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ROOT EXTRACT OF *Clerodendrum paniculatum* L. AS A NATURAL HEPATOPROTECTIVE AGENT

Wandaphi Kharbyngar*[1], Ramdas Bhat[2]

[1]PG Scholar, Department of Pharmacology, Srinivas College of Pharmacy, Valachil, Mangalore – 574143, Karnataka, India.
[2]Associate Professor, Department of Pharmacology, Father Muller College of Pharmaceutical Sciences, Deralakatte, Mangalore – 575018, Karnataka, India.

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***Corresponding Author: Wandaphi Kharbyngar**
PG Scholar, Department of Pharmacology, Srinivas College of Pharmacy, Valachil, Mangalore – 574143, Karnataka, India.
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ABSTRACT

Objective: This study investigated whether the root extract of *Clerodendrum paniculatum* L. is effective as a hepatoprotective agent in rat models. **Methods:** The experiment utilized Wistar rats randomly assigned to five groups and treated with either paracetamol (2 g/kg, p.o.) or CCl_4 (0.7 ml/kg, i.p.) to induce hepatotoxicity. Test groups received *Clerodendrum paniculatum* root extract at doses of 200 mg/kg and 400 mg/kg, while a standard group received silymarin (25 mg/kg, p.o.). Liver function was assessed through biochemical markers— ALT, AST, ALP, and total bilirubin. Histopathological analysis of liver tissues was also performed. **Results:** Rats treated with the extract showed a significant, dose-dependent decrease in ALT, AST, ALP, and total bilirubin levels compared to toxic controls. The 400 mg/kg dose exhibited effects nearly comparable to silymarin. Histology confirmed reduced necrosis, inflammation, and fatty changes in liver tissue, indicating structural recovery. Preliminary phytochemical analysis revealed the presence of flavonoids, alkaloids, and tannins, supporting the extract's antioxidant potential. **Conclusion:** Our results suggest that *Clerodendrum paniculatum* L. root extract offers substantial protection against liver toxicity, making it an effective natural agent against liver injury.

KEYWORDS: *Clerodendrum paniculatum*, hepatoprotective, paracetamol, Carbon tetrachloride, silymarin.

INTRODUCTION

Liver disease refers to many different diseases, infections and other disorders that affect the cells, tissues, structures and functions of the liver, and liver diseases are seen as a serious health problem worldwide. Therefore, it is critical to develop new compounds that may either prevent or treat liver diseases. The realisation of this fact has prompted research on natural and/or synthetic compounds that may have hepatoprotective effects.[1]

As our largest solid organ and gland, the liver plays an essential role in maintaining health and well-being. Many vital functions are performed by the liver including: production of biliary secretions; conversion of bilirubin; coordination of

different types of blood-circulating systems; digestion; detoxification; and storage of minerals and vitamins. The liver is an incredibly important organ because if a person loses all function of his or her liver, they could die within minutes.[2] Although the most commonly associated liver functions are not immunological in nature, the liver provides other important immune features. Examples include: foetal liver haematopoiesis; strong innate immunity; low adaptive immune response, e.g., compared with over-aggressive autoimmune responses; and the establishment of immune tolerance.[3]

The human body treats nearly all drugs/medications as foreign substances (normally, known as xenobiotics) and utilizes a variety of chemical processes (metabolism) in order to modify these substances into a form which can be excreted from the body. There are also many types of chemical modifications that occur during metabolism, including: (a) changing a substance's biological activity and (b) decreasing the fat solubility of a substance. The primary location of the metabolism of drugs and medications is in the smooth endoplasmic reticulum of the liver; however, almost all organs within the body can metabolize drugs/medications at some level. Because the liver performs such an important role in removing/modifying drugs and other foreign substances, it is particularly susceptible to drug-induced injury.[4] The process of metabolite biotransformation at a rapid rate can also cause the constant production of free radicals. The oxygen reactive species were responsible for decreased ATP levels, increased lipid peroxidation and oxidative damage to proteins and DNA, which are primarily responsible for the damage caused to the liver by most hepatotoxic drugs.[5]

Liver disease is responsible for 2 million deaths each year worldwide. The causes are as follows: 1 million deaths from complication from cirrhosis; 1 million deaths from viral hepatitis; and 1 million deaths from HCC (hepatocellular carcinoma). Liver injury is a common reason for drug withdrawals during development, during preclinical testing and following marketing.[6] Drugs account for a significant portion of the causes of liver injury. Approximately 75% of idiosyncratic drug reactions now lead to liver transplantation or death and, furthermore, over 1,000 medications, poisons and herbs have been documented to induce liver injury. In fact, acute fatty liver, cholestatic jaundice, liver granulomas, active chronic hepatitis, cirrhosis, liver tumours and other liver diseases are linked to drug-induced liver injury and are all dependent on the amount of drug taken at the time it was taken. This supplement identifies the list of medications that can lead to drug-induced hepatotoxicity, discusses how they affect the liver and outlines some of the signs and symptoms that may occur when taking medication that can damage the liver.[7]

A condition referred to as "Herb-Induced Liver Injury" (HILI) refers to liver injury caused by consumption of herbal products, which may occur infrequently among select populations vulnerable to developing this syndrome. Clinical presentations for both DILI and HILI are virtually identical. Both forms of liver injurious reactions can be attributed to the presence of either synthetic or naturally occurring chemicals that produce harmful metabolites during metabolization of ingested substances into non-toxic metabolic byproducts. Furthermore, there is evidence to support that metabolization of synthetic and naturally occurring chemicals into non-toxic metabolic by-products creates an opportunity for kidney and/or liver damage to occur in at-risk populations. Most cases of HILI will resolve without treatment; however, there are reports of patients experiencing liver transplantation, acute liver failure (ALF), chronic liver injury, or death resulting from their use of herbal medicines.[8]

Hepatic disorders continue to increase despite advancements in the fields of medical treatment and the introduction of new hepatoprotective drugs. Protecting the liver from hepatotoxic substances or reversing the changes caused by them on the body’s antioxidant defences is essential; substances that protect the liver from these harmful compounds are

termed hepatoprotective agents.[5] Currently, despite many pharmaceutical products being researched, no pharmaceutical product exists that protects your liver from harm. Instead of being a pharmaceutical product, herbal remedies are widely available, more affordable, culturally acceptable, more closely assimilated into the body, and produce fewer negative side effects.[1] Liver injury is aggravated by free radicals and oxidative stress, both of which may be neutralized by the antioxidant system. One of the best sources of antioxidants is through the use of plant extracts, which may also provide hepatoprotective activity.[9] Thus, extensive research has been carried out to find out natural/synthetic substrates with hepatoprotective properties.[5] Historically, herbal medicines have been used for thousands of years in India — from the time of Ayurvedic medicine, through the practice of alternative medicine practices in China to Europe; this exemplifies the strong association between plants and medicine. Many hepatoprotective drugs are derived from plants. A conservative estimate indicates that there are approximately 700 different mono- and poly-herbal preparations available for treating numerous medical ailments associated with the liver, such as syrups, powders, tablets, tinctures, and decoctions.[4]

