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SHORT COMMUNICATION

Hypolipidemic Potentials of Methanolic Extract of *Cymbopogon flexuosus*

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Abstract

Background: Hyperlipidemia is a condition marked by high levels of Total Cholesterol (TC), Low-Density Lipoprotein (LDL), and Very Low-Density Lipoprotein (VLDL), along with reduced levels of High-Density Lipoprotein (HDL) in the blood. **Aim:** This study aimed to evaluate the cholesterol-lowering (hypolipidemic) effect of methanolic extract from *Cymbopogon flexuosus* leaves in rats fed a high-cholesterol diet. **Methods:** The anti-hyperlipidemic effects of the extract were tested in rats over 45 days. Antioxidant activity was also assessed using DPPH and hydrogen peroxide free radical scavenging assays. **Results:** When cholesterol was given to rats for 45 days, the activities of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase significantly increased, and the amounts of lipids in the tissues and plasma increased while HDL cholesterol dropped. Additionally, it lowered superoxide dismutase activity in the tissues and increased malondialdehyde levels. Co-administration of *Cymbopogon flexuosus* extract at doses of 100 and 200 mg/kg, however, dramatically restored lipid levels to nearly normal in rats fed high cholesterol. HDL cholesterol levels increased by 225.44% and 110.39%, while plasma, liver, heart, kidney, and lung cholesterol decreased by 23.13% and 56.43%, 30.09% and 20.90%, 38.21% and 74.53%, 12.61% and 32.49%, and 37.11% and 29.90%, respectively. While SOD activity in the liver, heart, kidney, and lung increased by 89.35% and 149.21%, 74.91% and 68.35%, 56.76% and 114.77%, and 204.91% and 274.62%, respectively, the levels of AST, ALT, and ALP in the plasma and MDA in the tissues similarly reduced. **Conclusions:** This study's findings corroborate previous research showing that *Cymbopogon flexuosus* can reduce lipid levels in rats given a high-cholesterol diet.

Major Finding: The study found that *Cymbopogon flexuosus* (lemongrass) can help lower fat levels in the blood and also acts as an antioxidant. These results suggest that the plant could be a promising natural treatment for high cholesterol.

Keywords: Cymbopogon *flexuosus,* Malondialdehyde, High Cholesterol, Hyperlipidemia, Scavenge

1. Introduction

Total cholesterol, low-density lipoprotein, and very low-density lipoprotein levels in the blood are elevated in hyperlipidemia, whereas HDL levels are decreased. According to the American Heart Association, hyperlipidemia is characterised by an abnormally high blood fat content. Triglycerides and cholesterol make

