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"Preclinical Evaluation of Hepatoprotective Potential of *Codiaeum Variegatum* Plant Extract in Ccl4-Induced Liver Damage"

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Abstract

The present study aimed to evaluate the hepatoprotective potential of the methanolic extract of *Codiaeum variegatum* against carbon tetrachloride (CCl_4)-induced liver damage in Wistar rats. The plant extract was subjected to phytochemical screening, determination of total phenolic and flavonoid content, and antioxidant evaluation using the DPPH radical scavenging assay. Acute oral toxicity studies were performed according to OECD guideline 423 to assess the safety profile of the extract. Hepatoprotective activity was investigated using CCl_4-induced hepatotoxicity by evaluating serum biochemical parameters such as SGPT, SGOT, ALP, and total bilirubin along with histopathological examination of liver tissues. Phytochemical analysis revealed the presence of flavonoids, phenolics, alkaloids, glycosides, and saponins. The extract exhibited considerable antioxidant activity due to its rich phenolic and flavonoid content. Treatment with *Codiaeum variegatum* extract significantly restored altered liver enzyme levels and improved hepatic architecture in a dose-dependent manner. The higher dose (400 mg/kg) demonstrated hepatoprotective effects comparable to the standard drug silymarin. The findings suggest that *Codiaeum variegatum* possesses promising hepatoprotective activity, possibly mediated through its antioxidant and membrane-stabilizing properties.

Keywords: Codiaeum variegatum, Hepatoprotective activity, Carbon tetrachloride, Antioxidant activity, Phytochemicals, Liver toxicity

I. Introduction

The liver is a vital metabolic organ responsible for detoxification, protein synthesis, carbohydrate metabolism, bile secretion, and biotransformation of xenobiotics. Due to its central role in metabolism and exposure to toxic chemicals, the liver is highly susceptible to injury caused by drugs, alcohol, environmental pollutants, and infectious agents. Liver disorders such as hepatitis, cirrhosis, fibrosis, and hepatocellular carcinoma represent major global health concerns and are associated with significant morbidity and mortality worldwide **(Asrani *et al.,* 2019)**. Although several synthetic drugs are available for the management of hepatic diseases, their long-term use is often limited by adverse effects, high cost, and incomplete therapeutic efficacy. Therefore, the search for safer and effective hepatoprotective agents from natural sources has gained considerable scientific attention. Carbon tetrachloride (CCl_4) is one of the most widely used experimental hepatotoxins for inducing liver injury in laboratory animals. Hepatic metabolism of CCl_4 by cytochrome P450 enzymes generates reactive free radicals such as trichloromethyl (CCl_3) and trichloromethyl peroxy radicals, which initiate lipid peroxidation, oxidative stress, inflammation, and necrosis of hepatocytes **(Weber *et al.,* 2003)**. The CCl_4-induced hepatotoxicity model closely resembles human liver injury and is commonly employed for evaluating the hepatoprotective activity of medicinal plants and phytoconstituents. Medicinal plants have been used traditionally for centuries in the treatment of liver disorders because of their antioxidant, anti-inflammatory, and membrane-stabilizing properties. Among various medicinal plants, *Codiaeum variegatum* (family: Euphorbiaceae), commonly known as croton, has attracted interest due to its diverse pharmacological activities. The plant is rich in bioactive compounds including flavonoids, alkaloids, tannins, phenolics, terpenoids, and glycosides, which are known to possess strong antioxidant and cytoprotective effects **(Gupta & Gupta, 2019)**. Previous studies have reported antimicrobial, anti-inflammatory, antioxidant, and wound-healing activities of *Codiaeum variegatum* extracts, suggesting its therapeutic potential in oxidative stress-mediated disorders.Oxidative stress plays a crucial role in the pathogenesis of hepatic injury by promoting lipid peroxidation, cellular degeneration, and inflammatory responses. Natural antioxidants derived from medicinal plants can neutralize reactive oxygen species and enhance endogenous antioxidant defense systems, thereby protecting hepatic tissue from toxic insults. Considering the phytochemical richness and biological properties of Codiaeum variegatum, it may serve as a promising candidate for hepatoprotective therapy. However, limited scientific evidence is available regarding its protective effect against chemically induced liver damage. Therefore, the present study aims to evaluate the hepatoprotective potential of *Codiaeum variegatum* plant extract against CCl_4-induced liver damage using suitable preclinical experimental models. The study is expected to provide scientific validation for the traditional medicinal use of the plant and contribute toward the development of plant-based therapeutic agents for hepatic disorders.