The purpose of hepatoprotective models is to assist the compounds, fractions, or extracts being tested in preventing or reversing the damage that hepatotoxins produce. The degree of the hepatoprotective effect can be measured using liver histology, survival rate, or biochemical markers.[5] A rat model of high-dose paracetamol-induced hepatotoxicity is commonly used to study the biochemical and histological features of liver injury and potential preventative medications.[9] When considering the evaluation of liver function with liver tests, usually the following tests are discussed: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP), Gamma-Glutamyl Transferase (GGT), 5'nucleotidase, Total Bilirubin, Conjugated (direct) Bilirubin, Unconjugated (indirect) Bilirubin, Prothrombin time (PT), International Normalised Ratio (INR), Lactate Dehydrogenase, Total Protein (TP), Globulins and Albumin. These tests will assist in determining the region (location) of the hepatic injury and the differential diagnosis can also be sorted based on the elevation pattern observed. If there is an elevation in bilirubin, ALT, and AST out of proportion to ALP, then this would indicate a hepatocellular disease. If a pattern is seen where there is an increase in ALP and bilirubin out of proportion to the AST and ALT, then this would indicate a cholestatic pattern. An increase in AST/ALT and alkaline phosphatase will be referred to as a mixed damage pattern. An isolated hyperbilirubinemia is when there is an increase in bilirubin with normal AST/ALT and alkaline phosphatase.[10]

Need of the Study

The liver is a central hub for many essential body functions. It helps regulate fats and cholesterol, supports the immune system, manages blood volume, and influences growth-related hormones. One of its biggest jobs is handling macronutrients—processing and distributing carbs, fats, and proteins to fuel the body. It stores glucose as glycogen after meals and can make new glucose during fasting. The liver also breaks down fats or packages them for storage elsewhere. And because it processes amino acids, removes nitrogen waste through urea production, and produces most blood proteins, it plays a major role in protein and amino-acid metabolism.[11] The liver has a large supply of blood and is the primary site in the body where most materials go through metabolism. Because of these two factors, it is easily affected by several different types of pathogens (bacteria, viruses, and parasites) located outside and within the body. This susceptibility can lead to liver injury and/or illness.[12]

More than two million people die from liver diseases each year—about 4% of all deaths worldwide, or 1 in every 25. Liver cancer alone accounts for an estimated 600,000 to 900,000 of those deaths. Although liver disease ranks as the

11th leading cause of death, experts believe the true number may be higher. Cirrhosis also contributes heavily to global illness, ranking 15th in disability-adjusted life years (DALYs). Liver disease hits younger adults especially hard, ranking as the 12th leading cause of DALYs in people aged 25 to 49.[13]

Liver diseases have many causes, including viral infections, immune problems, cancer, alcohol misuse, and drug overdose.[14] While viral infections often lead to acute liver problems, long-term conditions are more commonly linked to hepatitis B or C, heavy alcohol use, and the growing global burden of metabolic dysfunction–associated steatotic liver disease (MASLD). When these chronic conditions progress to cirrhosis or liver cancer, the risks of illness and death rise sharply.[15] Whether triggered by chemicals or infections, ongoing liver injury can lead to fibrosis, which may eventually progress to cirrhosis and liver failure.[16]

Despite significant advancements in modern medicine, there are still no reliable medications that can fully enhance liver function, provide complete protection, or stimulate liver cell regeneration. Although some drugs, such as steroids and antivirals, as well as immunization, are available to manage and prevent liver diseases, they are often expensive and associated with undesirable side effects. Furthermore, despite the persistent occurrence, high morbidity, and mortality of hepatic disorders, current medical prevention remains inadequate, and no therapy has yet successfully halted disease progression. This highlights the urgent need for innovative, suitable alternatives—particularly classical or herbal medicines—that could replace synthetic drugs.[16,17] There is strong evidence that medicinal plants and their natural compounds can help treat and manage liver diseases.[17] For centuries, nature has provided a rich source of healing substances, with many plants producing useful phytochemicals. These plant-based compounds play important roles in treatments for a wide range of conditions, including arthritis, cancer, neurodegenerative and heart diseases, and skin disorders. In fact, most modern medicines originally came from medicinal plants, highlighting how closely human health is tied to plant biodiversity.[18] Medicinal plants demonstrate liver-protective effects through multiple mechanisms. These include strengthening antioxidant defenses by enhancing the activity of enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, reducing lipid peroxidation, and reversing liver fibrosis by upregulating matrix metalloproteinase expression and removing collagen deposits, while simultaneously suppressing the activation of hepatic stellate cells. Additionally, they exhibit anti-inflammatory effects by modulating various inflammatory pathways, possess antifibrotic properties, and stimulate the degradation of the extracellular matrix.[19] Studies on whole plant extracts and isolated compounds have shown many positive liver effects. The most researched plant families include Asteraceae, Lamiaceae, Fabaceae, Apiaceae, Araliaceae, Ericaceae, and Zingiberaceae.[18] It has been demonstrated that more than 160 phytoconstituents from 101 plants have liver-protective qualities.[20]

Criteria for Selection of Plant

The use of herbs to treat liver diseases has been a practice for centuries. Studies, including both animal and human studies, have proven that herbs can positively affect liver tissue through several different methods including by: (1) preventing inflammation, (2) decreasing peroxidation, (3) preventing/cutting collagen buildup and having antifibrotic effects, and (4) scavenging free radicals and having antioxidant effects. The investigation of the mechanism(s) and mode(s) of action(s) of various plants and the confirmation of the medicinal effects of some herbs or plant extracts through clinical studies has validated the experiences and knowledge passed down from earlier generations. The truth is that how plants help in liver damage is not dependent upon the cause; thus, plants improve many types of liver injury, such as acute liver injuries (developed acutely), chronic liver inflammation (developed over a period of time), fibrosis, steatosis, etc., regardless of the underlying cause.[21]

Clerodendrum is a genus of approximately 500 species within the Verbenaceae or Lamiaceae family, consisting of small trees and herbs. According to various studies, *Clerodendrum* species exhibit anti-inflammatory, anti-diabetic, anti-cancer, anti-malarial, and other properties. The main constituents of *Clerodendrum* include flavonoids, phenolics, terpenes, steroids, volatile substances, and several other types.[22] *Clerodendrum paniculatum* is one of the most important species of *Clerodendrum* L., which is grown in various Asian countries. The ethnomedical significance of *Clerodendrum paniculatum* is that it is used to treat a variety of ailments, including wounds, typhoid fevers, snakebites, jaundice, dizziness, malaria, anaemia and pile diseases. Researchers have isolated over 30 phytochemicals from the parts of this plant, many of which are known to possess antibacterial, antioxidant anthelmintic, anti-inflammatory, antimutagenic, cytotoxic, hypolipidemic, insecticidal and anti-ageing properties.[23]

It has been demonstrated that *Clerodendrum paniculatum* has anti-oxidant, anti-inflammatory, hepatoprotective, and anti-diabetic qualities in its leaves, roots, and other parts. Phytochemical examination of the floral extract identified proteins, steroids, flavonoids, phenolics, carbohydrates, and tannins.[22] Chemicals such as β-amyrin, β-sitosterol, and (24s)ethylcholesta–5,22,25-triene–3β–ol were extracted from *Clerodendrum paniculatum* roots. The standard phytochemical investigation of the leaves of *Clerodendrum paniculatum* revealed the presence of alkaloids, coumarins, flavonoids, glycosides, phenolic compounds, phytosterols, saponins and terpenoids.[24]

Since there are insufficient studies evaluating the hepatoprotective activity of the root of the *Clerodendrum paniculatum* plant, this is the purpose of this paper.