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up these lipids. Blood lipids and fatty compounds increase the risk of atherosclerosis and cardiovascular disease[1]. More than 17 million people die every year from cardiovascular diseases, which encompass conditions such as atherosclerosis and myocardial infarction[2]. Cardiovascular illnesses are linked to several cardiometabolic risk factors, including hypercholesterolemia, diabetes, hypertension, obesity, and an inactive lifestyle[3]. It is well-established that atherosclerosis, hypertension, and renal failure are all influenced by hypercholesterolemia[4]. A prior study has also shown that hypercholesterolemia increases oxidative stress and lipid peroxidation[5]. Hence, a feasible way to manage the harmful effects of hypercholesterolemic situations is to decrease oxidative stress. There is a well-documented high incidence of side effects in synthetic medications with cholesterol-lowering properties. Thus, there is a growing need for natural items that may reduce lipid levels and act as antioxidants, all while causing little or no adverse effects. Plants are recognised as a reliable source of beneficial medications that are safer than synthetic alternatives. Essential oil from lemongrass (*Cymbopogon flexuosus*) has pharmacological properties, which is why it is widely farmed. Citral, isoneral, isogeranial, geraniol, citronellal, citronellol, germacrene-D, and elemol are some of the bioactive chemicals found in lemongrass essential oil. Geranyl acetate is another one of these molecules. The essential oil of lemongrass also includes other compounds that are good for you. This is because these components provide LEO with its antifungal, antibacterial, antiviral, anticancer, and antioxidant characteristics. For financial gain, businesses in the pharmaceutical, cosmetic, and food preservation industries utilise LEO characteristics. When used for cancer treatment, LEO paves the way for novel pharmaceutical approaches. Although there is little evidence of their impact on humans, certain LEO components have demonstrated encouraging anticancer capabilities *in vitro*. Therefore, the anticancer effects of LEO components require more investigation. The function of LEO in the fields of oncology, medicine, and agriculture is covered in this article. Within the *Poaceae* family, *Cymbopogon* stands out as a significant essential oil producer. Commercially, the most popular species are *Citronella, Palmarosa,* wild lemongrass, and lemongrass. Essential oils such as lemongrass, ginger grass, *Citronella, Palmarosa,* and *rusa* are produced by the plant. Cyclic and acyclic monoterpenoids make up the majority of lemongrass essential oil. One of the most valuable components of lemongrass, citral, is responsible for the herb's distinctive lemon aroma. It is clinical that generates ionone and vitamin A. The *Cymbopogon* family of plants has several uses, including warding off worms, inflammation, pain, pesticides, pathogens, and even Alzheimer's disease[6].

2. Materials and Methods

2.1 Plant Material

The plant *Cymbopogon flexuosus* leaves were obtained from Maharana Pratap College of Pharmacy's herbal Garden and validated by CSIR and Authentication Number-CIMAP-HA-1000520251.

2.2 Extraction

The collected plant leaves were first removed and dried in an oven at 45°C. The dried leaves were then ground into a fine powder using a mechanical grinder. A portion of the powdered material (52 g) was subjected to extraction using a Soxhlet apparatus with methanol (500 mL) as the solvent. The resulting extract was concentrated by evaporating the solvent in a water bath. The yield of the soluble constituents was determined gravimetrically by weighing the dried extract after solvent removal.

$$Yield\ \% = \frac{Weight\ of\ dry\ extract}{Weight\ of\ dry\ leaves\ powder} \times 100$$

The dried extracts were kept at a temperature of 4°C for future research.

2.3 *In Vitro* Anti-oxidant

2.3.1 DPPH Assay

For the DPPH assay, a mixture of 0.1 mL of different sample concentrations and 0.4 mL of a 0.3M DPPH reagent in ethanol was used. After a thorough shaking, the mixture was left to incubate for 30 minutes in the dark at room temperature. Spectrophotometric measurements of the reaction's absorbance at 517 nm were made both before and after incubation. The following equation was used to compute the DPPH free radical scavenging effect.

(% Scavenging activity = Abs (control) - Abs (standard) / Abs (control) × 100)

A blank reaction containing all reagents except the test extract is called a control, whereas the absorbance of the test extract is called the sample absorbance. The 50% inhibition (IC_{50}) level was determined by doing the tests three times. Making use of butylated hydroxy toluene.

2.3.2 Hydrogen Peroxide Assay

We used Ruch's approach to find out how well the extracts scavenged hydrogen peroxide. Hydrogen peroxide was dissolved in phosphate buffer (pH 7.4) at a concentration of 2 mmol/l. A hydrogen peroxide solution (0.6 ml) was mixed with extracts at concentrations ranging from 10 to 100 µg/ml. After 10 minutes, the absorbance at 230 nm was measured in comparison to a control solution that included phosphate buffer but did not contain hydrogen peroxide[7].

(% Scavenging activity = Abs (control) - Abs (standard) / Abs (control) × 100)

2.3.3 Experimental Design

After the animals were given three weeks to adjust to their environment, they were divided into six groups, with six animals in each group.

