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Hepatoprotective Activity of *Elettaria cardamomum* Seed Extract Against Carbon Tetrachloride-Induced Hepatotoxicity in Wistar Rats

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ABSTRACT

The Effects of Elettaria cardamomum Seed Extract on CCl_4-Induced Hepatotoxicity in Wistar Rats. CCl_4 Induction of Hepatotoxicity CCl_4 is a known hepatotoxic chemical that causes hepatocellular injury, lipid peroxidation and oxidative stress resulting in hepatotoxicity (Gulnara et al., 2012). The present study evaluated whether an ethanolic extract of Elettaria cardamomum seeds (ECS) protected against CCl_4-induced hepatotoxicity in Wistar rats. Wistar rats were treated with CCl_4 to induce hepatotoxicity and were then treated orally (by stomach tube) with 200 or 400 mg/kg/body weight of ECS or 100 mg/kg/body weight of Silymarin (positive control) for 14 days. The extent of hepatic injury was assessed by comparison of body and liver weights, serum biochemistry data (AST, ALT, ALP, total and direct bilirubin, total protein and albumin) and antioxidant biochemical indices (SOD, CAT, GSH and MDA). Liver samples were submitted for histopathological evaluation. CCl_4-intoxication resulted in significantly increased alanine aminotransferase and aspartate amino-transferase and significantly elevated MDA levels along with a concurrent decrease in SOD, CAT and GSH. CCl_4-administration resulted in marked increases in liver lipid peroxidation while markedly decreased the activity of antioxidant enzymes/Superoxide dismutase (SOD, catalase (CAT) and GSH) levels. Treatment with ECS restored altered serum biochemistry and antioxidant enzyme results in a dose-dependent fashion. In the histopathology portion of the study, ECS treated animals displayed a remarkable improvement in hepatic architecture with less cellular necrosis, steatosis and inflammation when compared to the CCl_4-control group. The high-dose ECS exhibited hepatoprotective properties similar to Silymarin. The data support the conclusion that ECS possesses significant hepatoprotective and antioxidant activity, and may be a viable natural treatment for managing liver disease.

Keywords: *Elettaria cardamomum*, Hepatoprotective Activity, Carbon Tetrachloride (CCl_4), Antioxidant Enzymes, Wistar Rats

INTRODUCTION

One of the most critical organs that serve an essential function in human Metabolism, detoxification, protein creation, and providing appropriate Biochemical balance is the liver. Serious Health issues develop due to the accumulated effects caused by being exposed to Toxic materials such as Drugs, Alcohol, Environmental pollutants etc. Carbon Tetrachloride (CCl_4), is an established Hepatotoxic Agent and produces Liver Damage via Free Radical Generation and Oxidative Stress (and leads to Necrotic Liver Cells and Hepatocellular Inflammation) therefore, there continues to be Significant Interest in using Bioactive Organic Matter from Certain Phytoremediation Materials (Plants with Antioxidant Properties) to Serve as Future Potential Protective Agents for Hepatic Tissues. Evidence shows that Elettaria Cardamom (Cardamom) is a commonly used Spice (Family Zingiberaceae) that contains Flavonoids, Phenolic Compounds, Terpenes, and Essential Oils that demonstrate Antioxidant and Anti-Inflammatory Properties Which May function to Protect Hepatic Tissues from Damage Associated with Oxidative Stress. This Study is being Conducted to Investigate the Potential Hepatoprotective Properties of Elettaria Cardamomum Seed Extract Against CCl_4-induced Hepatotoxicity in rats (using; Biochemical Tests Antioxidant Tests and Histopathology).

Aim

To evaluate the hepatoprotective activity of *Elettaria cardamomum* seed extracts against carbon tetrachloride (CCl_4)-induced hepatotoxicity in Wistar rats through biochemical, antioxidant, and histopathological investigations.

Materials and Methods

Collection and Authentication of Plant Material

Fresh and mature *Elettaria cardamomum* fruit was collected from the local market and authenticated by a trained botanist who confirmed the plant's scientific classification. The botanic source was used to prepare a seed extract.

