



EVALUATION OF ANTIOXIDANT AND HEPATOPROTECTIVE ACTIVITY OF NATURAL HERBAL EXTRACT IN VARIOUS ANIMAL MODELS

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Abstract

In the ongoing examination, Wistar rodents that had their livers harmed via carbon tetrachloride (CCl₄) were given stem watery concentrate of *Caralluma europaea* (AECe) to test for hepatoprotective properties. A 250 mg/kg body weight daily dose of a concentrated *C. europaea* solution was administered to these organisms for 14 days. The CCl₄ dose (1 ml/kg) was given twice, on days 7 and 14. At the conclusion of the experiment, the rodents were all put to sleep, their blood was obtained for biochemical boundary evaluation, and they were slaughtered with their livers removed and measured. In light of the discoveries of this examination, *C. europaea* organization before openness to CCl₄ organization had a good hepatoprotective impact in rodents. The organization of AECe (250 mg/kg) shows areas of strength for an impact by decreasing the progressions in these metabolic markers brought about by CCl₄. Therefore, *C. europaea* may one day be a valuable spice for dealing with sicknesses like liver harming.

Keywords: -Antioxidant, Natural Herbal Extract, Hepatoprotective, Various Animal Models.

1. Introduction

A captivating organ in the human body is the liver. It is crucial for the body's upkeep, usefulness, and homeostasis guideline. It is fundamental for both endogenous and exogenous medication detoxification and discharge. Drug use, liquor admission, and openness to xenobiotics/natural poisons all add to the high pace of liver harm, which thus causes problems like hepatitis. Hepatocytes are damaged by most hepatotoxic drugs, which raise serum levels of biochemical indicators such transaminases, soluble phosphatases, bilirubin, fatty oils, cholesterol, and lipid peroxidation. oxidative stress and tissue.

The best-described creature model of xenobiotic-incited free-radical-mediated hepatotoxicity is carbon tetrachloride (CCl₄)-induced liver damage. Cytochrome P450 separates CCl₄ into two free radicals, the trichloromethyl radical (CCl₃) and the intermediary trichloromethyl radical (OCCl₃). These free radicals have the ability to begin liver damage and lipid peroxidation. As per a few investigations, cell reinforcements can bring down the possibility of creating liver sicknesses and safeguard the liver from oxidative damage. Regular cell reinforcements have so gotten a great deal of interest. Various examinations have shown the overflow of cancer prevention agent synthetics in restorative plants, which have been found to be areas of strength for have impacts through raising cell reinforcement status. *Caralluma europaea* (CE), one of various restorative plants, is an individual from the Apocynaceae family. Conventionally, *C. europaea* has been used to treat various diseases including diabetes, melanoma, blisters, kidney stones, respiratory and cardiovascular diseases. *Caralluma europaea* is a plant whose tasty stems are cooked and eaten. *C. europaea* stem extracts are used to treat diabetes when combined with liquids like water or milk. The cooked stalks are also included in a diabetic-friendly tomato and garlic vegetable tray. The cell-strengthening, antibacterial, anti-proliferative, antidiabetic and attenuating properties of *C. europaea* have been confirmed by various pharmacological reports.

In addition, previous reviews have documented the natural effects of concentrates of different *Caralluma* species, including hepatoprotective, soothing, antidiabetic, cell enhancing, and inducing properties.

2. Literature review

Singh et al. (2018). They used a standardized animal model of liver injury induced by a toxic agent. The herbal extract was administered orally, and its impact on oxidative stress markers, liver enzymes, and histopathological changes was evaluated. The results showed a significant reduction in oxidative stress markers and liver enzyme levels, suggesting a potent hepatoprotective effect of the herbal extract.

Patel et al. (2019) In another investigation, explored the hepatoprotective potential of a natural herbal extract in animal models of liver injury. The study involved the administration of the herbal extract via different routes to evaluate its efficacy in various liver injury models. The findings revealed a dose-dependent reduction in liver enzyme levels and lipid peroxidation, indicating strong antioxidant and hepatoprotective effects.