Objectives of the Study

The objectives of this study are as follows:

1. Collection and authentication of the roots of *Clerodendrum paniculatum*
2. Extraction of the roots of *Clerodendrum paniculatum*
3. Preliminary phytochemical study
4. Study the potential protective effect of the root extract of *Clerodendrum paniculatum* on the liver using following animal models:

a) Paracetamol induced liver toxicity.
b) Carbon tetrachloride induced liver toxicity.

5. Biochemical estimation : Blood collection from the animals for

- Aspartate aminotransferase (AST)
- Alanine aminotransferase (ALT)
- Alkaline phosphatase (ALP)
- Total bilirubin

6. Assess histopathological examination of liver

MATERIALS AND METHODS

Experimental animals

The study included both male and female Wistar rats weighing from 150-250 g. These animals were placed in several groups and individually housed in standard polyacrylic cages (38 × 23 × 10 cm) with a maximum of three animals per cage under uniform environmental conditions including air temperature (22 ± 2°C), relative humidity (55 ± 5%), and a

standard dark-light cycle (12/12 hours). The rats had unrestricted access to food and water for the entire duration of this experiment. All the rats were allowed to become accustomed to their new housing conditions for a 10-day period prior to the commencement of testing.[25] The usage of animals in this study has the approval of the Committee for Control and Supervision of Experiments on Animals, and was carried out in compliance with the Institutional Animal Ethics Approval, no. SCP/IAEC/JUL/2024-249.

Collection of plant material and preparation of extract

The collected roots of *Clerodendrum paniculatum* were dried in the shade, and the resulting product was then ground into a coarse powder (250 g) before being extracted using 95% ethanol. This extraction process involved a thorough soaking of the powdered plant material in the ethanol, followed by filtering through Whatman No. 4 filter paper, and then evaporating off the solvent. The final weight of the extract was recorded and kept in a refrigerator to prevent degradation of the active constituents.[26]

Preliminary phytochemical screening:[27,28]

The extract of *Clerodendrum paniculatum* root was subjected to phytochemical screening for the detection of alkaloids, glycosides, tannins, proteins, carbohydrates, flavonoids, steroids, saponins, etc.

I. Tests for Alkaloids

0.5 g of the extract was dissolved in 10 ml of dilute hydrochloric acid and then filtered. The resulting filtrate was used for the following tests:

Mayer's test

To one ml of filtrate, few ml of Mayer's reagent was added in a test tube. Formation of yellow cream precipitate indicates the presence of alkaloids.

Wagner's test

One ml of the filtrate was treated with a few drops of Wagner's reagent. A reddish-brown precipitate confirms the presence of alkaloids.

Hager's test:

One ml of filtrate was treated with few drops of Hager's reagent. Formation of yellow precipitate indicates the presence of alkaloids.

Dragendorff's test

One ml of filtrate was treated with few drops of Dragendorff's reagent. Formation of reddish-brown precipitate indicates the presence of alkaloids.

II. Tests for glycosides

Bromine water test:

The extract was dissolved in bromine water. The formation of a yellow precipitate indicates the presence of glycosides.

Keller-Killiani test:

0.5 g of the dried extract was dissolved in 2 ml of glacial acetic acid containing a drop of ferric chloride solution. Concentrated sulfuric acid (1 ml) was carefully added beneath the mixture to form two layers. A reddish-brown lower layer and a bluish-green upper layer indicate the presence of glycosides.

III. Tests for Tannins

Extract was treated with vanillin hydrochloride reagent. Formation of purplish red colour indicates the presence of

tannins.

Gelatin test:

Extract was treated with gelatin solution. Formation of white precipitate indicates the presence of tannins.

IV. Tests for Proteins

Biuret test:

To 1ml extract, 5-8 drops of 10% w/v copper sulphate solution was added and heated. Formation of violet red colour indicates the presence of proteins.

Millon's test:

To 1ml of extract, added 5-6 drops of Millon's reagent. Formation of white precipitate which turns to red on heating indicates the presence of proteins.

V. Tests for Carbohydrates

Extracts were dissolved individually in 5ml of distilled water and filtered. The filtrates were used to test the presence of carbohydrates.

Molisch's Test:

Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube and 2ml concentrated sulphuric acid was added carefully along the sides of the test tubes. Formation of violet ring at the junction indicates the presence of carbohydrates.

Benedict's test:

Filtrates were treated with Benedict's reagent and heated on water bath. Formation of an orange red precipitate indicated the presence of reducing sugars.

Fehling's Test:

Filtrates were hydrolyzed with dilute hydrochloric acids, neutralized with alkali and heated with Fehling's A and B solutions. Formation of red precipitate indicates the presence of reducing sugars.

VI. Tests for Flavonoids

Shinoda test:

A little quantity of extract was dissolved in alcohol with few fragments of Mg turnings and con: HCl drop wise. Formation of pink or crimson-red colour indicates the presence of flavonoids.

Ferric chloride test:

Extract was treated with ferric chloride solution. Formation of Intense green to black colour indicates the presence of flavonoids.

VII. Tests for Steroids

Salkowski reaction:

2mg of dry extract was shaken with $CHCl_3$, to the $CHCl_3$ layer, H_2SO_4 was added slowly by the sides of test tube. Formation of red colour indicates the presence of steroids.

Lieberman Burchard's test:

2mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1ml of conc. H_2SO_4. Formation of red violet or green colour indicates the presence of Steroids.

Evaluation of Hepatoprotective Activity

A. Paracetamol Induced Liver Toxicity in Rats[29]

Wistar rats of either sex was randomly assigned into five groups of six animals each. The different groups were assigned as follows:

Group I: Normal control (vehicle 1ml/kg; p.o)

Group II: Toxic control (Vehicle 1ml/kg; p.o. + Paracetamol 2g/kg, p.o. on 9th day)

Group III: Standard group (Silymarin 25 mg/kg, p.o + Paracetamol 2g/kg, p.o. on 9th day)

Group IV: Test group (*Clerodendrum paniculatum* root extract 200mg/kg, p.o. + Paracetamol 2g/kg, p.o. on 9th day)

Group V: Test group (*Clerodendrum paniculatum* root extract 400mg/kg, p.o. + Paracetamol 2g/kg, p.o. on 9th day)

Procedure

Clerodendrum paniculatum root extract and other drug preparations were suspended in an appropriate solvent for oral administration. Throughout the course of treatment, all medications were administered orally once every day. On the ninth day, oral paracetamol (2 g/kg body weight) intoxicated all groups except group I.