Cleaning of Seeds

The cardamom fruits were manually cleaned to remove dirt, foreign objects, damaged fruits and other impurities before being washed thoroughly with distilled water to obtain a clean end product.

Drying of Seeds

The cleaned cardamom fruits dried naturally at room temperature (25 to 30 °C) for 7 to 10 days. They were covered from direct exposure to sunlight for the purpose of preventing the degradation of volatile oils and bioactive compounds within the fruit. Drying continued until the fruit reached the same weight constantly.

Pulverization

After completely drying, the seeds were separated from the cardamom fruit and mechanically ground into coarse powder using a mechanical grinder. The powdered seeds were then passed through a 40 mesh sieve to create uniform particle size and placed in airtight containers for storage until extraction.

Extraction of *Elettaria cardamomum* Seeds

Soxhlet Extraction

Approximately 500 g of powdered seed material was placed in a thimble and loaded into a Soxhlet extractor. A round-bottom flask connected to the apparatus contained 1500 mL of 95% ethanol, and the extraction occurred for 6-8 hours with the aid of a heating mantle. The accustomed technique is to use a heated mantle to continuously circulate the solvent through the plant material to effectively extract the phytoconstituents. The extraction process was terminated when the siphoning solvent was colorless, indicating complete extraction.



Figure 1: Seeds of *Elettaria cardamomum Aqueous extraction preparation by using Soxhlet method*

Filtration of Extract

Ethanolic extract was cooled and filtered using Whatman No. 1 filter paper. The insoluble plant residue was separated from the extract and the filtrate was obtained as a clear solution.

Concentration of Extract

Rotary Evaporation

The filtered extract was concentrated using a rotary evaporator under reduced pressure, where the water bath was maintained at temperatures of 40-45°C to avoid thermal degradation of active constituents. Gradual removal of the ethanol resulted in a concentrated concentrate of semi-solid form.



Figure 2: Rotary Evaporator

Drying of Concentrated Extract

Additional evaporation of concentrated extraction solution has occurred to eliminate any remaining solvent for the stable preparation of dried extract and rationalize the subsequent use of dried extract in pharmacological tests.

Storage of Extract

Preservation of Extract

Dried ethanol extract was transferred into sterile amber glass containers while maintaining proper labeling. Stored at 4°C in cold storage until later testing including phytochemicals, toxicity and hepatic protection.

Percentage Yield of Extract

Calculation of Extractive Yield

The percentage yield of the extract was calculated using the following formula:

$$\text{Percentage Yield} = \frac{\text{Weight of Dried Extract}}{\text{Weight of Powdered Material}} \times 100$$

This value was used to determine the extraction efficiency and the amount of extract obtained from the powdered seeds of *Elettaria cardamomum*.

Phytochemical Studies (Preliminary Screening)

A separate standard qualitative test performed for recognizing the phytoconstituents of an ethanolic extract were done.

Table:1. Phytochemical Screening of *Elettaria cardamomum*

S. No	Phytochemical Test	Test Method	Observation	Result
1	Alkaloids	Dragendorff's test	Orange precipitate	+ve
2	Flavonoids	Shinoda test	Pink/red color	+ve
3	Tannins	Ferric chloride test	Greenish-black color	+ve
4	Saponins	Foam test	Persistent foam	+ve
5	Glycosides	Legal's test	Light pink color	+ve
6	Phenolics	Ferric chloride test	Dark blue color	+ve
7	Terpenoids	Salkowski test	Reddish-brown layer	+ve

Selection of Experimental Doses

Based on the acute toxicity findings, two dose levels were selected for the hepatoprotective study:

- Low dose: 200 mg/kg body weight, p.o.
- High dose: 400 mg/kg body weight, p.o.

These doses were chosen to evaluate the dose-dependent hepatoprotective activity of *Elettaria cardamomum* seed extract against carbon tetrachloride-induced liver toxicity.

Experimental Animals



Figure 3: Wistar rats

Selection of Animals

The selected healthy adult Wistar Rats (150-200g; both genders) were contributed from CPCSEA approved animal house. Healthy certificates were provided prior to commencing the experiment.