- **Group I** received only clean (purified) water.
- **Group II** was given only Cymbopogon flexuosus (a type of lemongrass) at a dose of 200 mg for every kilogram of body weight.
- **Group III** was given cholesterol at a dose of 30 mg/kg of body weight.
- **Group IV** received the same amount of cholesterol (30 mg/kg) along with 100 mg of Cymbopogon flexuosus per kg of body weight.
- **Group V** received both cholesterol (30 mg/kg) and a higher dose of Cymbopogon flexuosus (200 mg/kg).
- **Group VI** was given cholesterol (30 mg/kg) and Questran (a cholesterol-lowering drug) at 0.26 g/kg of body weight[8,9].

Rats were euthanised by heart puncture under mild ether anaesthesia after being orally supplied with cholesterol, the extract, and Questran for 45 days. To conduct biochemical tests, we extracted and preserved animal samples of blood, liver, kidney, heart, and lungs.

2.3.4 Plasma Biochemical Parameter Determination

Enzymatic kits were used to measure the plasma concentrations of proteins, cholesterol, triglycerides, aspartate aminotransferase, and alanine aminotransferase.

2.3.5 Preparation of Tissue Homogenates

To create a 10% (w/v) tissue homogenate, samples of the heart, kidneys, and lungs are homogenised in phosphate buffer saline. The homogenates were centrifuged at 12,000 rpm for 15 minutes, and the resulting supernatant was utilised for the biochemical experiment.

2.3.6 Antioxidant Enzyme Activity and Malondialdehyde Level Determination

Per the methods outlined by *Misra and Fridovish*, *Buege and Aust*, the concentrations of superoxide dismutase, thiobarbituric acid-reactive product, and malondialdehyde were assessed in the kidney and liver homogenates, respectively. Each enzyme's activity was reported in milligrams of protein[10].

2.3.7 Statistical Analysis

The mean±S.E.M. was used to describe the outcomes of this investigation. Statistical analysis was conducted using One-way Analysis of Variance and Tukey's test. Statistical significance was determined by a pvalue less than 0.05.

3. Results

3.1 Antioxidant Activities

Multiple studies have linked oxidative stress to age-related illnesses. The beneficial effects of antioxidants on reducing or blocking neuronal death in the pathophysiology of this condition have also been the subject of a great deal of research. Furthermore, a compound's radical scavenging capability and total antioxidant activity determine its antioxidant capacity. Two activities—the hydrogen peroxide scavenging test and the capacity to scavenge DPPH—were assessed to ascertain the antioxidant capabilities of the plant extracts.

3.2 DPPH Radical Scavenging Activity

Various extracts were studied for their antioxidant activity using 1,2-diphenyl-2-picryl hydrazyl radical (DPPH). Results are discussed in Table 1.

Table 1. DPPH radical scavenging activity of the methanol extract

S. No.	Con. (µg/mL)	Percentage Inhibition	
		Standard (Butylated hydroxytoluene)	Methanol
1	20	53.95	45.94
2	40	64.81	59.86
3	60	76.62	68.18
4	80	85.21	76.95
5	100	91.45	88.87
IC_{50} (µg/mL)		10	25

Table 2. Hydrogen peroxide radical scavenging activity of methanol extract

S. No	Con. (µg/mL)	Percentage Inhibition	
		Standard (Ascorbic acid)	Methanol
1	20 µg/mL	32.23	40.43
2	40 µg/mL	38.13	45.65
3	60 µg/mL	56.34	50.81
4	80 µg/mL	73.32	57.65
5	100 µg/mL	88.34	65.56
IC_{50} (µg/mL)		47	54

You may evaluate the free radical scavenging action with the help of the DPPH, a stable free radical that turns any chemical that donates a proton into a deep purple colour. A change in colour causes DPPH's absorbance at 517 nm to drop as its concentration drops. The IC_{50} value (µg/mL) for the methanolic extract of *Cymbopogon flexuosus* was 25. Compared to conventional BHT, which had an IC_{50} value of 10. *Cymbopogon flexuosus* methanolic extract outperformed the standard in scavenging tests. Table 2 displays the findings of the hydrogen peroxide radical scavenging assay that was performed on the methanolic extract.