Evaluation

On day 11, blood was drawn via heart puncture while under mild anaesthesia. The serum was then separated by centrifugation at 2500 rpm for 20 minutes in order to analyse different biochemical markers. The liver was removed from the animals after they were sacrificed by overdosing on ketamine anaesthesia. A portion of the liver was put in a 10% formalin solution for histological examination.

Biochemical Estimation

AST, ALT, ALP, and total bilirubin concentration were estimated from the separated blood serum samples using commercially available diagnostic kits.

Histopathological Studies

10% formalin was used to preserve the isolated liver. A microtome was used to take the sections. Haematoxylin and eosin staining was used to assess the various histopathological indicators.

B. Carbon Tetrachloride (CCl_4) Induced Liver Toxicity[29]

Wistar rats of either sex was randomly divided into five groups of six animals each. The different groups were assigned as follows:

Group I: Normal control (vehicle 1ml/kg, p.o.)

Group- I Normal control (animals shared with paracetamol-induced model)

Group II: Toxic control (Vehicle 1ml/kg, p.o. + CCl_4/olive oil 1:1 v/v 0.7ml/kg, i.p.)

Group III: Standard group (Silymarin 25 mg/kg, p.o + CCl_4/olive oil 1:1 v/v 0.7ml/kg, i.p.)

Group IV: Test group (*Clerodendrum paniculatum* root extract 200mg/kg, p.o. + CCl_4/olive oil 1:1 v/v 0.7ml/kg, i.p.)

Group V: Test group (*Clerodendrum paniculatum* root extract 400mg/kg, p.o. + CCl_4/olive oil 1:1 v/v 0.7ml/kg, i.p.)

Procedure

For seven days, all treatments were administered orally once a day. On the second, fourth, and sixth days of treatment, CCl_4 (1:1 of CCl_4 in olive oil 0.7ml/kg, i.p.) was intoxicated to all animals except group I.

Evaluation

On day 11, blood was drawn via heart puncture while under mild anaesthesia. The serum was then separated by centrifugation at 2500 rpm for 20 minutes in order to analyse different biochemical markers. The liver was removed from the animals after they were sacrificed by overdosing on ketamine anaesthesia. A portion of the liver was put in a 10% formalin solution for histological examination.

Biochemical Estimation

AST, ALT, ALP, and total bilirubin concentration were estimated from the separated blood serum samples using commercially available diagnostic kits.

Histopathological Studies

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Methods For Estimation of Biomarkers:[30]

Using a semi-auto analyser, the biochemical parameters were estimated in accordance with the standard procedure specified by the manufacturer's instruction manual included in the kit.

1. Estimation of ALP

Principle: Serum alkaline phosphatase (ALP) catalyzes the breakdown of p-nitrophenyl phosphate into p-nitrophenol and phosphate in the presence of magnesium ions (Mg^{2+}). The amount of p-nitrophenol formed is measured by its absorbance, which directly reflects the ALP activity.

Reaction

ALP, Mg^{2+} P-Nitro phenyl phosphate + H_2O $\longrightarrow$ p-nitrophenol + Phosphate

ALP: Alkaline phosphate

The formation of 4-nitrophenol causes an increase in absorbance, which is measured photometrically. This change in absorbance is directly proportional to the ALP activity in the sample.

Procedure

Pipette	Sample
Working reagent	1000 µl
Sample	20 µl

Mix and incubate for 1 min at 37°C. Read the change in absorbance per 20 sec, during 1 min.

2. Estimation of Alanine Transaminase (U.V. kinetic) (ALT)

Principle: Alanine aminotransferase (ALT) catalyzes the transfer of an amino group from alanine to α-ketoglutarate (α-KG), producing glutamate and pyruvate. The pyruvate is then reduced to lactate by lactate dehydrogenase (LDH), during which an equivalent amount of NADH is oxidized to NAD^+.

Reactions

$$\text{L-Alanine} + \alpha\text{-keto glutarate} \xrightarrow{\text{GPT}} \text{Pyruvate} + \text{L-glutamate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{Lactate} + NAD^+$$

Procedure

Pipette	Sample
Working reagent	1000 µl
Sample	100 µl

Mix and incubate for 1 min at 37°C read the change in absorbance per 20 sec during 1min using semi auto analyzer.

3. Estimation of Aspartate Amino Transaminase (U.V. kinetic) (AST)

Principle: Aspartate aminotransferase (AST) catalyzes the transfer of an amino group from L-aspartate to α-ketoglutarate, producing oxaloacetate and L-glutamate. The oxaloacetate is then converted to malate by malate dehydrogenase (MDH) in the presence of NADH. The reaction is monitored by measuring the decrease in absorbance at 340 nm, which reflects the oxidation of NADH and is directly proportional to AST activity.

Reactions

$$\text{L- Aspartate + α- Ketoglutarate} \longrightarrow \text{Oxaloacetate + L glutamate}$$

$$\text{Oxaloacetate + NADH + } H^{+} \xrightarrow{\text{MDH}} \text{L- Malate + } NAD^{+}$$

Procedure

Pipette	Sample
Working reagent	1000 µl
Sample	100 µl

Mix and incubate for 1 min at 37°C read the change in absorbance per 20 sec, (ΔOD/20sec) during 1 min using semi auto analyzer.

4. Estimation of Serum Bilirubin

In an acidic environment, bilirubin reacts with diazotized sulfanilic acid to form azobilirubin, a pink-colored compound whose absorbance is directly proportional to the bilirubin concentration. In this acidic medium, water-soluble direct bilirubin reacts readily. However, indirect or unconjugated bilirubin must first be solubilized with a surfactant before it can react in the same way as direct bilirubin.

Procedure

	Total bilirubin		**Direct bilirubin**	
	Sample blank	Test	Sample blank	Test
Total bilirubin reagent	1000 µL	1000 µL		
Direct bilirubin reagent			1000 µL	1000 µL
Activator total / direct	50 µL	20 µL		20 µL
Serum		50 µL	50µL	50 µL

Histopathological Studies

The livers were removed from the animals and preserved in 10% formalin. Histopathological analysis of the liver samples was then conducted at Nandikur Clinical Lab in Balmatta, Mangalore.

Statistical Analysis

All data are presented as mean ± SEM. Differences between groups were analyzed using one-way ANOVA, followed by Dunnett's test for multiple comparisons to determine statistical significance.

RESULTS

Extraction of Plant Material

The percentage yield of root extract of *Clerodendrum paniculatum* was found to be 4% as shown in Table 1.

Preliminary Phytochemical Screening

The following phytochemicals were found in the extract displayed in Table 2 according to preliminary phytochemical analysis: carbohydrates, alkaloids, flavonoids, phenolic compounds, tannins, steroids, proteins and amino acids, and terpenoids.

Pharmacological Activities

A. Hepatoprotective Effects of *Clerodendrum paniculatum* Root Extract Against Paracetamol Induced Liver Damage in Rats

Paracetamol administration in rats led to significant liver damage, reflected by changes in serum biochemical markers. However, pre-treatment with the root extract of *Clerodendrum paniculatum* provided substantial protection against this paracetamol-induced hepatotoxicity (as shown in Table 3). The protective effects of the extract were comparable to those observed with the standard treatment, Silymarin.