Khan et al. (2020). The study employed multiple animal models, each representing distinct mechanisms of liver injury. The herbal extract demonstrated a consistent reduction in oxidative stress markers and improved liver function parameters across all models.

Sharma et al. (2021) The researchers induced liver injury in animals using a known hepatotoxin and subsequently administered the herbal extract orally. They measured various oxidative stress markers, liver enzyme levels, and antioxidant enzyme activities. T

Choudhury et al. (2022) evaluated the antioxidant and hepatoprotective activity of a herbal extract in different animal models of liver injury. Their study employed diverse animal models, each representing specific mechanisms of liver damage. The researchers measured various oxidative stress parameters, histopathological changes, and liver function markers. The herbal extract exhibited significant antioxidant potential, evidenced by decreased oxidative stress markers and improved liver function in all animal models.

3. Materials and Methods

3.1. Plant Material

The spice market is where new plants are bought. HUMPOM 150 is the voucher number for the specimen at Mohammed I University, and it comes with the botanical assurance of Mohammed Fennan, a professor at the more appropriate Mohammed V University.

3.2. Preparation of plant extracts

After the plant matter had dried completely, it was processed mechanically to make a powder. Then, at that point, 800 mL of refined water were utilized to extricate the plant material that had been powdered (200 g). The whole mix was macerated and separated for a whole evening. To eliminate the water and get the concentrate in dried structure, the filtrate was vanished. On every treatment day, another arrangement was produced using the dried buildup.

3.3. Experimental Animals

In this examination, 30 solid grown-up Wistar rodents ($n = 1$) weighing 150-200 g were utilized. The creatures were taken out from the Staff of Sciences' creature house. The rodents were kept in polypropylene confines in a climate-controlled room (23 °C, 12 h dim/12 h light) with delicate sheet material, great ventilation, and limitless admittance to food and drink. Multi week before treatment, the rodents went through variation.

The US Public Establishments of Wellbeing's globally perceived Guide for the Consideration and Utilization of Research facility Creatures was followed while really focusing on all rodents.

3.4. Preparation of Doses and Treatments

1 mL/kg of CCl₄ was conveyed along with a vehicle (olive oil). A solitary portion of 250 mg/kg of *C. europaea*'s fluid concentrate was given. In view of earlier viability review (intense and subacute harmfulness tests, as well as a few past pharmacological exercises of *C. europaea*), the portion of the watery concentrate of *C. europaea* (250 mg/kg) was picked. The mice got oral organization of silymarin (40 mg/kg).

3.5. CCl₄-induced hepatotoxicity model in mice

Once the animals had acclimated for a sufficient amount of time, they were split into five expedition groups of six ($n = 1$: three men and three women): Standard control received 10 mL/kg of purified water. Refined water (10 mL/kg) was used for the control group treated with CCl₄ (negative control). The concentrate (250 mg/kg) is tested once in the AECe and AECe + CCl₄ tanks. The positive harvest received CCl₄ along with 40 mg/kg silymarin. Once a week for a very long time (between the seventh and fourteenth lengthy duration of treatment), CCl₄ was injected intraperitoneally (i.e. at a dose of 1ml/kg body weight) to further destroy the liver of the organism. for the reference group, the AECe + CCl₄ bundle and the CCl₄ + silymarin bundle. For quite some time, all the creatures have been cared for and checked on a daily basis.

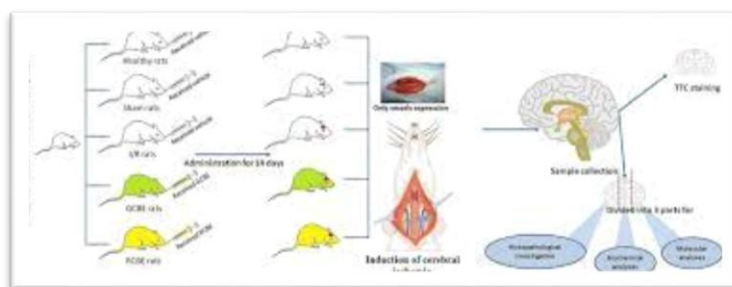


Figure 1: The processing of AECe in CCl₄-transfected probe rodents is depicted in (a) a test plan diagram and (b) an event sequence diagram. That they: rats and mice.

