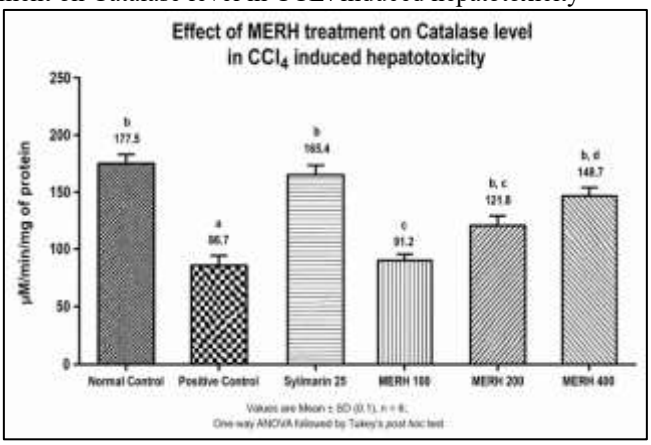
Graph 33: Effect of MERH treatment on GSH level in CCl_4-induced hepatotoxicity. Values are expressed as mean ± SD (n = 6). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test.

Chinese Journal of Health Management
Volume 20 Issue 6, Year of Publication 2026, Page 1689-1709 | DoI-10.564220/1674-0815.2026.167

Effect of MERH treatment on SOD level in CCL_4 induced hepatotoxicity

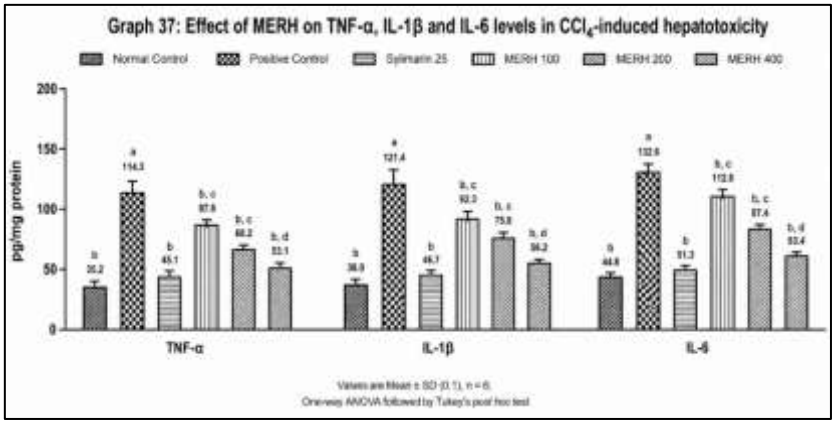


Graph 34: Effect of MERH treatment on SOD level in CCl_4-induced hepatotoxicity. Values are expressed as mean ± SD (n = 6). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test.Effect of MERH treatment on Catalase level in CCL_4 induced hepatotoxicity



Graph 35: Effect of MERH treatment on Catalase level in CCl_4-induced hepatotoxicity. Values are expressed as mean ± SD (n = 6). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test.

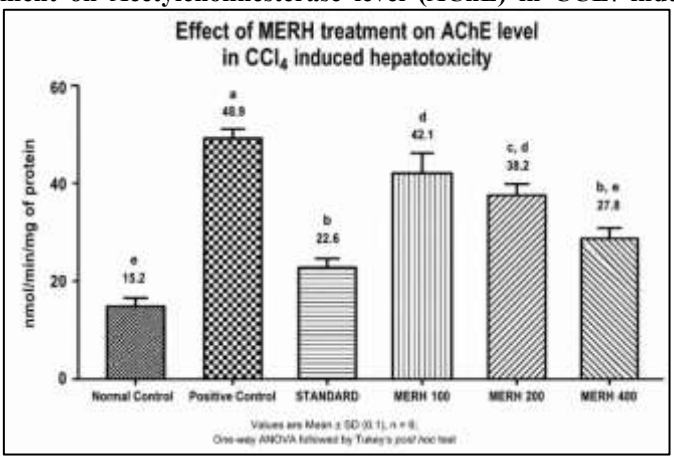
Effect of MERH treatment on pro-inflammatory cytokines levels (TNF-α, IL-1β, IL-6) in CCL_4 induced hepatotoxicity