Biochemical parameters

The effect of the root extract of *Clerodendrum paniculatum* on various biochemical parameters are shown in Table No.3 and Figure No. 1 and 2.

Group I (Control)

The measured levels of ALP, AST, ALT, and total bilirubin were 105.70 ± 2.26, 30.98 ± 1.50, 33.83 ± 2.22, and 0.46 ± 0.03, respectively. Histopathological examination revealed a normal liver structure, with intact portal triads, well-organized sinusoids, and properly arranged hepatocyte cords (Fig. 3A).

Group II

In the paracetamol-treated group, levels of ALP, AST, ALT, and total bilirubin increased significantly to 249.31 ± 2.19, 191.50 ± 1.44, 155.08 ± 1.52, and 1.79 ± 0.17, respectively, compared to the control group. Histopathological analysis revealed inflammation in the portal tracts (Fig. 3B).

Group III

Treatment with Silymarin (25 mg/kg) resulted in a significant ($p < 0.001$) reduction in ALP, AST, ALT, and total bilirubin levels to 110.21 ± 1.68, 34.63 ± 1.23, 38.94 ± 1.60, and 0.51 ± 0.09, respectively. Histopathological examination showed hepatocytes that appeared nearly normal (Fig. 3C).

Group IV

Treatment with 200 mg/kg of *Clerodendrum paniculatum* root extract led to a significant ($p < 0.05$) reduction in ALP, AST, ALT, and total bilirubin levels, which were measured at 133.83 ± 2.29, 48.63 ± 1.57, 50.03 ± 2.25, and 0.66 ± 0.10, respectively. Histopathological analysis showed mild inflammation in the portal tracts (Fig. 3D).

Group V

Treatment with 400 mg/kg of *Clerodendrum paniculatum* root extract resulted in a significant ($p < 0.01$) reduction in ALP, AST, ALT, and total bilirubin levels to 121.50 ± 2.3, 39.53 ± 1.94, 42.73 ± 1.43, and 0.57 ± 0.09, respectively. Histopathological examination revealed only minimal inflammation in the portal tracts (Fig. 3E).

Histopathological studies of the liver

Histopathological evaluation of all groups following paracetamol (PCM) toxicity is shown in Fig. 3. Liver sections from the normal group displayed intact liver architecture, with most hepatocytes appearing healthy. In contrast, the toxic control group showed inflammation, centrilobular degeneration, and necrosis. Treatment with *Clerodendrum paniculatum* root extract (200 mg/kg and 400 mg/kg) markedly reduced inflammation, centrilobular damage, and bridging necrosis. Liver sections from these treated groups showed largely normal hepatocytes with significantly smaller areas of necrosis compared to the toxic group, demonstrating the protective effect of the extract against PCM-induced liver damage.

B. Hepatoprotective Effects of *Clerodendrum paniculatum* Root Extract Against Carbon Tetrachloride (CCl_4) Induced Liver Damage in Rats

CCl_4 administration in rats led to significant liver damage, reflected by changes in serum biochemical markers. However, pre-treatment with the root extract of *Clerodendrum paniculatum* provided substantial protection against this CCl_4-induced hepatotoxicity (as shown in Table 4). The protective effects of the extract were comparable to those observed with the standard treatment, Silymarin.

Biochemical parameters

The effect of the root extract of *Clerodendrum paniculatum* on various biochemical parameters is shown in Table 4 and Fig. 4 and 5.

Group I (Control)

The measured levels of ALP, AST, ALT, and total bilirubin were 98.31 ± 1.61, 33.51 ± 1.19, 27.36 ± 1.13, and 0.44 ± 0.12, respectively. Histopathological examination revealed a normal liver structure, with intact portal triads, well-organized sinusoids, and properly arranged hepatocyte cords (Fig. 6A).

Group II

In the CCl_4-treated group, levels of ALP, AST, ALT, and total bilirubin increased significantly to 239.15 ± 2.19, 105.68 ± 1.62, 99.20 ± 0.16 and 1.28 ± 0.16, respectively, compared to the control group. Histopathological analysis revealed inflammation in the portal tracts (Fig. 6B).

Group III

Treatment with Silymarin (25 mg/kg) resulted in a significant ($p < 0.001$) reduction in ALP, AST, ALT, and total bilirubin levels to 107.51 ± 2.40, 39.88 ± 1.13, 31.46 ± 2.2 and 0.47 ± 0.03, respectively. Histopathological examination showed hepatocytes that appeared nearly normal (Fig. 6C).

Group IV

Treatment with 200 mg/kg of *Clerodendrum paniculatum* root extract led to a significant ($p < 0.05$) reduction in ALP, AST, ALT, and total bilirubin levels, which were measured at 122.60 ± 2.26, 52.73 ± 2.2, 49.01 ± 1.26 and 0.64 ± 0.21,

respectively. Histopathological analysis showed mild inflammation in the portal tracts (Fig. 6D).

Group V

Treatment with 400 mg/kg of *Clerodendrum paniculatum* root extract resulted in a significant ($p < 0.01$) reduction in ALP, AST, ALT, and total bilirubin levels to 16.75 ± 1.41, 44.91 ± 1.57, 36.83 ± 1.37 and 0.53 ± 0.03, respectively. Histopathological examination revealed only minimal inflammation in the portal tracts (Fig. 6E).

Histopathological studies of the liver

Histopathological evaluation of all groups following CCl_4 toxicity is shown in Fig. 6. Liver sections from the normal group displayed intact liver architecture, with most hepatocytes appearing healthy. In contrast, the toxic control group showed inflammation, centrilobular degeneration, and necrosis. Treatment with *Clerodendrum paniculatum* root extract (200 mg/kg and 400 mg/kg) markedly reduced inflammation, centrilobular damage, and bridging necrosis. Liver sections from these treated groups showed largely normal hepatocytes with significantly smaller areas of necrosis compared to the toxic group, demonstrating the protective effect of the extract against CCl_4-induced liver damage.

Table 1: Percentage Yield of Crude Extract of *Clerodendrum Paniculatum* Root.

Solvent	Extraction method	Colour	Percentage yield
Ethanol	Maceration	Black	4% (w/w)

Table 2: Preliminary Phytochemical Screening of Extract Of *Clerodendrum Paniculatum* Root.

Sl. No.	Test	Result
1	Carbohydrates	+
2	Alkaloids	+
3	Flavonoids	+
4	Phenolic compounds	+
5	Tannins	+
6	Terpenoids	+
7	Steroids	+
8	Proteins and Amino acids	+
9	Saponins	-

(+ = Present in test, - = Absence in test)

Table 3: Effect of *Clerodendrum Paniculatum* Root Extract on Ast, Alt, Alp And Total Bilirubin In Pcm Induced Liver Toxicity.