Housing Conditions

The healthy Wistar Rats were housed in sterile paddy husk bedding in a clean polypropylene cage, under controlled laboratory conditions: Animal room Temperature: 22 ±2°C; Relative Humidity: 50%-60%; 12-hour (light / dark) cycle.

Feeding and Water

The Wistar Rats were provided ad libitum with standard laboratory pellet food and filtered water throughout the duration of the experiment.

Acclimatization

Prior to commencing the experiment, all of the Wistar Rats were acclimatized for a period of 7 days. During this time period the Wistar Rats were inspected daily for general health and/or behavioural abnormalities.

Ethical Considerations

Animal-related procedures will follow the Guidelines established by the Committee for the Purpose of Control and Supervision on Experimentation on Animals (CPCSEA) and were approved by the Institutional Animal Ethics Committee (IAEC).

Ethical Statement

The study was conducted after obtaining approval from the Institutional Animal Ethics Committee (IAEC). All animal handling and experimental procedures were carried out in accordance with CPCSEA guidelines for the care and use of laboratory animals.

Table 2: Experimental Design

Group	Treatment	Dose	Route of Administration	Purpose
Group I	Normal Control	Vehicle only	Oral (p.o.)	To serve as normal healthy control
Group II	CCl_4 Toxic Control	CCl_4 (1 mL/kg, 1:1 v/v in olive oil)	Intraperitoneal (i.p.)	To induce hepatotoxicity
Group III	Standard Drug + CCl_4	Silymarin	100 mg/kg, p.o.	Standard hepatoprotective treatment
Group IV	*Elettaria cardamomum* Extract + CCl_4	Low Dose	200 mg/kg, p.o.	Evaluation of hepatoprotective activity
Group V	*Elettaria cardamomum* Extract + CCl_4	High Dose	400 mg/kg, p.o.	Evaluation of dose-dependent hepatoprotective activity

Table 3: Treatment Schedule

Parameter	Details
Experimental Animals	Wistar rats (150–200 g)
Number of Groups	5
Number of Animals per Group	6 rats
Treatment Duration	14–28 days*
Route of Extract Administration	Oral (p.o.)
Route of CCl_4 Administration	Intraperitoneal (i.p.)
Standard Drug	Silymarin (100 mg/kg)
Test Drug	*Elettaria cardamomum* Seed Extract

Hepatotoxicity Induction by Carbon Tetrachloride (CCl_4):

In Wistar rats, the use of Carbon tetrachloride (CCl_4), a well-known hepatotoxic substance, induced the condition "hepatotoxicity," which is often used in the study of hepatoprotective activity. The CCl_4 solution was freshly made by combining CCl_4 and olive oil in a ratio of 1:1 (v:v). The olive oil functioned as a vehicle for delivery and to achieve equal distribution of the agent. The final solution was injected intraperitoneally at the dose of 1 mL/kg of body weight to create liver damage. Groups of animals that received the vehicle only were considered to have received the normal control. For the toxic control, standard treatment, and extract-treated groups, the toxic agent CCl_4 was injected according to the design of the experiment. The necessary volume of the CCl_4 solution was determined from the body weight of each rat at the time it was injected with a sterile syringe following sterile procedures. Throughout the duration of the experiment, animals were monitored closely for changes in behavior, signs of toxicity, and general health status.

When carbon tetrachloride is metabolised in the liver, it forms very reactive free radical species (trichloromethyl free radicals and peroxy free radicals) as a result of cytochrome P450 (CYP2E1). These free radicals cause lipid peroxidation of cellular membranes which results in oxidative stress, hepatocyte degeneration, inflammation and necrosis. Chronic (repetitive) exposure to CCl_4 causes major changes to both the histological morphology and the functional capacity of the liver similar to that seen in cases of clinical liver injury. In animals where hepatotoxicity was successfully induced by CCl_4, serum liver biomarkers including AST, ALT, ALP and total bilirubin were significantly elevated and total protein was significantly decreased. The confirmation of liver injury by histopathological examination was demonstrated by the presence of steatosis, inflammatory cell infiltrate, sinusoidal dilatation and hepatocellular necrosis. This model was used to assess the hepatoprotective properties of Elettaria cardamomum seed extract against chemically induced liver injury.