II. Materials and methods

2.1 Plant collection

Codiaeum variegatum (250.546 g) was collected from a localized area in Bhopal. The botanical identity and authenticity of the selected traditional medicinal plant, *Codiaeum variegatum* leaves were confirmed by a qualified plant taxonomist. Authentication ensured the purity and correct identification of the plant material prior to its use in experimental or therapeutic applications **(López *et al.*, 2020)**

2.2 Extraction process

The extraction of the plant material in this study was performed using the Soxhlet apparatus, employing the continuous hot percolation method to ensure efficient recovery of phytochemicals. Initially, the Soxhlet thimble was loaded with powdered *Codiaeum variegatum* leaves. After the completion of the Soxhlet extraction, the solvent containing the extracted compounds was concentrated by evaporating the solvent under reduced pressure. The remaining crude extract was then weighed using an analytical balance. The extract yield was calculated as a percentage based on the initial dry weight of the plant material **(Joshi *et al.*, 2013).**

2.3 Phytochemical investigation

Using a thorough qualitative phytochemical analysis, the experiment was conducted to determine if certain phytoconstituents were present or absent. Medical reactions to tests were measured by the precipitate formation or the color intensity. Standard operating procedures were applied **(Goyal *et al.*, 2012, Prasad et al., 2012).**

2.4 Determination of Total Phenolic and Flavonoid Content

The total phenolic content of *Codiaeum variegatum* leaves extracts was quantified using the Folin–Ciocalteu colorimetric assay. The total phenolic content in the plant extract was expressed as milligrams of gallic acid equivalent (GAE) per gram of extract **(Babbar *et al.*, 2011).** The total flavonoid content of *Codiaeum variegatum* leaves extract was determined using the aluminum chloride colorimetric method. The total flavonoid content of the extract was then calculated from this standard curve and expressed as milligrams of Rutin equivalent per gram of dry extract (mg RE/g) **(Ghafar *et al.*, 2017).**

2.5 DPPH Radical Scavenging Assay

The antioxidant potential of *Codiaeum variegatum* leaves extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. The decrease in absorbance of DPPH in the presence of the extract, compared to the control, was used to calculate the free radical scavenging activity. This method allowed for quantitative assessment of the extract's antioxidant capacity **(Xie & Schaich, 2014).**

2.6 Acute oral toxicity

The acute oral toxicity of the methanolic extract of *Codiaeum variegatum* leaves extract was evaluated according to OECD Guideline 423 using a stepwise procedure. Groups of three animals of the same sex received oral doses of 5, 50, 300, and 2000 mg/kg body weight, with 50 mg/kg as the starting dose. Animals were observed for signs of toxicity and mortality immediately after dosing, during the first 24 hours, and daily for 14 days. Parameters assessed included behavioral changes, physical appearance, and neurological symptoms **(Bhandary *et al.*, 2013).**

2.7 In vivo study of Hepatoprotective activity in rats (Singh *et al.*, 2012)

Male Wistar rats (180 ± 35 g) were used for the hepatoprotective study following approval from the Institutional Animal Ethics Committee (IAEC). Animals were maintained under standard laboratory conditions with free access to food and

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water. The rats were randomly divided into five groups (n=6): normal control, CCl_4-induced toxic control, silymarin-treated standard group (100 mg/kg), and two treatment groups receiving methanolic extract of *Codiaeum variegatum* at doses of 200 and 400 mg/kg, respectively. All treatments were administered orally once daily for 14 days. Hepatotoxicity was induced using CCl_4, and the protective effects of the extract were assessed through biochemical and histopathological evaluations. At the end of the study, blood samples were collected through the retro-orbital plexus for serum biochemical analysis, including AST, ALT, ALP and total bilirubin levels, using an automatic clinical chemistry analyzer. The study protocol enabled comprehensive assessment of liver function and the hepatoprotective efficacy of the plant extract.

2.8 Histopathological Examination

The Liver tissue sections that had been fixed with formalin were serially dehydrated in alcohol, cleaned in xylene, and then embedded in paraffin blocks. After cutting and staining the micro sections (4-5 microns thick) with hematoxylin and eosin (H and E) according to usual procedure, the sections were checked for histopathological alterations.