This approach was used to test the extract's capacity to scavenge hydrogen peroxide. The IC_{50} value (µg/mL) for the methanolic extract was determined to be 54, and the absorbance was measured at 230 nm. The ascorbic acid standard has an IC_{50} value of 47.

3.3 Effects of the Extract on Rat Plasma and HDL Lipids

Cymbopogon flexuosus alone, at 200 v body weight, had no discernible effect on plasma or HDL lipid parameters compared to the control group. In contrast to the control group, animals given cholesterol alone had a 39.81% rise in plasma cholesterol and a 26.55% increase in triglyceride concentrations, a 66.42% decrease in HDL cholesterol, and a 36.05% increase in HDL triglyceride. In contrast to rats given high cholesterol alone, rats given both cholesterol and *C. flexuosus* extract or Questran at the same doses had a 23.13% and 56.43% reduction in plasma cholesterol concentration, a 36.76% and 21.39% reduction in plasma triglyceride concentration, an increase of 225.44% and 110.39% in HDL cholesterol concentration, and a 48.52% and 24.42% reduction in HDL triglyceride concentration, respectively. Figures 1 and 2 display the results of the examinations of cholesterol and triglycerides, and HDL lipids.

3.4 Effects of the Extract on Liver, Heart, Kidney, and Lungs of Rats

Cholesterol alone considerably raised concentrations in the liver (by 36.73%), heart (by 68.70%), kidneys (by 66.82%), and lungs (by 63.71%), in comparison to the control group. Cholesterol administration alone substantially raised triglyceride concentrations across the board, although phospholipid concentrations were considerably elevated, mainly in the kidneys and lungs, compared to the control group. When rats with high cholesterol were given 100 milligrams/Kilogram and 200 milligrams/Kilogram of *C. flexuosus* extract, the concentrations of lipids in their tissues were found to be lower than in rats treated with high cholesterol alone. Specifically, the hepatic triglyceride concentration was reduced by 35.54% and 32.89%, the cardiac triglyceride concentration by 18.02% and 18.02%, the renal triglyceride concentration by 19.14% and 20.86%, and the pulmonary triglyceride concentration by 51.12% and 48.88%, respectively. Table 3 displays the results of the studies of different tissues.

Effect of extract on activities of ALP, ALT and AST

Cholesterol alone raised ALP, ALT, and AST enzymatic activity by 20.52%, 44.65%, and 18.90%, respectively, compared to normal rats (Table 3). Plasma ALP, ALT, and AST activity was lower in rats treated with cholesterol and given 100 mg/kg and 200 mg/kg body weights of CF+ extract and Questran, respectively, compared to rats given high cholesterol alone. Table 4 represented the results of ALP, ALT and AST.