Groups	Treatment	ALP(U/I)	AST(U/I)	ALT(U/I)	TB (mg/dl)
Normal Control	Vehicle (1ml/kg)	105.70±2.26	30.98±1.50	33.83±2.22	0.46±0.03
Toxic Control	PCM (2g/kg)	249.31±2.19[a]	191.50±1.44[a]	155.08±1.52[a]	1.79±0.17[a]
Standard	ilymarin (25mg/kg)	110.21±1.68***	34.63±1.23***	38.94±1.60***	0.51±0.09***
Dose 1	*Clerodendrum paniculatum* root *extract* (200 mg/kg)	133.83±2.29*	48.63±1.57*	50.03±2.25*	0.66±0.10*
Dose 2	*Clerodendrum paniculatum* root *extract* (400mg/kg)	121.50±2.3**	39.53±1.94**	42.73±1.43**	0.57±0.09**
All the values are Mean± SEM, n=6. One way ANOVA followed by Dunnett‟s t test. [a] p< 0.001 when compared with vehicle treated control group. *p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.					

Table 4: Effect of *Clerodendrum Paniculatum* Root Extract on Ast, Alt, Alp & Tb In Carbon Tetrachloride (Ccl_4) Induced Liver Toxicity.

Groups	Treatment	ALP(U/I)	AST(U/I)	ALT(U/I)	TB (mg/dl)
Normal ontrol	Vehicle (1ml/kg)	98.31 ± 1.61	33.51 ± 1.19	27.36 ± 1.13	0.44 ± 0.12
Toxic Control	Carbon tetrachloride	239.15 ± 2.19[a]	105.68 ± 1.62[a]	99..20 ± 0.16[a]	1.28 ± 0.16[a]
Standard	Silymarin (25mg/kg)	107.51±2.40***	39.88±1.33***	31.46± 2.2***	0.47±0.03***
Dose 1	*Clerodendrum paniculatum* root *extract* (200 mg/kg)	122.60± 2.26*	52.73± 2.27*	49.01± 1.26*	0.64± 0.21*
Dose 2	*Clerodendrum paniculatum* root *extract* (400 mg/kg)	116.75± 1.41**	44.91± 1.57**	36.83± 1.37**	0.53±0.03**
All the values are Mean± SEM, n=6. One way ANOVA followed by a Dunnett's t test. [a]p< 0.001 when compared with vehicle treated control group. *p<0.05, **p<0.01, ***p<0.001 when compared with toxic control.					

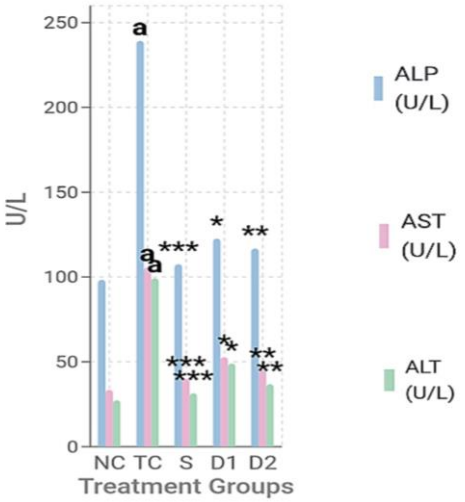


Fig. 1: Effect of *Clerodendrum paniculatum* root extract on Serum ALT, AST& ALP in PCM induced liver toxicity.

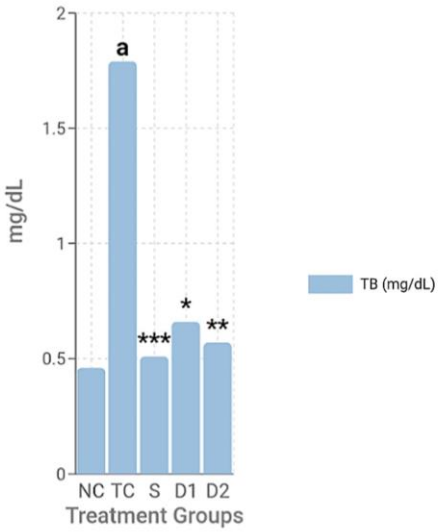


Fig. 2: Effect of *Clerodendrum paniculatum* root extract on TB in PCM induced liver toxicity.

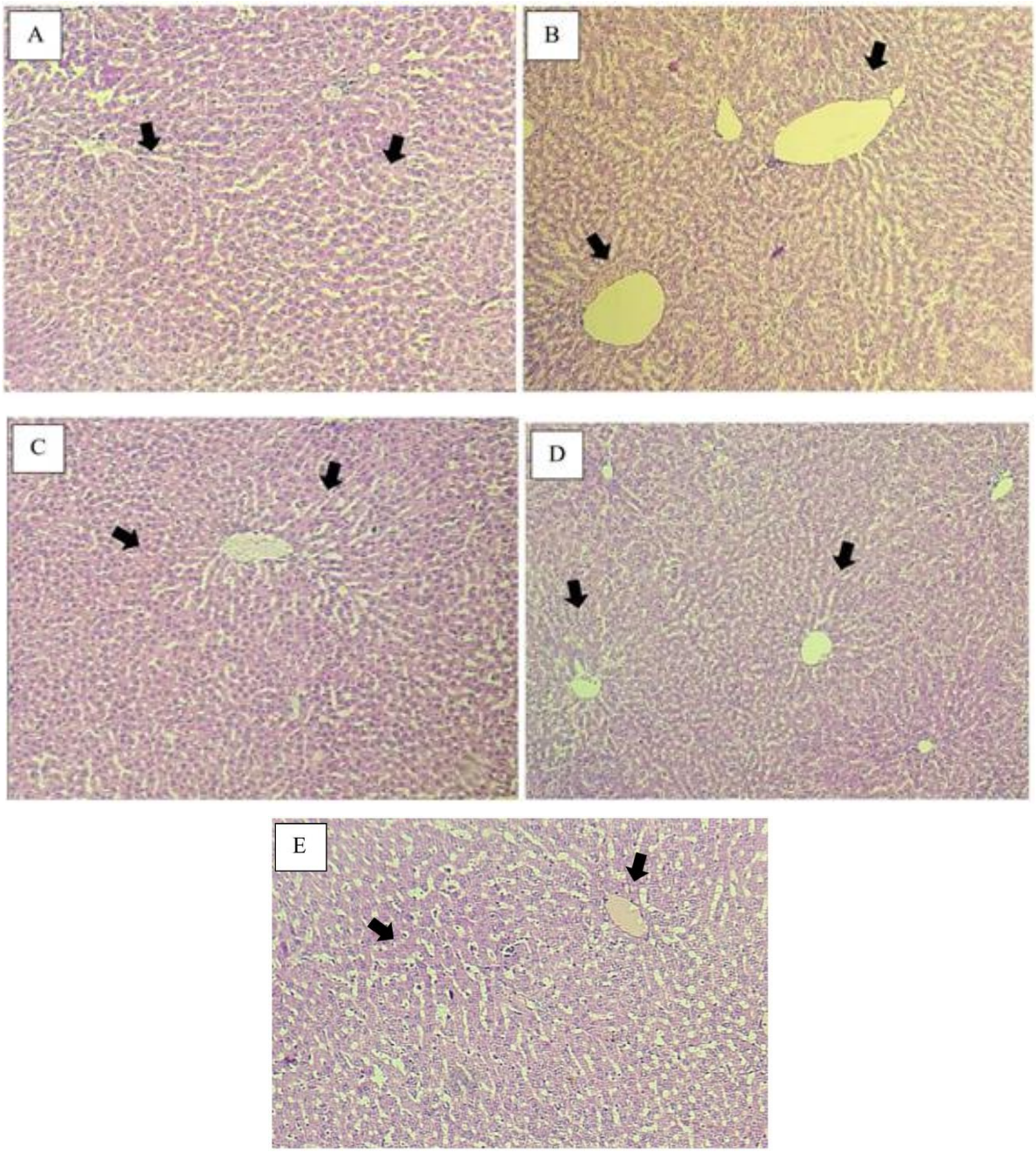


Fig. 3: Effect of *Clerodendrum paniculatum* root extract on liver histology in PCM-induced liver toxicity.