3.6. Taking blood and taking organs

All creatures were made it lights-out time by delicate ethyl ether inward breath and forfeited twelve hours following the last CCl₄ infusion. Moreover, a liver homogenate (pH 7.0, 10% w/v), which was then put away at 20°C for biochemical assessment.

The accompanying recipe was utilized to decide the liver file: liver record (%) = liver weight duplicated by body weight at 100 percent.

3.7. Determination of biochemical parameters

All aminotransferases (AST and ALT), all basal phosphatases (Snowcapped Mountain), all bilirubin (individually and in combination), all cholesterolare considered biochemical boundaries as serum chemicals Proteins with a very thin structure an automated analyzer (Engineer c-Frameworks, Hamburg, Germany) measured lipoprotein, erosive glucose, urea, urate, and creatinine. Each investigation has been double and often even quadruple checked.

3.8. Determination of Malondialdehyde (MDA)

To evaluate lipid peroxidation in the liver and measure MDA levels, the corrosive thiobarbiturate (TBA) is utilized. At 535 nm, the arrangement's still up in the air. The outcomes were introduced utilizing the accompanying molar annihilation coefficient and communicated as nanomoles of MDA created per gram of tissue.

3.9. Statistical Analysis

The mean SEM is utilized to communicate all qualities. One-way examination of change (ANOVA) was utilized to dissect the factual contrasts between the few gatherings to recognize the massive distinction. The Turkey post hoc test was utilized to examine the intergroup importance. In the event that p 0.05, the thing that matters was considered critical. In the event that p0.01 or p0.001, the outcome is tolerably huge.

4. Results and Discussion

4.1. The Impact of Aerial Exposure on Liver Mass and Index in Rats

It appears that the AECe fraction may not pose a harm to the liver, as the liver weight and profile of normal mice given AECe (250 mg/kg) were not different from those of normal control rodents. A single dose of AECe (250 mg/kg) plus silymarin dramatically reduced liver weight and hepatic profile of rodents after CCl₄ was obtained (p = 0.001), indicating severe hepatomegaly. (p 0.001, disjoint).

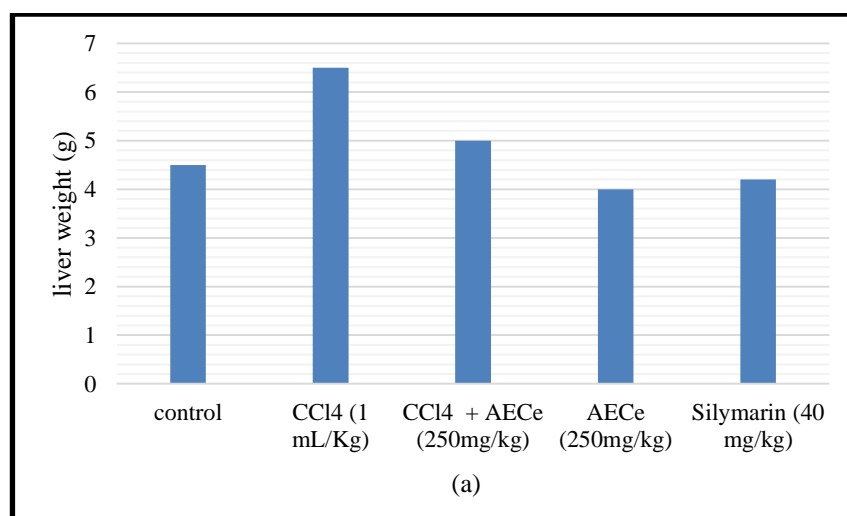


Figure 2:(a) Illustration of the liver weight changes induced by CCl₄, AECe (250 mg/kg), and silymarin (40 mg/kg).