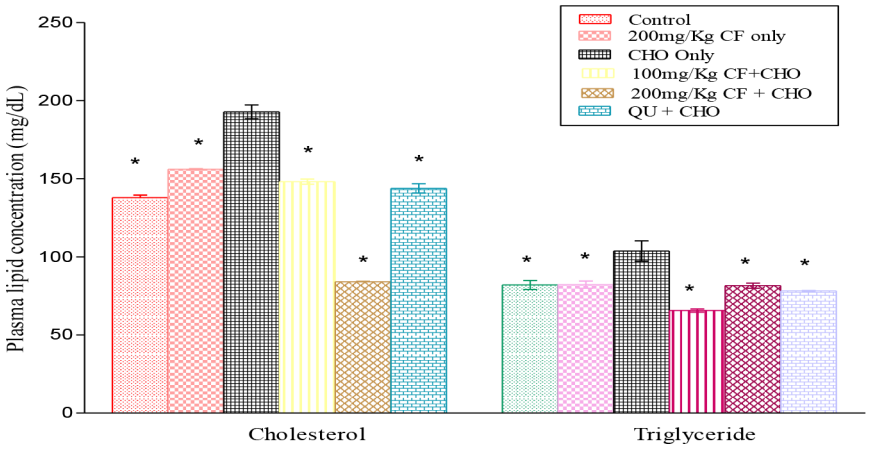


Figure 1. Impact of *C. flexuosus* leaf extract in methanol on rat plasma lipids. *Significantly different from the CHO group at p<0.05; values are averages ± SEM (n = 6).

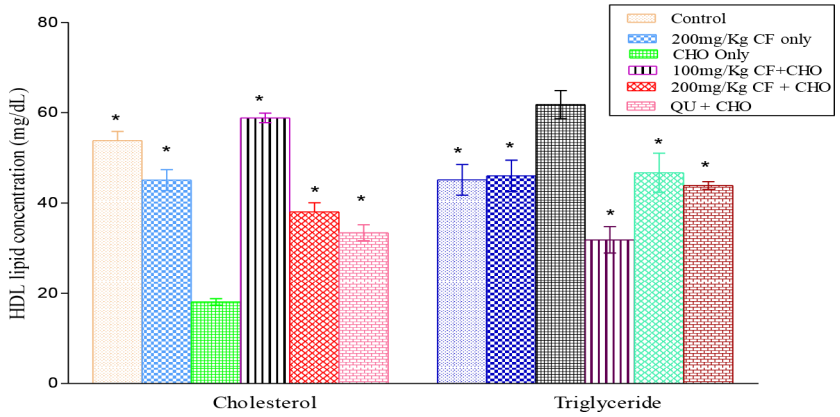


Figure 2. Effects of *Cymbopogon flexuosus* leaf extract in methanol on HDL lipids in rats. * Significantly different from the CHO group at p<0.05; values are averages ± SEM (n = 6).

3.5 Effect of *Cymbopogon flexuosus* Extract on Tissue SOD Activity

Although the liver, heart, kidney, and lung tissue SOD levels of rats given high cholesterol alone were 35.03%, 43.29%, 71.47%, and 79.51% lower than those of normal rats, respectively, administering 200 mg/kg of *C. flexuosus* on its own had no discernible effect on SOD levels. But when rats given cholesterol also received 100 mg/kg and 200 mg/kg of *C. flexuosus* extract, the levels of SOD in their livers and hearts were elevated by 89.35% and 149.21%, respectively; in their kidneys, they were raised by 56.76% and 114.77%, and in their lungs, they were raised by 204.91% and 274.62%, compared to the rats given high cholesterol alone. Figure 3 represents the *C.n flexuosus* extract on tissue SOD activity.

3.6 Effect of *Cymbopogon flexuosus* on Tissues Malonaldehyde Levels

While normal rats showed considerably lower levels of Malondialdehyde (MDA) in the liver, heart, kidneys, and lungs after receiving high-cholesterol diets, mice given 200 milligrams/Kilogram of *C. flexuosus* had no discernible effect. But when rats given cholesterol and *C. flexuosus* extract at doses of 100 mg/kg and 200 mg/kg body weight were compared to rats given cholesterol alone, the results showed that the extract reduced hepatic MDA levels by 27.20% and 23.29%, cardiac MDA levels by 55.76% and 73.14%, renal MDA levels by 46.23% and 49.56%, and pulmonary MDA levels by 68.13% and 50.46%, respectively. Figure 4 represents the *C. flexuosus* extract on tissue MDA activity.