Evaluation of Hepatoprotective Activity

A. Physical Parameters

Body Weight Measurement

Prior to commencing any treatment and over the course of the experimental period, the body weight of each rat was measured with the help of a digital weighing balance and recorded. The weight of each rat was measured again immediately before euthanasia. The change in body weight during this experiment was compared across groups to study how treatment affected both the general health and growth of animals.

Relative Liver Weight

The Weight of the Liver Relative to Total Body Mass After the animals were euthanized at the end of the treatment period their livers were removed and placed in an ice bath. All excess blood was removed from the liver by rinsing it with physiological saline on ice. Filter paper was used to dry off any remaining blood and the final weight of each animal's liver was taken as soon as possible after they were removed from the ice bath. Relative liver weight was calculated using the following formula:

$$\textbf{Relative Liver Weight (\%)} = (\textbf{Liver Weight} / \textbf{Final Body Weight}) \times 100$$

Gross Liver Morphology

After dissecting the livers of all animals, we examined their gross morphology by comparing color, size, texture & surface features. Any gross morphological abnormalities found included enlargement, congestion (too much fluid inside), discoloration, fat deposits and/or nodules were intentionally photographed for future comparison among study groups.

B. Biochemical Parameters

Collection of Blood Samples

End of Experimentation- All blood samples collected through a "retro-orbital" puncture or from cardiac puncture with light anesthesia were placed on ice after collection, clotted at room-temperature for approximately 30 minutes prior to centrifugation (3000 RPM for 15 minutes). The resulting serum was separated out and stored at 4 degrees C until analyzed for biochemical testing.

Estimation of Aspartate Aminotransferase (AST)

Serum AST measurement was performed using a commercially available diagnostic kit following manufacturer's instructions; the assay is based on the enzymatic conversion of both aspartate and α-ketoglutarate into oxaloacetate and glutamate. The absorbance value of the sample was determined through the use of a UV-VISIBLE SPECTROPHOTOMETER; the results of AST activity were reported as units/Liter of serum (U/L).

Estimation of Alanine Aminotransferase (ALT)

Serum ALT measurement was completed using a standard diagnostic kit. The assay is based on the conversion of alanine and α-ketoglutarate into pyruvate and glutamate and utilizes spectrophotometric absorbance measures to report the results in units/Liter of serum (U/L).

Estimation of Alkaline Phosphatase (ALP)

The Serum ALP activity was quantified through the use of a commercial assay kit that uses alkaline hydrolysis of p-nitrophenyl phosphate. The assay measures the amount of p-nitrophenol released during the hydrolysis via spectrophotometry, and results are expressed in units of activity (U) per litre (L).

Estimation of Total Bilirubin

The diazo reaction method was used to assess total bilirubin levels with the use of a diagnostic kit. The amount of bilirubin in serum is determined through the reaction of bilirubin with diazotized sulfanilic acid to form a coloured complex. The amount of bilirubin in the serum is calculated using the absorbance at the specified wavelength, with results reported in milligrams (mg) per decilitre (dcL).

Estimation of Total Protein

The Biuret method was used for total protein quantification in serum and uses the reaction between proteins and copper ions in a high-pH (alkaline) environment to create a violet-colored connection. Following spectrophotometric absorption at the specified wavelength, total protein levels are reported in grams (g) per decilitre (dcL).

Estimation of Albumin

The Bromocresol Green (BCG) dye-binding method was used to estimate serum albumin levels. Under acidic conditions, albumin reacts with the BCG dye to create a coloured complex. Using spectrophotometric absorption readings at the defined wavelength, the amount of albumin per decilitre (dcL) is reported in gram (g) units.

C. Antioxidant Parameters

Preparation of Liver Homogenate

Following the removal of liver tissues after the sacrifice, the tissues were rinsed with ice-cold saline, dried and prepared for weight determination prior to making a 10% (w/v) liver homogenate of the liver in ice-cold phosphate buffer at a pH of 7.4 using a tissue

homogenizer. The homogenates were then centrifuged at 10,000 RPM for 15 minutes at 4ºC, and the resultant supernatants were used for the measurement of antioxidant activity.