III. Results and discussion

The methanolic extract of *Codiaeum variegatum* yielded 4.90%, with 12.3 g obtained from 250.546 g of plant material. This indicates that methanol is an efficient solvent for extracting a variety of bioactive compounds from the plant. The significant yield suggests that the plant contains a considerable amount of methanol-soluble phytochemicals, including phenolics and flavonoids, which are known for their antioxidant and therapeutic properties. These findings highlight the potential of *Codiaeum variegatum* as a source of biologically active compounds for further pharmacological studies.

3.1Phytochemical Test

Table 1: Phytochemical test of extract of methanol

S. No.	Experiment	Presence or absence of phytochemical test Methanol extract
1.Alkaloids		
1.1	Mayer's reagent test	Absent (- ve)
1.2	Wagner's reagent test	Present (+ ve)
1.3	Hager's reagent test	Present (+ ve)
1.4	Dragondrof test	Present (+ ve)
2.Glycoside		
2.1	Borntrager test	Present (+ ve)
2.2	Killer-Killiani test	Present (+ ve)
3.Carbohydrates		
3.1	Molish's test	Present (+ ve)
3.2	Fehling's test	Present (+ ve)
3.3	Benedict's test	Present (+ ve)
3.4	Barfoed's test	Absent (- ve)
4.Flavonoids		
4.1	Alkaline reagent test	Present (+ ve)
4.2	Lead Acetate test	Present (+ ve)
5.Tannin and Phenolic Compounds		
5.1	Ferric Chloride test	Present (+ ve)
5.2	Lead Acetate	Present (+ ve)
5.3	Gelatin test	Present (+ ve)
6.Saponin		
6.1	Foam test	Present (+ ve)
7.Test for Triterpenoids and Steroids		
7.1	Salkowski's test	Absent (-ve)
7.2	Libbermann-Burchard's test	Present (- ve)

8.Test for Protein and Amino Acid		
8.1	Biuret's Test	Present (+ ve)
8.2	Ninhydrin test	Absent (- ve)

Phytochemical analysis of the methanolic extract of *Codiaeum variegatum* revealed the presence of alkaloids, glycosides, carbohydrates, flavonoids, tannins, phenolic compounds, saponins, and proteins. Most tests for triterpenes, steroids, and amino acids were negative, indicating these compounds are either absent or present in very low amounts. The abundance of phenolic and flavonoid compounds suggests strong antioxidant potential. Alkaloids, glycosides, and saponins are known for their diverse pharmacological activities. Overall, the extract appears to be rich in bioactive compounds, supporting its potential therapeutic value. These findings justify further investigation into its medicinal and biochemical properties.

3.2 Quantitative Estimation of Phytoconstituents

3.2.1 Total Phenolic content (TPC) and Total flavonoid content TFC estimation

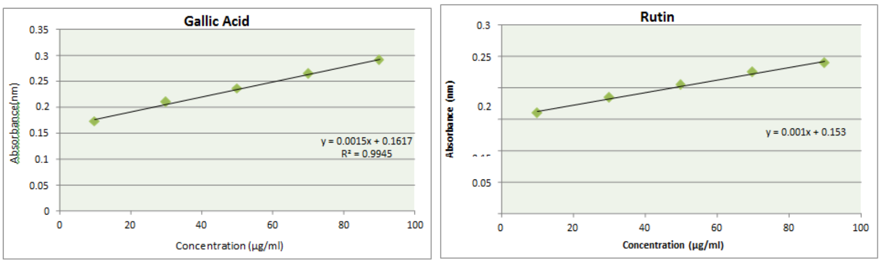


Figure 1: Represent standard curve of Gallic acid and Rutin

Table 2: Total Phenolic Content and Total Flavonoid Content in *Codiaeum variegatum* extract

S. No	Absorbance	TPC in mg/gm equivalent of Gallic Acid
1	0.179	66.3mg/gm
2	0.235	
3	0.270	
S. No	Absorbance	TFC in mg/gm equivalent of Rutin
1	0.172	43 mg/gm
2	0.192	
3	0.225	

The quantitative examination of *Codiaeum variegatum's* methanolic extract revealed a significant phenolic and flavonoid content. The total phenolic content was found to be 66.3 mg/g, expressed in gallic acid equivalents, showing a high concentration of phenolic compounds. The total flavonoid concentration was measured at 43 mg/g, expressed as rutin equivalents. These findings imply that the extract has high antioxidant potential, as phenolics and flavonoids are recognized for their free radical scavenging properties.