Table 3. *Cymbopogon flexuosus* methanolic leaf extract and its effects on lipids in rat tissues

Group	Liver	Heart	Kidney	Lung
Cholesterol				
Control	2.94 ± 0.02*	3.77 ± 0.51*	2.14 ± 0.09*	2.37 ± 0.10*
Cymbopogon flexuosus (CF) only	1.94 ± 0.02*	2.71 ± 0.36*	2.30 ± 0.09*	2.48 ± 0.09*
Cholesterol only	4.02 ± 0.03	6.36 ± 0.67	3.57 ± 0.12	3.88 ± 0.07
100 mg/kg CF + Cholesterol	2.81 ± 0.30*	3.93 ± 0.32*	3.12 ± 0.04*	2.44 ± 0.01*
200 mg/kg CF + Cholesterol	3.18 ± 0.11*	1.62 ± 0.13*	2.41 ± 0.14*	2.72 ± 0.12*
Questran+Cholesterol	3.59 ± 0.06	2.24 ± 0.02*	2.97 ± 0.06*	3.18 ± 0.08*
Triglycride				
Control	2.61 ± 0.08*	2.90 ± 0.01*	2.59 ± 0.12*	3.29 ± 0.03*
Cymbopogon flexuosus (CF) only	2.02 ± 0.17*	2.78 ± 0.24*	2.11 ± 0.01*	3.37 ± 0.06*
Cholesterol only	3.77 ± 0.06	3.83 ± 0.14	3.50 ± 0.03	9.35 ± 0.29
100 mg/kg CF+Cholesterol	2.43 ± 0.02*	3.14 ± 0.21	2.83 ± 0.05*	4.57 ± 0.12*
200 mg/kg CF+Cholesterol	2.53 ± 0.09*	3.14 ± 0.11	2.07 ± 0.02*	4.78 ± 0.23*
Questran+Cholesterol	3.08 ± 0.06*	3.14 ± 0.21	3.07 ± 0.04*	5.71 ± 0.13*
Phospholipid				
Control	22.33 ± 0.98	19.00 ± 0.62	12.23 ± 0.28*	31.12 ± 0.93*
Cymbopogon flexuosus (CF) only	20.05 ± 1.18	21.10 ± 0.80	12.14 ± 0.17*	30.79 ± 0.35*
Cholesterol only	21.82 ± 0.16	23.47 ± 0.10	23.37 ± 0.51	35.68 ± 0.42
100 mg/kg CF+ Cholesterol	18.91 ± 0.03	20.70 ± 1.84	13.02 ± 0.24*	23.39 ± 0.51*
200 mg/kg CF+Cholesterol	17.48 ± 0.92*	20.26 ± 1.59	13.05 ± 0.49*	30.94 ± 0.78*
Questran+Cholesterol	21.20 ± 0.42	23.63 ±0.54	15.01 ± 0.77*	29.50 ± 0.40*

*Notably different from the CHO group at p<0.05; values are averages ± SEM (n = 6).

Table 4. *Cymbopogon flexuosus* affected the activities of ALP, ALT, and AST in rats fed a high-cholesterol diet

Blood Enzymes	Alkaline phosphatase	Alanine aminotransferase	Aspartate aminotransferase
Control	196.40 ± 9.33	14.49 ± 0.68*	81.35 ± 4.29
Cymbopogon flexuosus	170.20 ± 9.67*	18.17 ± 0.66*	69.42 ± 1.29*
Cholesterol only	236.70 ± 4.22	20.96 ± 0.26	96.73 ± 1.35
100 mg/kg CF+Cholesterol	207.00 ± 15.77	16.43 ± 0.67*	83.13 ± 5.12
200 mg/kg CF+Cholesterol	210.60 ± 5.07	16.78 ± 0.21*	65.41 ± 6.33*
Questran+Cholesterol	183.10 ± 13.68*	11.57 ± 0.57*	43.00 ± 0.42*

Values are means ± SEM (n = 6); *Significantly different from the CHO group at p<0.05.