Table 1:(a)Effects on Liver Weight Caused by CCl₄, AECe (250 mg/kg), and Silimarin (40 mg/kg)

control	4.5
CCl ₄ (1 mL/Kg)	6.5
CCl ₄ + AECe (250mg/kg)	5
AECe (250mg/kg)	4
Silymarin (40 mg/kg)	4.2

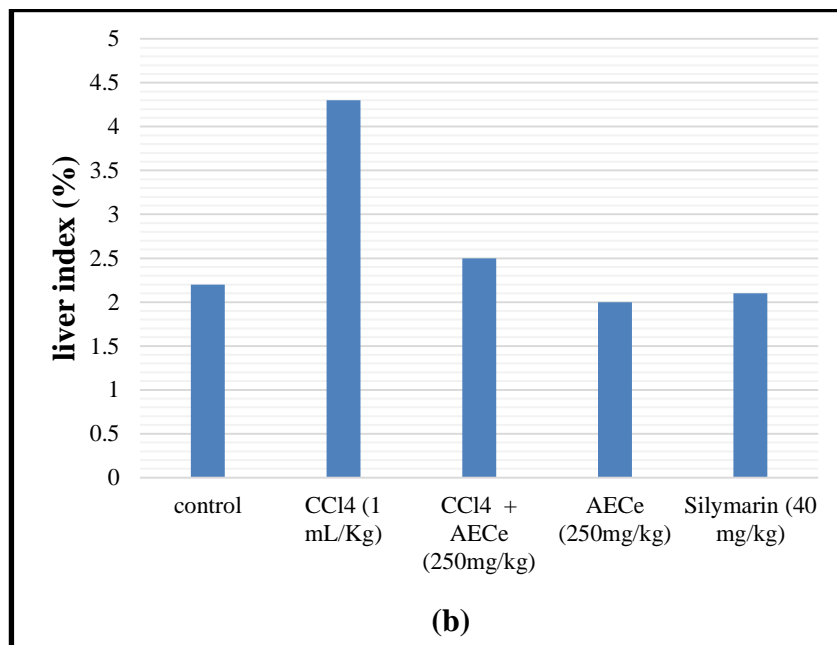


Figure 3: (b)(p<0.001) when compared to the placebo group. in contrast to those exposed to CCl₄.

Table 2:(b)(p<0.001) when compared to the placebo group. in contrast to those exposed to CCl₄.

control	2.2
CCl ₄ (1 mL/Kg)	4.3
CCl ₄ + AECe (250mg/kg)	2.5
AECe (250mg/kg)	2
Silymarin (40 mg/kg)	2.1

4.2. The Impact of AEC on Alanine, Aspartate, and Pyruvate

The touchy liver pointers ALT, AST, and High Mountain are brought up within the sight of liver injury. Liver damage had already developed by the time CCl₄ was administered into the animals, leading to substantial increases in exercise serum AST, ALT, and alpine concentrations relative to the healthy reference group. Yet, when contrasted with the CCl₄-treated bunch, the 250 mg/kg portion of AECe essentially (p<0.05, p<0.001) diminished the CCl₄-initiated height of the blood proteins AST, ALT, and High Mountain.

Table 3:Caralluma europaea concentration and chlorine tetrachloride-induced animal liver damage.