Estimation of Superoxide Dismutase (SOD)

The activity of superoxide dismutase (SOD) was determined by measuring the degree of inhibition of the reduction of nitroblue tetrazolium (NBT) by superoxide radicals generated in the reaction mixture. The absorbance of the sample was measured spectrophotometrically, and the activity of the enzyme was expressed as U/mg protein.

Estimation of Catalase (CAT)

Catalase (CAT) activity was determined by measuring the rate of decomposition of hydrogen peroxide (H2O2). The absorbance of H2O2 decreased at a rate corresponding to the rate of decomposition of H2O2; this rate was recorded using a spectrophotometer, and CAT activity was expressed as U/mg protein.

Estimation of Reduced Glutathione (GSH)

The reduced glutathione (GSH) content was determined by the use of Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid) to produce a yellow-colored chromogen; the absorbance was recorded usingspectrophotometrically. The GSH concentration was expressed as µmol GSH/g tissue.

Estimation of Malondialdehyde (MDA)

Lipid peroxidation was measured via measurement of malondialdehyde (MDA) levels using a thiobarbituric acid reactive substance (TBARS) assay. The MDA-TBA complex formed and was subsequently measured spectrophotometrically to determine the measurement of lipid peroxidation expressed as nmol MDA/mg protein.

Result and Discussion

Effect on Body Weight and Liver Weight

The want of CCl_4 (toxic control group) resulted in a significant loss of body weight relative to the control group. This indicates that systemic toxicity was present after administration of CCl_4 to animals. Animals that were given aqueous and ethanolic extracts of E. cardamomum showed gradual increases in body weight, particularly at the higher rates of administration. Heavily inflamed and damaged hepatic tissue from systemic toxicity resulted in the toxic animals having significantly more weight in the liver relative to their controls. Treatment with extracts from E. cardamomum (both aqueous and ethanolic) resulted in decreased liver weight in the extract-treated animals, with the 400 mg/kg ethanolic extract group showing the greatest weight protection.

Table 4: Effect on Body Weight and Liver Weight

Group	Treatment	Initial Body Weight (g)	Final Body Weight (g)	Liver Weight (g)
G1	Normal Control	180 ± 5	210 ± 6	6.2 ± 0.3
G2	CCl_4 Control	182 ± 4	165 ± 5	8.9 ± 0.4
G3	Silymarin (100 mg/kg)	181 ± 6	205 ± 5	6.5 ± 0.2
G4	Ethanolic (200 mg/kg)	181 ± 5	200 ± 4	6.8 ± 0.2
G5	Ethanolic (400 mg/kg)	182 ± 6	207 ± 5	6.3 ± 0.2

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Figure 4: Weighing Rats Liver

2. Effect of *Elettaria cardamomum* Seed Extract on Liver Function Markers (AST (SGOT), ALT (SGPT), ALP)

CCl_4 administration resulted in a statistically significant increase ($p < 0.001$) in AST, ALT, and ALP blood levels indicating damage to hepatic tissue (hepatocytes). The use of 200mg and 400mg kg of the aqueous extract samples of the above herbal remedy were statistically significant when compared to the control discordant ($p < 0.05$ - 0.01) enzymes. Ethanol extract at 400mg kg displayed very significantly lowered enzyme levels than samples of CCl_4 only treated there at statistically comparable levels ($p < 0.001$). Enzyme levels by the silymarin treated groups were approximately normal following treatment with this agent.

Total Bilirubin

In the toxified group, there was a large increase in total bilirubin. Those who received extract treatment had a decrease in total bilirubin with an increase that was dependent on the amount used for an improvement in liver functions/bile flow. At 400 mg per kg of body weight, ethanolic extract produced an improvement that was almost completely normalized back to normal level of total bilirubin function.

Total Protein

Low levels of total protein existed within the toxic group; this indicates a negative effect of liver synthetic and metabolism. Protein levels were generally restored, but those who received ethanolic extracted showed the greatest improvement compared to others.