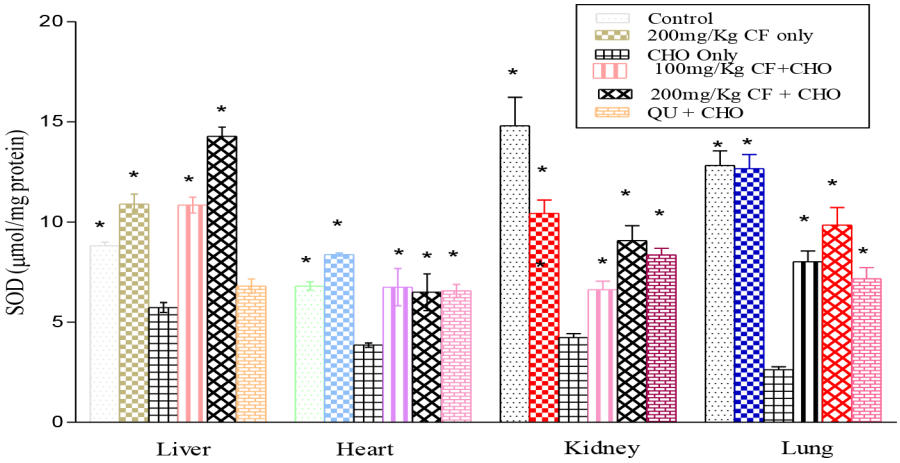


Figure 3. Impact of *C. flexuosus* methanolic leaf extract on Superoxide Dismutase (SOD) activity in rats given cholesterol. *Notably different from the CHO group at p<0.05; values are averages ± SEM (n = 6).

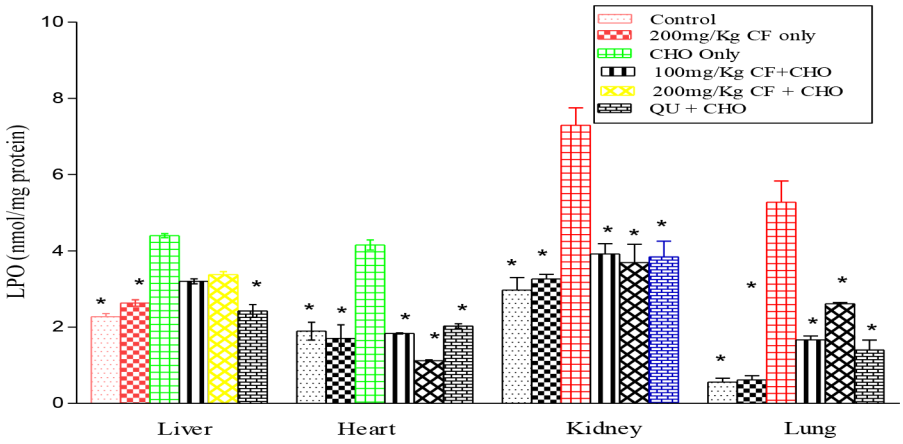


Figure 4. Results of administering a methanolic extract of *C. flexuosus* leaves to rats that were given a diet high in cholesterol and measuring their MDA levels. *Notably different from the CHO group at $p<0.05$; values are averages ± SEM (n = 6).