3.3Anti-Oxidant Activity

3.3.1 DPPH 2, 2- diphenyl-1-picryl hydrazyl Assay

Table 3: DPPH radical scavenging activity of Std. Ascorbic acid

Concentration (µg/ml)	**Absorbance**	**% Inhibition**
20	0.470	52.620
40	0.426	57.056
60	0.337	66.431
80	0.277	72.076
100	0.138	85.742

Control-0.992
IC50 - 18.69

Table 4: DPPH radical scavenging activity of methanol extract of *Codiaeum Variegatum*

Concentration (µg/ml)	Absorbance	% Inhibition
20	0.508	46.243
40	0.454	51.957
60	0.443	53.121
80	0.402	57.460
100	0.356	62. 328
Control-0.945		
IC50-37.57		

The DPPH radical scavenging assay showed that *Codiaeum variegatum's* methanolic extract had concentration-dependent antioxidant activity. At 100µg/ml, the extract inhibited 62.32% of the cells. The extract's IC_{50} value (37.57µg/ml) suggests moderate antioxidant activity compared to the standard ascorbic acid, which had a higher effect with an IC_{50} value of 18.69µg/ml. The extract's antioxidant activity may be related to its high phenolic and flavonoid content.

3.4 Acute Oral toxicity study on wistar rat (OECD 423)

The acute oral toxicity study of *Codiaeum variegatum* methanolic extract at 50 mg/kg showed no mortality, indicating it is non-lethal at this dose. Most clinical parameters, including behavior, sleep, urine, and motor activity, remained normal throughout the 14-day period. Minor transient changes, such as hair fall, flaky eyes, runny nose, and discolored stool, were observed but resolved without intervention. Body weight and food intake remained stable, suggesting no significant toxicity. Overall, the extract appears safe and well-tolerated at the tested dose.