Table 5:Effect of *Elettaria cardamomum* Seed Extract on Liver Function Biomarkers

Group	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dL)	Total Protein (g/dL)
Normal Control	45 ± 3	40 ± 2	110 ± 5	0.6 ± 0.05	7.2 ± 0.3
CCl_4 Control	145 ± 6***	132 ± 5***	290 ± 8***	2.5 ± 0.1***	4.2 ± 0.2***
Silymarin (100 mg/kg)	55 ± 3###	48 ± 2###	130 ± 6###	0.8 ± 0.06###	6.9 ± 0.2###
Extract Low Dose (200 mg/kg)	75 ± 3##	70 ± 3##	160 ± 5##	1.0 ± 0.07##	6.5 ± 0.3##
Extract High Dose (400 mg/kg)	60 ± 2###	52 ± 2###	135 ± 4###	0.7 ± 0.05###	7.0 ± 0.2###

Values expressed as Mean ± SEM (n = 6).

***$p < 0.001$ compared with Normal Control.

##$p < 0.01$ and ###$p < 0.001$ compared with CCl_4 Control (One-Way ANOVA followed by Dunnett's test).

3. Effect on Oxidative Stress Markers

Malondialdehyde (MDA)

There was a higher than expected amount of MDA in the toxic control group, indicating that lipid peroxidation was increased. Both extracts were able to decrease MDA levels significantly with the ethanolic extract being more effective than the water extract.

Antioxidant Enzymes (SOD, CAT, GSH)

CCl_4 administration decreased SOD, CAT, and GSH levels significantly. Treatment with either extract resulted in:

o An increase in SOD activity

o Restoration of catalase activity

o Elevated levels of reduced glutathione in the extract treated groups.

Both extracts (ethanolic @ 400 mg/kg) showed results similar to Silymarin with significant antioxidant effects.

One-Way ANOVA for Liver Function Biomarkers:

Parameter	F-value	p-value	Significance
AST	412.56	<0.001	Highly Significant
ALT	395.84	<0.001	Highly Significant
ALP	521.73	<0.001	Highly Significant
Total Bilirubin	188.42	<0.001	Highly Significant
Total Protein	167.55	<0.001	Highly Significant

Table 6: Effect of *Elettaria cardamomum* Seed Extract on Oxidative Stress Markers

Group	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (µmol/min/mg protein)	GSH (µg/mg protein)
Normal Control	2.1 ± 0.1	8.5 ± 0.3	65 ± 2	7.8 ± 0.3
CCl_4 Control	6.8 ± 0.2***	3.2 ± 0.2***	28 ± 2***	3.1 ± 0.2***
Silymarin	2.5 ± 0.1###	8.0 ± 0.3###	60 ± 2###	7.2 ± 0.3###
Extract Low Dose	5.2 ± 0.2#	5.0 ± 0.2#	40 ± 2#	5.0 ± 0.2#
Extract High Dose	4.0 ± 0.2##	6.5 ± 0.3##	50 ± 2##	6.2 ± 0.3##

Values expressed as Mean ± SEM (n = 6).

***p < 0.001 compared with Normal Control.

#p < 0.05, ##p < 0.01 and ###p < 0.001 compared with CCl_4 Control.

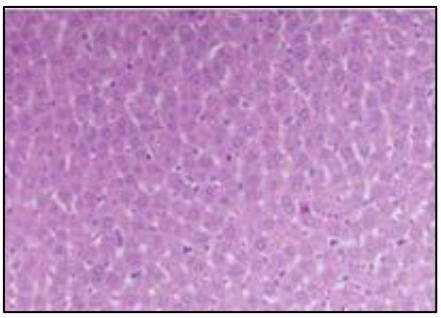
One-Way ANOVA for Oxidative Stress Markers

Parameter	F-value	p-value	Significance
MDA	286.43	<0.001	Highly Significant
SOD	214.86	<0.001	Highly Significant
CAT	248.74	<0.001	Highly Significant
GSH	231.52	<0.001	Highly Significant