4. Discussion

Evidence suggests that we can use lipid levels in plasma as indicators of hypertension, atherosclerosis, and coronary heart disease in animals. Many studies have shown that dietary treatment or medication therapy can reduce subsequent CVD-associated mortality and morbidity by controlling cholesterol. Because of the documented toxicity of synthetic lipid-lowering medications in earlier research, screening medicinal plants for lipid-lowering characteristics with a less harmful impact offers a potential new drug development pathway[9,11,12]. Since hypercholesterolemia is associated with an increase in free radical levels, this study used HPA and DPPH radical scavenging tests to evaluate *C. flexuosus's* antioxidant capacity. When testing the overall antioxidant activity of plants, these two assays are among the most commonly used choices. All of the extracts had their enzyme inhibition percentages reported in this experiment. The IC_{50} value and inhibitory activity were 25 and 54 µg/ml, respectively, for the methanolic extract. And when contrasted with the norm, therefore, the methanolic extract is suitable for use in future research. This finding is in line with another study that found that rats given high cholesterol levels increased their plasma cholesterol and decreased their HDL cholesterol levels compared to the control group. This strongly suggests that there are abnormalities in the metabolism of cholesterol. Consistent with earlier research, administering cholesterol leads to a significant accumulation of lipids in the tissues[13]. Animals that were given both high cholesterol and *C. flexuosus* extract simultaneously had lower cholesterol levels overall compared to animals that were given cholesterol alone. *C. flexuosus* leaf extract may have lowered cholesterol levels via influencing lipoprotein lipase activity or the liver's cholesterol metabolism; however, the exact mechanism is unclear[14]. It is likely that the chemical components found in *C. flexuosus* leaf extracts either inhibit the enzymatic pathways involved in cholesterol production or both. The high levels of these enzymes were reduced in rats that received both cholesterol and *Cymbopogon flexuosus* extract, compared to animals that received only cholesterol. This result suggests that the plasma membrane was stabilised and that the liver damage induced by cholesterol was repaired. This finding is in line with the generally held belief that transaminase serum levels normalise when hepatic parenchyma heals and hepatocytes regenerate[18]. The results of this investigation corroborate those of an earlier one that found that a cholesterol-rich diet causes hypercholesterolemia and an increase in lipid peroxidation in the tissues of animals treated with cholesterol alone[15]. Indicates the inability of an antioxidant defence system to prevent the generation of excessive free radicals. When rats were given cholesterol alone, the amount of lipid peroxidation was higher than when 100 and 200 mg of *C. flexuosus* leaves or the conventional medicine (Questran) were also administered to the animals. Therefore, the antioxidant potential of *C. flexuosus* methanolic extract may be the mechanism of protection. In this study, rats that

were fed a diet rich in cholesterol had a significant reduction in the activity of the antioxidant enzyme superoxide dismutase. Rats given cholesterol may have higher SOD utilisation against reactive oxygen species, which would explain why their tissues have lower SOD activity[8,15,16]. But when rats were given both cholesterol and *C. flexuosus* leaf extract at the same time, the SOD levels were restored to their original levels, showing that the former protects tissues from oxidative damage while the latter simply leads to a decline. According to our research, *C. flexuosus* has powerful free radical scavenging capabilities and includes a large number of phenolic compounds. An abundance of research has documented the antioxidant capabilities of phenolic compounds found in plant extracts in a variety of experimental settings[17]. The presence of phenolic chemicals in the extract of *C. flexuosus* may explain its capacity to protect rats given cholesterol against the formation of hypercholesterolemia. It is well-known that the phenolic compounds can serve as antioxidants by reducing oxidative damage. As a result of a cascade of processes involving antioxidant enzymes, they can either directly trap free radicals or scavenge them. Research like this provides credence to the use of *C. flexuosus* in traditional medicine for the treatment of hypertension and heart conditions.

5. Conclusion

This study's findings corroborate previous research showing that *C. flexuosus* can reduce lipid levels in rats given a high-cholesterol diet. The lipid levels in the plasma, liver, heart, kidneys, and lungs were reduced by *C. flexuosus* at 100 and 200 milligrams/Kilogram, while the HDL cholesterol levels were elevated. *C. flexuosus* may have beneficial effects on health due to its antioxidant properties, which allow it to neutralise harmful free radicals and aid in the treatment of diseases caused by these molecules, such as cardiovascular disorders and free radical-mediated illnesses.

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7. Author Contribution

S.S. Writing – review and editing, T.S. Writing – original draft, A.D. Investigation and review a editing, N.V. Supervision, S.S. and N.G.- Revision of Manuscript. All authors have read and agreed to the published version of the manuscript.

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