A (normal rat): Section of the liver tissue of control showing normal central vein and radiating hepatocytes.

B (PCM-induced rat): Section of the liver tissue of animal treated with PCM showing fatty degeneration, necrosis and fibrosis.

C (Silymarin-treated rat): Section of the liver tissue of animal treated with Silymarin showing normal hepatocytes with central hepatic vein.

D [*Clerodendrum paniculatum* root extract (200 mg/kg) treated rat]: Section of the liver tissue of animal treated with *Clerodendrum paniculatum* root extract showing absence of necrosis and minimal fatty change.

E [*Clerodendrum paniculatum* root extract (400 mg/kg) treated rat]: Section of the liver tissue of animal treated with extract of *Clerodendrum paniculatum* root showing absence of necrosis and moderate fatty change.

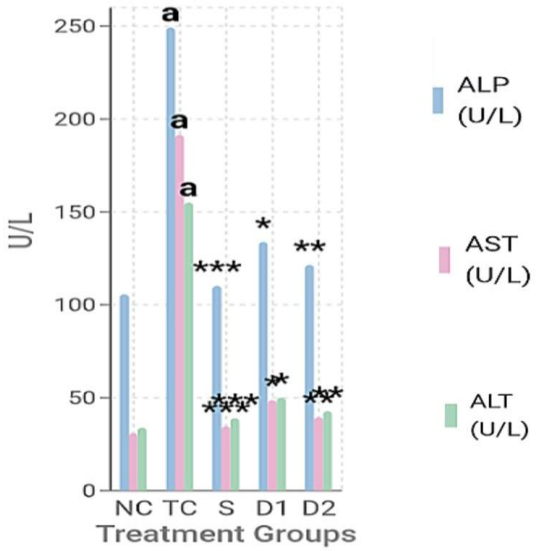


Fig. 4: Effect of *Clerodendrum paniculatum* root extract on Serum ALT, AST& ALP in CCl_4 induced liver toxicity.

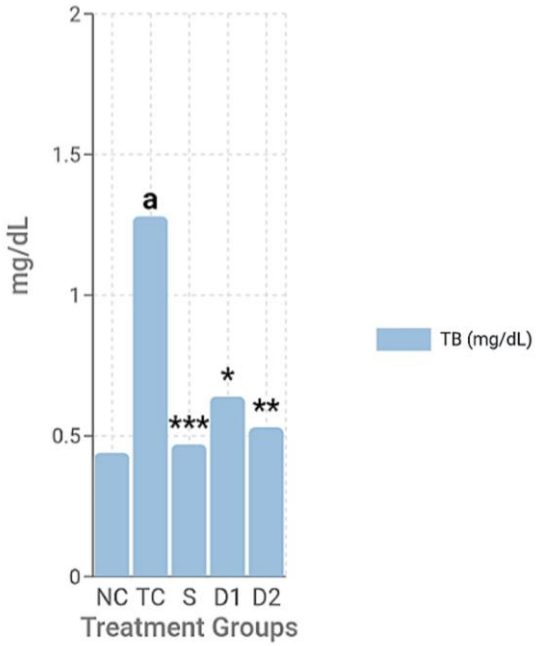
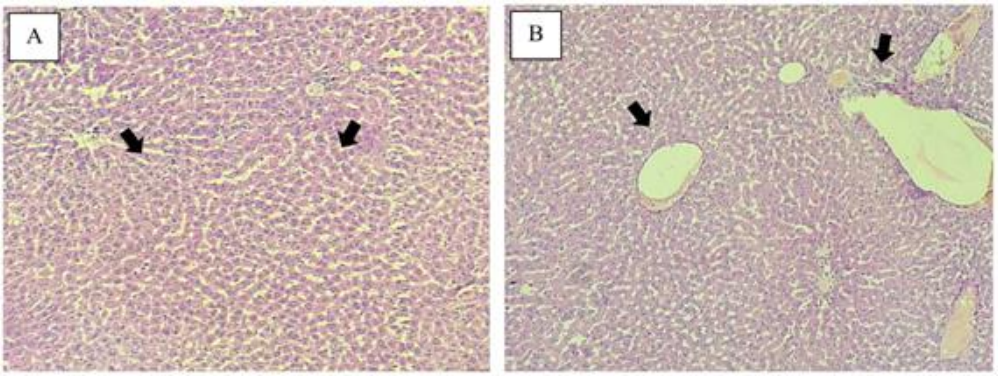


Fig. 5: Effect of *Clerodendrum paniculatum* root extract on TB in CCl_4 induced liver toxicity.



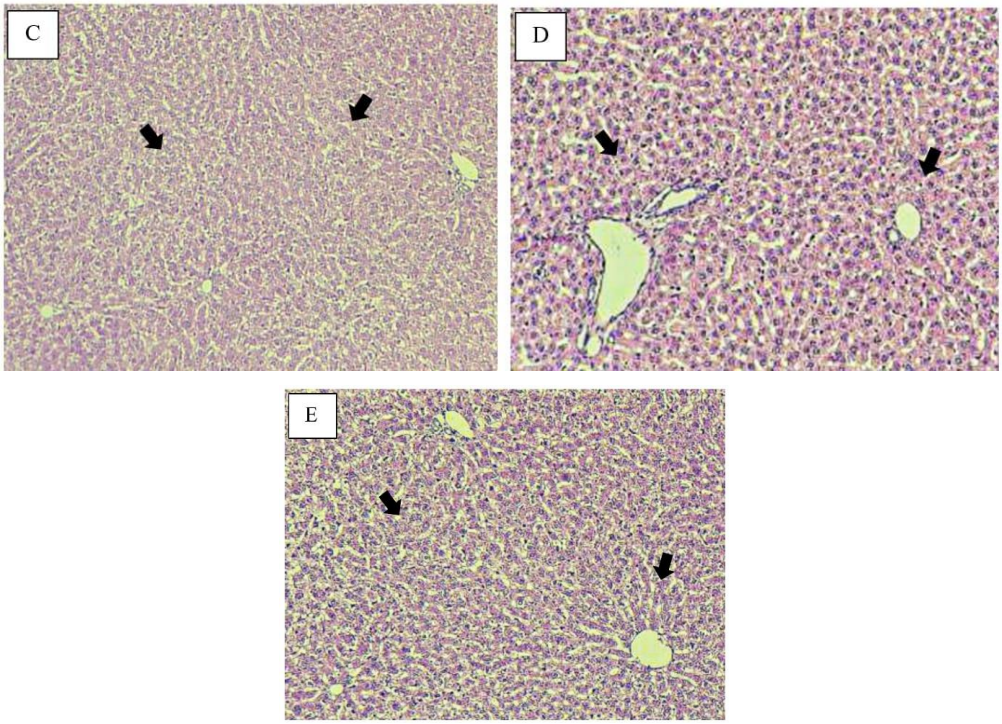


Fig. No. 6: Effect of *Clerodendrum paniculatum* root extract on liver histology in CCl_4 induced liver toxicity.