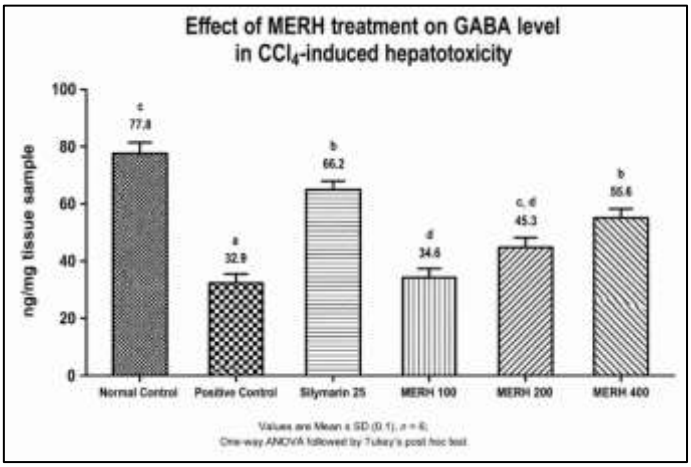
Graph 36: Effect of MERH treatment on TNF-α, IL-1β and IL-6 levels in CCl_4-induced hepatotoxicity. Values are expressed as mean ± SD (n = 6). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test.

Chinese Journal of Health Management
Volume 20 Issue 6, Year of Publication 2026, Page 1689-1709 | DoI-10.564220/1674-0815.2026.167

Effect of MERH treatment on Acetylcholinesterase level (AChE) in CCL_4 induced hepatotoxicity

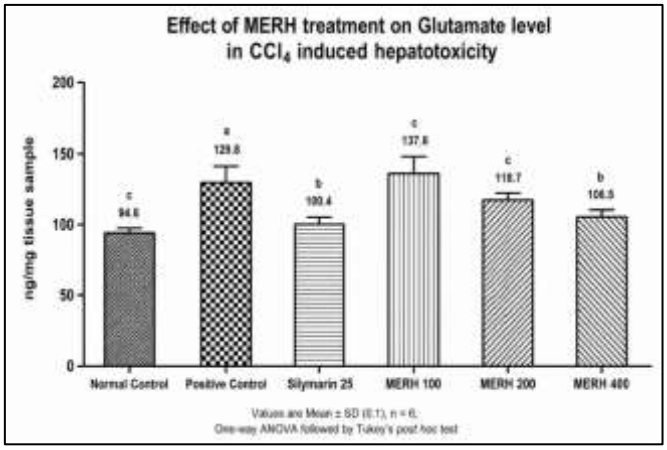


Graph 37: Effect of MERH treatment on AChE level in CCl_4-induced hepatotoxicity. Values are expressed as mean ± SD (n = 6). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test.



Graph 38: Effect of MERH treatment on GABA level in CCl_4-induced hepatotoxicity.

CCl_4 significantly decreased GABA levels, whereas Silymarin and MERH treatment restored them in a dose-dependent manner. Values are expressed as mean ± SD (n = 6). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test.Effect of MERH treatment on Glutamate level in CCL_4 induced hepatotoxicity.



Graph 39: Effect of MERH treatment on Glutamate level in CCl_4-induced hepatotoxicity.

CCl_4 significantly increased glutamate levels, while Silymarin and MERH treatment reduced them in a dose-dependent manner. Values are expressed as mean ± SD (n = 6). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test.