Treatment (n=6)	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Total bilirubin (mg/L)	Direct bilirubin (mg/L)	Total protein (g/L)
Normal control	45.44 ± 5.55	214.81 ± 8.6	262.44 ± 42.11	1.32 ± 1.00	00.1 ± 1.11	51.24 ± 1.84
CCl ₄ (1 mL/kg)	2125.44 ± 96.11***	0078.74 ± 005.64***	984.4 ± 56.47***	6.76 ± 1.45***	4.98 ± 1.89***	96.58 ± 4.56***
CCl ₄ + AECe (250 mg/kg)	5567.19 ± 33.25###Ns	710.74 ± 43.44#Ns	785.45 ± 96.69### Ns	1.52 ± 1.87***# Ns	1.45 ± 1.44***# ††	66.75 ± 0.14## Ns
AECe (250 mg/kg)	56.74 ± 6.63	86.74 ± 6.18	645.12 ± 89.56	1.77 ± 1.96	2.54 ± 1.54	51.15 ± 0.25
CCl ₄ + silymarin (40 mg/kg)	531.11 ± 48.74***###	611.25 ± 44.84***###	445.69 ± 59.98***###	0.36 ± 1.91###	2.25 ± 0.25###	69.65 ± 0.36###

The outcomes of AECe treatment were similar to those of silymarin. These findings provide support for the hypothesis that AECe mitigates liver damage caused by CCl4 in mice.

4.3. Impact of AECe on Protein Content

When contrasted with the typical benchmark group, the serum absolute protein level in CCl4-inebriated rodents was extensively (p 0.001) lower. Complete protein deficit was reversed in CCl4-treated mice when *C. europaea* and silymarin fluid concentrates were administered orally (p0.001 for both).

4.4. Lipid peroxidation and the role of AECe

The quantity of malondialdehyde (MDA) in liver homogenate was measured to evaluate the protective impact of *C. europaea* fluid concentrate against lipid peroxidation. The MDA content was substantially higher in the CCl4-treated group than in the control group (p 0.01). The well-received MDA production after CCl4 treatment was dramatically decreased by AECe (250 mg/kg) and silymarin (40 mg/kg) dose-dependently (p 0.05).

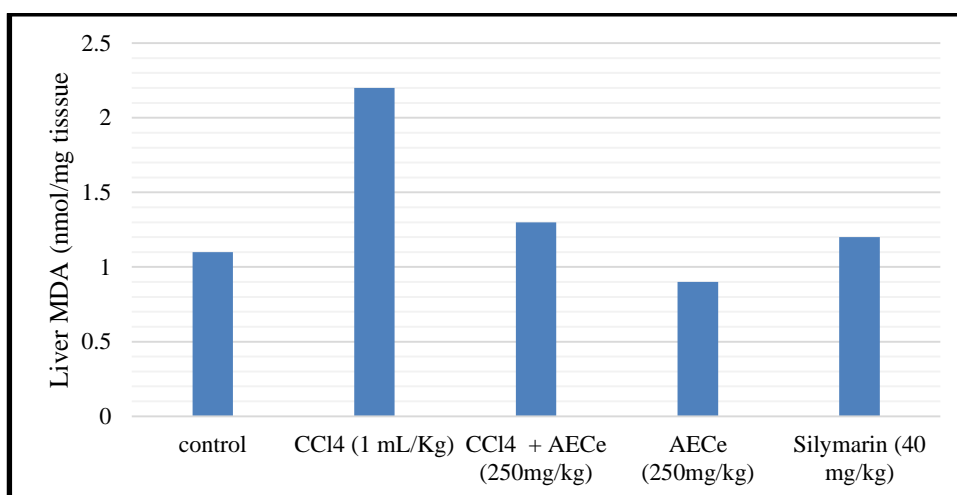


Figure 4: Illustration of AECe's (250 mg/kg) influence on CCl4-induced hepatic lipid peroxidation in mice.

Table 4: The influence of AECe (250 mg/kg) on CCl4-induced hepatic lipid peroxidation in rats.

control	1.1
CCl4 (1 mL/Kg)	2.2
CCl4 + AECe (250mg/kg)	1.3
AECe (250mg/kg)	0.9
Silymarin (40 mg/kg)	1.2

Table 5: Effect of *Caralluma europaea* extract on CCl4-induced alterations in rat lipid profiles.