A (normal rat): Section of the liver tissue of control showing normal central vein and radiating hepatocytes.

B (CCl_4 induced rat): Section of the liver tissue of animal treated with CCl_4 showing fatty degeneration, necrosis and fibrosis.

C (Silymarin treated rat): Section of the liver tissue of animal treated with Silymarin showing normal hepatocytes with central hepatic vein.

D [*Clerodendrum paniculatum* root extract (200 mg/kg) treated rat]: Section of the liver tissue of animal treated with *Clerodendrum paniculatum* root extract showing absence of necrosis and minimal fatty change.

E [*Clerodendrum paniculatum* root extract (400 mg/kg) treated rat]: Section of the liver tissue of animal treated with extract of *Clerodendrum paniculatum* root showing absence of necrosis and moderate fatty change.

DISCUSSION

The main aim of this study was to evaluate the hepatoprotective effects of *Clerodendrum paniculatum* root extract using two experimental models: paracetamol-induced liver toxicity and carbon tetrachloride induced liver toxicity. The protective effects were assessed by measuring serum enzymes (ALT, AST, ALP, and total bilirubin) and through detailed histopathological examination of the liver.

The liver, closely connected to the gastrointestinal tract, is frequently exposed to harmful substances such as drugs, chemicals, and oxidative stress from free radicals. Because it plays a central role in metabolizing and detoxifying these compounds, the liver can end up damaging itself in the process.[31]

Many medicinal herbs have long been used in traditional medicine to manage liver disorders and other health conditions.[22] In several Asian and African countries, *Clerodendrum paniculatum* has been used to treat a variety of ailments, including tumors, leprosy, fever, asthma, hypertension, rheumatism, and microbial infections. Koppilakal et al. extracted quercetin (3,3’,4’,5,7-pentahydroxy flavone) from the flowers and demonstrated both antioxidant and

hepatoprotective effects in CCl_4-induced liver damage in male rats, while Leena and Aley Kutty isolated quercetin from the plant's roots. Pertiwi et al., using LC-MS/MS, identified multiple flavonoids in the ethyl acetate extract and also reported strong antibacterial activity in the leaf extract.[32]

Preliminary phytochemical screening of the root extract of *Clerodendrum paniculatum* revealed the presence of carbohydrates, alkaloids, flavonoids, phenolic compounds, tannins, steroids, proteins, amino acids, and terpenoids.
Phenolic compounds are well known for their antioxidant and anti-inflammatory effects. They protect cells by neutralizing free radicals, stabilizing cell membranes, and reducing the production of inflammatory cytokines like TNF-α, TGF-β, and interleukins (IL-2, IL-6, IL-8). Flavonoids, including quercetin, rutin, catechin, and apigenin, are particularly important for their strong free radical–scavenging activity and have documented anti-hepatotoxic (liver-protective) properties.[22] Quercetin's antioxidant activity comes from its ability to bind transition metal ions and neutralize free radicals. Its 3',4'-diphenolic group makes it especially effective at scavenging superoxide anions. Studies have shown that quercetin offers hepatoprotective effects in rats on high-fat diets, reducing oxidative stress caused by diabetes and hyperglycemia by modulating pathways involved in cellular damage.[32]

Acetaminophen is safe at normal doses but can cause severe liver damage if overdosed. Normally, it's metabolized in the liver and eliminated after conjugation with glucuronic acid and sulfate. A small portion is converted by liver enzymes (CYP2E1 and CYP1A2) into a reactive compound called NAPQI, which is usually neutralized by glutathione and safely removed. Overdose overwhelms this detox system, depletes glutathione, and allows NAPQI to accumulate, leading to liver injury.[33]

Carbon tetrachloride (CCl_4) causes liver damage by raising enzymes like ALT, AST, ALP, and γ-GT.[22] Its toxicity arises from free radical formation, which triggers lipid peroxidation and covalent binding to cellular components, damaging cell membranes and increasing permeability. At low oxygen levels, CCl_3• radicals disrupt lipid metabolism, leading to fatty liver, while at high oxygen, CCl_3O• radicals drive lipid peroxidation and cell death. CCl_4 also inhibits protein synthesis in hepatocytes, and its metabolite phosgene may further contribute to liver injury.[34]

Biomarkers are crucial for diagnosing and monitoring liver damage. ALT and AST indicate hepatocyte necrosis and inflammation, while elevated ALP helps detect cholestatic liver disease. Bilirubin, a byproduct of heme metabolism, gives bile, feces, and urine their color and reflects liver function. Disruptions in bilirubin metabolism can cause hyperbilirubinemia, which in severe cases may lead to jaundice, liver failure, or the need for a transplant.[35]

Silymarin is widely used as a standard hepatoprotective agent due to its proven safety and effectiveness. Its phenolic groups neutralize reactive oxygen species (ROS) generated during liver metabolism, forming stable, harmless compounds. Silymarin also modulates liver enzymes, reduces oxidative stress, and scavenges free radicals. Clinically, it supports liver repair by preventing activation of fibrogenic stellate cells and limiting proinflammatory mediator release from hepatic macrophages. Additionally, silymarin is minimally toxic and poses no risk to embryos.[36]

The study showed that *Clerodendrum paniculatum* root extract has significant hepatoprotective effects in both paracetamol- and CCl_4-induced liver toxicity models. At doses of 200 mg/kg and 400 mg/kg, the extract helped normalize liver function markers such as ALT, AST, ALP, total bilirubin, and total protein. A dose-dependent reduction in these elevated biomarkers highlighted the extract's ability to protect the liver, likely due to the antioxidant

activity of its bioactive compounds. Silymarin, used as the standard, also demonstrated strong protective effects by stabilizing liver enzymes, reducing oxidative stress, and modulating inflammation. Notably, the higher dose of the extract (400 mg/kg) showed efficacy comparable to silymarin, while the lower dose (200 mg/kg) provided moderate but significant protection.

CONCLUSION

The root extract of *Clerodendrum paniculatum* shows strong protective effects on the liver against damage caused by paracetamol and CCl_4. It helps restore key liver markers like ALT, AST, ALP, and bilirubin, with better effects at higher doses. These benefits are likely due to its antioxidant and anti-inflammatory compounds. At 400 mg/kg, its effectiveness is similar to the standard drug Silymarin, while 200 mg/kg still offers noticeable protection. Overall, the study supports the potential of *Clerodendrum paniculatum* as a natural liver-protective agent.

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