Histopathology examination

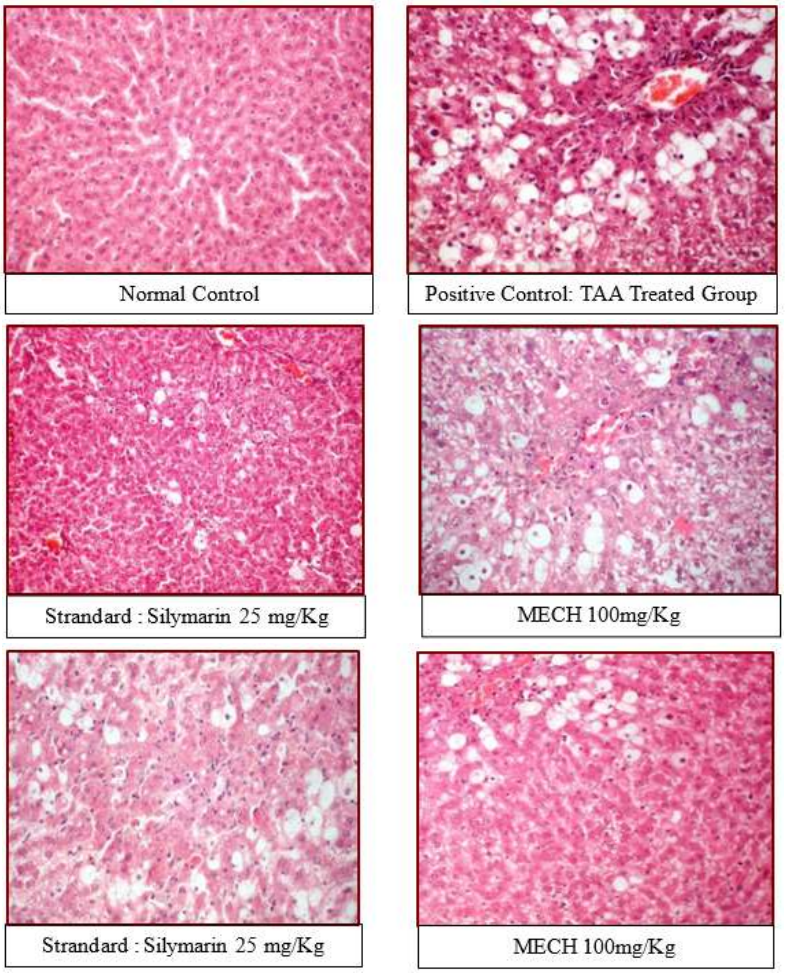


Figure 2: Effect of CCL_4 Silymarin and MERH on liver gross and histology in CCL_4- induced hepatotoxicity, (HEx 400).

4. DISCUSSION:

The present study demonstrated the significant hepatoprotective potential of the methanolic extract of Clidemia hirta (MERH) against ethanol and CCl_4-induced hepatotoxicity in Wistar rats. Ethanol and carbon tetrachloride are well-known hepatotoxic agents that induce liver injury through excessive production of reactive oxygen species, lipid peroxidation, inflammation, and oxidative stress, ultimately leading to hepatocellular degeneration and necrosis. In the present investigation, administration of ethanol and CCl_4 markedly elevated serum hepatic biomarkers such as SGOT, SGPT, ALP, total bilirubin, direct bilirubin, and ammonia levels while reducing albumin and total protein levels, indicating severe hepatic dysfunction and membrane damage.

Treatment with MERH significantly restored these altered biochemical parameters in a dose-dependent manner. The higher dose of MERH (400 mg/kg) showed pronounced hepatoprotective activity comparable to the standard drug silymarin. Reduction in serum transaminases and bilirubin levels suggests stabilization of hepatocyte membranes and improved functional integrity of the liver. Improvement in albumin and total protein levels further indicates restoration of synthetic capacity of hepatic cells.

The extract also demonstrated significant antioxidant activity both in vitro and in vivo. MERH reduced lipid peroxidation and significantly increased endogenous antioxidant enzymes such as GSH, SOD, and catalase. These findings indicate that the hepatoprotective activity of *Clidemia hirta* may be attributed to its free radical

scavenging and antioxidant properties. The phytochemical screening revealed the presence of flavonoids, phenolics, tannins, terpenoids, and glycosides, which are known for their antioxidant and anti-inflammatory activities.

Furthermore, MERH significantly reduced pro-inflammatory cytokines including TNF-α, IL-1β, and IL-6, suggesting suppression of inflammatory pathways associated with hepatic injury. Histopathological examination also confirmed the protective effect of MERH by showing restoration of normal hepatic architecture with reduced necrosis, fatty degeneration, and inflammatory infiltration in treated groups.

Overall, the findings suggest that *Clidemia hirta* possesses potent hepatoprotective, antioxidant, and anti-inflammatory activities and may serve as a promising natural therapeutic agent for the management of liver disorders.