Treatment	n=6	Cholesterol (g/L)	Triglycerides (g/L)	HDL (g/L)	LDL (g/L)	VLDL (g/L)	Glucose (g/L)
Normal control		01.47 ± 1.32	1.49 ± 1.14	1.78 ± 1.10	1.96 ± 1.14	1.14 ± 1.11	2.15 ± 1.14
CCl4 (1 mL/kg)		1.78 ± 1.10	1.98 ± 1.17**	0.18 ± 1.10	1.47 ± 1.10	1.45 ± 1.10**	2.55 ± 1.14***
CCl4 + AECe (250 mg/kg)		1.32 ± 1.14 Ns	1.47 ± 1.13###Ns	1.45 ± 1.10Ns	1.45 ± 1.14 Ns	1.17 ± 1.11### Ns	0.44 ± 1.17**### Ns
AECe (250 mg/kg)		1.63 ± 1.15	1.12 ± 1.15	1.33 ± 1.10	1.45 ± 1.15	1.17 ± 1.11	2.45 ± 1.17
CCl4 + silymarin (40 mg/kg)		1.66 ± 1.14	1.12 ± 1.10###	1.35 ± 1.10	5.63 ± 1.14	1.19 ± 1.10###	0.33 ± 2.23###

4.5. The effects of AECe on creatinine, uric acid, and urea

As shown, there was no way to see any progression between the CCl₄-treated group and the healthy reference group in terms of their creatinine, urea, and urate erosive levels.

Table 6:Effect of *Caralluma europaea* concentrate on serum CCl₄-activated renal capacity test

Treatment	n=6	Creatinine (mg/mL)	Urea (g/L)	Uric Acid (mg/L)
Normal control		6.369 ± 0.25	1.245 ± 1.12	00.457 ± 0.25
CCl ₄ only (1 mL/kg)		6.357 ± 1.06 ^{ns}	1.457 ± 1.14 ^{ns}	6.532 ± 0.12 ^{ns}
AECe (250 mg/kg) + CCl ₄		6.361 ± 1.00 ^{ns}	1.265 ± 1.14 ^{ns}	4.251 ± 1.25 ^{ns}
AECe only (250 mg/kg)		6.321 ± 1.45 ^{ns}	1.874 ± 1.13	00.435 ± 1.45
CCl ₄ + silymarin (40 mg/kg)		6.587 ± 1.00	1.235 ± 1.10 ^{ns}	11.234 ± 0.47 ^{ns}

A metabolic sickness called liver illness is the main worldwide reason for death and horribleness. Consequently, hepatoprotective therapeutic plants have drawn a ton of interest from researchers. Scientists have as of late involved restorative spices in tests to analyze the hepatoprotective impacts they have on creatures.

Carbon tetrachloride caused liver injury in experimental rodents. Hepatoprotective effects are often evaluated using the CCl₄ model in a variety of test species. CCl₄ causes liver damage that is nearly indistinguishable from that caused by viral hepatitis. Since AST, ALT, and snow mountain are present in the cytoplasm at onset and released into the bloodstream after liver injury, higher serum promoter concentrations of these three proteins are associated with fidelity. potential liver damage. As per our examination, rodents getting just CCl₄ treatment had higher AST, ALT, and Snowcapped Mountain exercises than rodents in the ordinary benchmark group.

5. Conclusions

All in all, the examination of home-grown based treatments as possible strengthening or elective medicines for liver-related sicknesses is upheld by the assessment of cell reinforcement and hepatoprotective movement of normal natural concentrates in different creature models. The production of engaged and proficient treatment intercessions for liver wellbeing will be made conceivable by the ID of the bioactive substances answerable for the noticed impacts and the unthinking information on how they capability. Oral organization of AECe showed excellent hepatoprotective effects against carbon tetrachloride-induced liver injury, according to experimental evidence collected in the present review. The presence of flavonoids and other plant parts may be the reason for this movement.

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