3.5 Ccl4 induced Hepatotoxicity Model in Rats

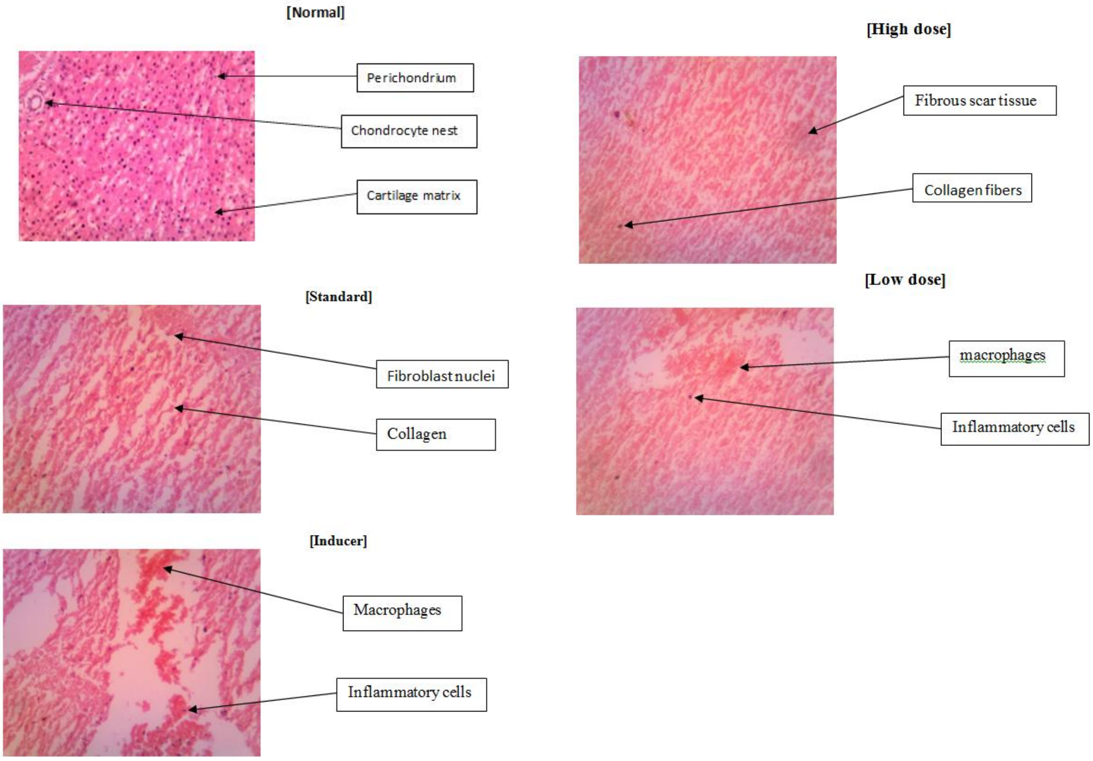
3.5.1 Analysis of General parameters

Table 5: Serum Glutamic Pyruvic Transaminase Test results

S.No.	TREATMENT GROUP	SGPT	SGOT	ALP	T. Billiuribin
1.	**Normal saline treated group**	54.60±1.30 u/l	71.61± 1.74u/l	153±7.58u/l	0.31±0.06mg/dl
2.	**Inducer group (CCl4 3 ml/kg)**	80.40±1.74 u/l	110.91±1.76 u/l	204±2.85 u/l	0.71±0.08mg/dl
3.	**Standard treatment group (Silymarin at 100 mg/kg)**	51.46±1.69 u/l	82.62± 1.74u/l	162±4.09u/l	0.39±0.03mg/dl
4.	**Low dose of *Codiaeum variegatum* extract (200 mg/kg)**	65.64±1.40 u/l	91.69± 1.87u/l	178±2.36u/l	0.50±0.04mg/dl
5.	**High dose of *Codiaeum variegatum* extract (400mg/kg)**	58.67±1.42 u/l	82.76±2.09 u/l	168±2.28u/l	0.43±0.07mg/dl

Biochemical investigation shows *Codiaeum variegatum* methanolic extract has hepatoprotective properties against CCl_4-induced liver injury. The inducer group had higher levels of SGPT, SGOT, ALP, and total bilirubin, indicating liver damage. The extract lowered these enzyme levels dramatically in a dose-dependent manner. The high dose (400 mg/kg) brought SGPT (58.67 u/l), SGOT (82.76 u/l), ALP (168 u/l), and total bilirubin (0.43 mg/dl) closer to the standard Silymarin group, demonstrating efficient liver protection. These findings imply that the extract can help restore normal liver function and minimize.

3.6 Histopathology Study



Histopathological analysis revealed that the normal control group (G-1) displayed intact tissue architecture, confirming healthy baseline tissue. The standard treatment group (G-2) maintained normal histology, indicating no adverse effects. Both the low-dose (G-3) and high-dose (G-4) *Codiaeum variegatum* extract-treated groups showed preserved tissue morphology; however, the high-dose group (G-4) exhibited the most pronounced protective effect, with tissues appearing closest to normal. In contrast, the inducer group (G-5) showed significant tissue alterations, reflecting pathological damage. These findings suggest that the methanolic extract is safe and well-tolerated, and that higher doses may provide enhanced protective effects against tissue injury, highlighting its potential therapeutic value.

IV. Discussion

The present study demonstrated the significant hepatoprotective potential of the methanolic extract of *Codiaeum variegatum* against CCl_4-induced liver toxicity in rats. Phytochemical analysis revealed the presence of several bioactive constituents, particularly phenolics and flavonoids, which contributed to notable antioxidant activity as confirmed by the DPPH assay. Acute toxicity evaluation established the safety of the extract up to 2000 mg/kg, with no major toxic manifestations observed during the study period. In the hepatotoxicity model, treatment with the extract effectively restored altered biochemical markers, including SGPT, SGOT, ALP, and total bilirubin, in a dose-dependent manner. Histopathological findings further supported the biochemical results by showing marked protection of hepatic architecture and reduction in necrotic and degenerative changes. The higher dose (400 mg/kg) exhibited effects comparable to the standard drug Silymarin. Overall, the findings suggest that the hepatoprotective activity of *Codiaeum variegatum* may be attributed to its antioxidant-rich phytoconstituents, which help in reducing oxidative stress and stabilizing hepatocellular membranes, thereby supporting its potential as a natural therapeutic agent for liver disorders.

V. Conclusion

The methanolic extract of *Codiaeum variegatum* demonstrated significant hepatoprotective activity along with a favorable safety profile at higher doses. The protective effects are likely attributed to its rich phenolic and flavonoid content and strong antioxidant potential, which help reduce oxidative stress and restore normal hepatic function against chemically induced liver injury. These findings suggest that *Codiaeum variegatum* may serve as a promising natural therapeutic candidate for liver disorders. Further mechanistic investigations and clinical studies are warranted to validate and expand its therapeutic applications.

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