5. CONCLUSION:

The present study demonstrated that the methanolic extract of *Clidemia hirta* (MERH) possesses significant hepatoprotective and antioxidant activity against ethanol- and CCl_4-induced hepatotoxicity in Wistar rats. MERH treatment effectively restored altered biochemical markers such as SGOT, SGPT, ALP, bilirubin, albumin, and total protein levels toward normal values. The extract also improved antioxidant enzyme status by reducing lipid peroxidation and enhancing GSH, SOD, and catalase activities. Furthermore, MERH reduced pro-inflammatory cytokines and improved neurochemical parameters including GABA, glutamate, and AChE levels. Histopathological studies further confirmed the protective effect of MERH by showing restoration of normal hepatic architecture. Overall, the study suggests that *Clidemia hirta* possesses promising hepatoprotective potential, which may be attributed to its rich phenolic and flavonoid content with strong antioxidant properties.

6. REFERENCES

1. Hall P, Cash J. What is the real function of the liver 'function'tests? The Ulster medical journal. 2012 Jan;81(1):30.
2. Friedman SL. Liver fibrosis–from bench to bedside. Journal of hepatology. 2003 Jan 1;38:38-53.
3. Recknagel RO, Glende Jr EA, Dolak JA, Waller RL. Mechanisms of carbon tetrachloride toxicity. Pharmacology & therapeutics. 1989 Jan 1;43(1):139-54.
4. Halliwell B, Gutteridge JM. Free radicals in biology and medicine. Oxford university press; 2015.
5. Li S, Tan HY, Wang N, Zhang ZJ, Lao L, Wong CW, Feng Y. The role of oxidative stress and antioxidants in liver diseases. International journal of molecular sciences. 2015 Nov 2;16(11):26087-124.
6. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Frontiers in pharmacology. 2014 Jan 10;4:177.
7. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. The scientific world journal. 2013;2013(1):162750.
8. Kokate CK. Practical pharmacognosy 3rd ed Vallabh Prakashan New Delhi.
9. Harborne AJ. Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media; 1998 Apr 30.
10. Singleton VL, Rossi Jr JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American journal of Enology and Viticulture. 1965;16(3):144-58.
11. Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods. American journal of enology and viticulture. 1977 Jan 1;28(1):49-55.
12. Woisky RG, Salatino A. Analysis of propolis: some parameters and procedures for chemical quality control. Journal of apicultural research. 1998 Jan 1;37(2):99-105.
13. Oyaizu M. Studies on products of browning reaction antioxidative activities of products of browning reaction prepared from glucosamine. The Japanese journal of nutrition and dietetics. 1986;44(6):307-15.
14. Dinis TC, Madeira VM, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Archives of biochemistry and biophysics. 1994 Nov 1;315(1):161-9.
15. Butterworth RF. Pathogenesis of hepatic encephalopathy and brain edema in acute liver failure. Journal of clinical and experimental hepatology. 2015 Mar 1;5:S96-103.
16. Recknagel RO, Glende Jr EA, Dolak JA, Waller RL. Mechanisms of carbon tetrachloride toxicity. Pharmacology & therapeutics. 1989 Jan 1;43(1):139-54.
17. Fiseha T, Alemayehu E, Mohammed Adem O, Eshetu B, Gebreweld A. Reference intervals for common clinical chemistry parameters in healthy adults of Northeast Ethiopia. PLoS One. 2022 Nov 2;17(11):e0276825.
18. Bergmeyer HU, Horder M, Rej R. International Federation of Clinical Chemistry (Ifcc) 1), 2). J. clin. Chem. clin. Biochem. 1986 Jan 1;24(7):481-95.
19. Ellman GL. Tissue sulfhydryl groups. Archives of biochemistry and biophysics. 1959 May 1;82(1):70-7.
20. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys. 1984 Apr 1;21(2):130-2.
21. Aebi H. Catalase. InMethods of enzymatic analysis 1974 Jan 1 (pp. 673-684). Academic press.
22. Donzanti BA, Yamamoto BK. An improved and rapid HPLC-EC method for the isocratic separation of amino acid neurotransmitters from brain tissue and microdialysis perfusates. Life sciences. 1988 Jan 1;43(11):913-22.
23. Elenkov IJ, Iezzoni DG, Daly A, Harris AG, Chrousos GP. Cytokine dysregulation, inflammation and well-being. Neuroimmunomodulation. 2005 Sep 21;12(5):255-69.

24. Doumas BT, Watson WA, Biggs HG. Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta. 1971;31(1):87–96.