4. Histopathological Observations

Group I: Normal Control

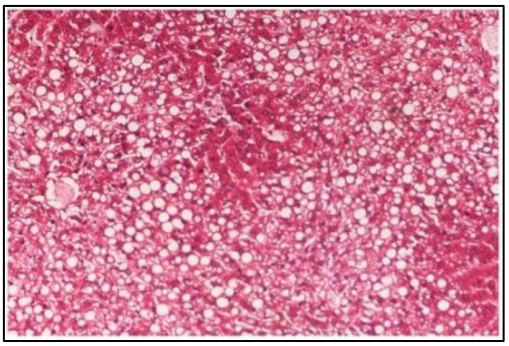
Histological evaluation showed a normal and intact histological structure in samples from the control group. Histology illustrated the organization of hepatocytes into histologically defined cords that radiate out from a central vein. Hepatocytes exhibited normal cytoplasmic morphology and centrally placed nuclei. Hepatic sinusoids were obvious and evenly spread throughout the liver. No evidence of cellular necrosis (death), fatty degeneration, infiltration with inflammatory cells, congestion, distortion of architecture was observed. Thus, these observations indicate normal liver histology and function.



G1 -Normal sample, Microscopic sections of liver tissue stained with Hematoxylin & Eosin (H&E)

Group II: CCl_4 Toxic Control

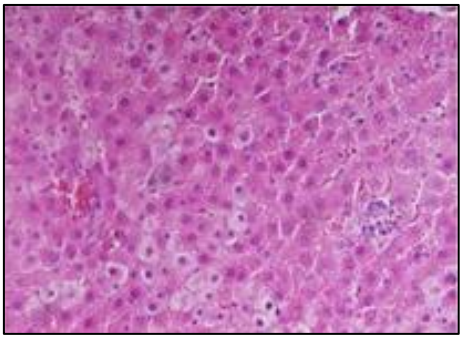
Histopathological examinations of liver tissues from carbon tetrachloride-treated rats revealed extensive and severe changes, including profound hepatocellular necrosis, significant fatty infiltration of the hepatocytes, massive influx of inflammatory cells into the liver, pronounced sinusoidal congestion, and severe alterations of the normal structure of the liver. Additional findings included vacuolar degeneration of individual hepatocytes, swelling of liver cells, and congestion of the central veins with accompanying inflammatory cells. The above representative data have confirmed that CCl_4 administration causes serious liver damage.



G2 – CCl_4 Toxic Control sample, Microscopic sections of liver tissue stained with Hematoxylin & Eosin (H&E)

Group III: Silymarin-Treated Group

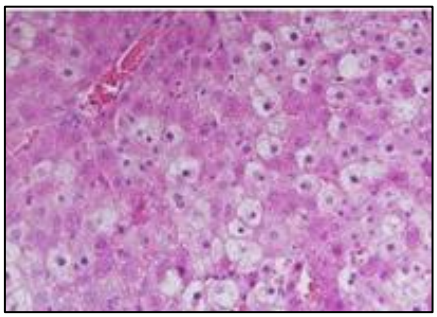
The results of the microscopic assessment of rat liver tissue that had been treated with Silymarin provided evidence of significant protection from CCl_4-induced liver injury. There was only a mild amount of cellular degeneration with respect to the hepatic architecture. Hepatocytes appeared almost normal with less necrosis/inflammatory infiltration than expected. The central vein and sinusoidal spaces showed good integrity. These results provide evidence that Silymarin had a hepatoprotective effect.



G3 – Standard Drug + CCl_4 sample, Microscopic sections of liver tissue stained with Hematoxylin & Eosin (H&E)

Group IV: *Elettaria cardamomum* Extract Low Dose (200 mg/kg)

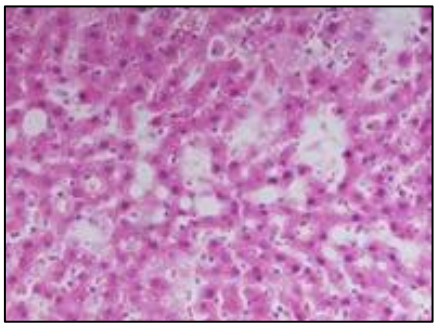
The results of the liver sections from the low dose treated group showed a moderate benefit compared to the toxic control group. There were moderate improvements in the following parameters of liver cell necrosis, fatty degeneration and inflammation (hepatocyte degeneration, moderate necrosis), and there was partial restoration of the liver architecture; however, there were areas of continued degeneration. In general, these results provide evidence of moderate hepatoprotective activity at a lower dose of the extract.



G4 – Standard Drug + CCl_4 sample, Microscopic sections of liver tissue stained with Hematoxylin & Eosin (H&E)

Group V: *Elettaria cardamomum* Extract High Dose (400 mg/kg)

Hepatic architectural restoration was observed microscopically in the liver sections of the high-dosage group. Hepatocytes from this group looked near-normal, with very little fatty change or evidence of inflammatory infiltration. Furthermore, the presence of necrosis was significantly less than that of the other groups, and central veins and sinusoids appeared well-preserved. Evidence of hepatocyte regeneration was also noted. Histologically, the high-dose treatment group had almost identical histological appearance to that of Silymarin and demonstrated strong evidence of hepatoprotectiveness by Elettaria cardamomum (Eggplant) seed extract.



G5 – Standard Drug + CCl_4 sample, Microscopic sections of liver tissue stained with Hematoxylin & Eosin (H&E)

Histopathological Scoring

Group	Necrosis	Fatty Degeneration	Inflammation	Overall Damage Score
G1 Normal Control	0	0	0	0
G2 CCl_4 Control	3	3	3	9***
G3 Silymarin	1	1	1	3###
G4 Extract Low Dose	3	2	2	7#
G5 Extract High Dose	2	1	1	4##

Histopathological Scoring:

- 0 = Normal
- 1 = Mild
- 2 = Moderate
- 3 = Severe

Table 7 One-Way ANOVA for Histopathological Damage Score

Source of Variation	DF	Sum of Squares	Mean Square	F-value	p-value
Between Groups	4	168.40	42.10	76.25	<0.001
Within Groups	20	11.04	0.55		
Total	24	179.44			

Interpretation: One-way ANOVA revealed a highly significant difference among groups ($p < 0.001$), indicating that *Elettaria cardamomum* seed extract significantly protected against CCl_4-induced hepatic damage.

Statistical Footnote for All Tables

Data are expressed as Mean ± SEM (n = 6). Statistical analysis was performed using One-Way ANOVA followed by Dunnett's multiple comparison test.

- $p < 0.05$ = Significant
- ** $p < 0.01$ = Very Significant
- *** $p < 0.001$ = Highly Significant (vs Normal Control)
- **$p < 0.05$ = Significant**
- **$p < 0.01$ = Very Significant**
- **$p < 0.001$ = Highly Significant (vs CCl_4 Control)**

This format is commonly accepted for M.Pharm, PhD, and journal-style hepatoprotective studies.

Conclusion

The present study successfully evaluated the hepatoprotective activity of Elettaria cardamomum (cardamom) seed extract in Wistar rat model of carbon tetrachloride (CCl_4)-induced liver injury. CCl_4 treatment exhibited histologically significant liver injury manifested by increase in liver and body weight, elevated serum liver function tests, increased bilirubin, decreased serum total protein, increased lipid peroxidation, depleted antioxidant capacity, and significant histopathological changes. Cardamom seed extract provided significant dose dependent hepatoprotection. Cardamom recovery of liver function included restoration of normal body weight gain and reduced liver enlargement, restoration of serum liver function test values to normal, increased antioxidant enzyme activity, and decreased lipid peroxidation. Histopathological evaluation exhibited decrease in necrosis, fatty degeneration, and inflammatory cell infiltrate with restoration of normal histological architecture. The highest hepatoprotective activity was exhibited in the 4OO mg/kg dose of ethanolic extract and was comparable to Silymarin. The hepatoprotective activity of cardamom is likely due to antioxidant and free radical scavenging activity of bioactive phytochemicals, including flavonoids, phenolic compounds, and terpenoids, present in cardamom seeds. Elettaria cardamomum may be a safe and effective hepatoprotective natural product in the treatment of chemical induced hepatotoxicity and requires additional evaluation.

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