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Original Article

PROPHYLACTIC LIPID LOWERING EFFECTS OF AQUEOUS EXTRACT OF *EMILIA PREATEMISSA* ON CARBON TETRACHLORIDE (CCL_4)-INDUCED HYPERLIPIDEMIC ALBINO RATS

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ABSTRACT

Objective: Hyperlipidemia has been described as one of the greatest risk factors contributing to the prevalence and severity of cardiovascular diseases which has been identified as a primary cause of death. This study evaluates the lipid-lowering effects of aqueous extract of *Emilia praetermissa* (EP) leaves.

Methods: The investigation was carried out on rats induced with Carbon tetrachloride (CCl_4 1 ml/kg body weight), pretreated with the aqueous extracts of *Emilia praetermissa* and then compared with a standard hypolipidemic drug, Simvastatin. The effects of *Emilia praetermissa* on the lipid profile were assessed by measuring the levels of total cholesterol, triglyceride, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol. The effect on HMGCoA Reductase and Lecithine Cholesterol Acyltransferase (LCAT), which are important enzymes in lipid metabolism were also evaluated in the plasma, liver and heart of albino rats.

Results: Administration of 50, 100 and 200 mg/kg doses of aqueous extract of *Emilia praetermissa* leaves led to a significant reduction (P<0.05) in total cholesterol, LDL cholesterol, triglyceride levels and a significant increase (P<0.05) in HDL cholesterol in the tissues in a manner close to that of Normal and Standard control group. *Emilia praetermissa* aqueous extract led to a significant increase (P<0.05) in the activity of LCAT and inhibition of HMGCoA reductase.

Conclusion: These results suggest that *Emilia praetermissa* leaves could play a cardioprotective role and probably serve as a new potential natural product for the management of hyperlipidema. Further investigations are warranted to elucidate the mechanism of its lipid-lowering action.

Keywords: Hyperlipidemia, *Emilia praetermissa*, Lipid Profile, HMG-CoA reductase, LCAT

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INTRODUCTION

In recent time, there has been a great deal of interest in the use of herbal remedies for treatment of human ailments. These remedies are used based on the fact that these medicines contain a large amount of natural substances which alleviate illness and promote health. World Health Organization (WHO) reported that more than 80% of the world population depends on traditional medicines for primary health care and they utilize the plant extracts or bioactive components present in them [1]. Hyperlipidemia is considered as one of the major risk factors causing cardiovascular diseases (CVDs). CVDs accounts for one third of total deaths around the world, it is believed that CVDs will turn out to be the main cause of death and disability worldwide by the year 2020 [2, 3].

Abnormal high dietary cholesterol which leads to hypercholesterolemia is strongly associated with cardiovascular diseases because it promotes atherosclerosis [4]. Indeed, it has been demonstrated that the development of coronary artery disease (CAD) is a function of the particle size of LDL-C and HDL-C, with the small particle size exhibiting great atherogenic potential [5]. Abundant evidence has accumulated relating the concentrations of lipids (total cholesterol and triglycerides) and their associated blood transporting lipoproteins (HDL-C, LDL-C, VLDL-C) with the occurrence of atherosclerosis in general and coronary artery disease (CAD) in particular. The strong association between the risk of coronary artery diseases (CAD), high levels of LDL-C and low levels of HDL-C has been well established [5].

The enzyme HMG-CoA reductase plays a vital role in the *de novo* cholesterol synthesis by catalyzing the conversion of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) to mevalonate, a rate-limiting step. Inhibition of HMG-CoA reductase alters the synthesis of cholesterol, proving beneficial in hypercholesterolemic conditions [6]. There are several drugs for the treatment of hypercholesterolemia which are majorly HMG CoA reductase inhibitors and most of these cholesterol lowering drugs are associated with some side effects; medicinal plants are being explored as natural hypocholesterolemic agents [6]

Emilia praetermissa is a species of flowering plant in the asteraceae family. It is a straggling herb common in farmlands and open cool places in the forest, especially along paths and roadsides. It is a forest plant distributed throughout western, central and southeast Africa [7]. In Nigeria, it is called (Igbo= nti-ele; Yoruba=Odundun). *Emilia pratermissa* is a useful plant of west tropical Africa generally used as food and medicine for general healing. In West Africa the leaves are occasionally eaten as a vegetable, either fresh in salads or cooked. This study was designed to evaluate the preventive and curative lipid-lowering effect of *Emilia pratermissa* as a source of a potential drug for the management of hyperlipidemia.

MATERIALS AND METHODS

Sample collection

The *Emilia praetermissa* (*Milne Redh*) leaves were harvested beside College of Health Technology in Ijero-Ekiti and it was identified at the Herbarium section of Plant Science and Biotechnology Department, Ekiti State University, Ado-Ekiti, Nigeria. A voucher specimen was deposited in the Departmental Herbarium with this voucher Number: UHAE-2017/105.

Apparatus/Equipment and reagents

Standard apparatus and equipment were used for the study. Standard chemicals and reagent kits was purchased from Randox Laboratories and Fortress kit, which was of analytical grade.

Aqueous extract preparation

The leaves of *Emilia praetermissa* was air-dried at room temperature, pulverized with an electric blender and then stored in a plastic container in the laboratory prior to analysis. The powdered leaves were extracted with 100g in 1000 ml of distilled water for 72h as described by the method of Farooq [8].

Experimental rats

Adult female albino Wistar rats, weighing 150–200g each were purchased from the breeding colony of Department of Biochemistry, Ekiti State University College of Medicine. They were acclimatized at 25 °C, on a 12h light/12h dark cycle for 2weeks before the experiment. The handling was guided by NIH Guide for the care and use of laboratory animals, and the ethical Committee for Animal Experimentation of the Ekiti State University.

CCl_4 preparation and induction

Carbon tetrachloride (CCl_4) was diluted with olive oil in ratio 1:1 dilution and 1 ml/kg body weight was administered intraperitonially, to induce hyperlipidemia. The CCl_4 dose followed was according to the dose that can induce oxidative stress as described by [9].

Experimental design

The animals were pre-treated with aqueous extract of *Emilia praetermissa* for a period of 19days before CCl_4 induction. The CCl_4 was given as a single acute dose after 19days of pretreatment with the extract and the animals were observed for 48 h before they were sacrificed on the 21st day of the experiment. Thirty (30) albino rats were divided into six (6) groups of five (5) rats each and subjected to different dosages of aqueous extract of *Emilia praetermissa* leaves: Group I (normal control) received an equivalent volume of water, group II served as Test control, Group III served as the Standard Control and received 100 mg/kg Simvastatin, Group IV received 50 mg/Kg body weight of extract, group V was pre-treated with 100 mg/kg of the extract and group VI was pre-treated with 200 mg/Kg body weight of extract orally daily. Animals were sacrificed after 21days by cervical dislocation, the blood sample was collected into a heparinised tube and organs (Liver and Heart) were excised and homogenized and centrifuged at 6,000rpm for 10 min. The dose was decided afer preliminary acute and subacute toxicity studies. The authors decided to use the above doses to see the effectiveness at not too low or too high doses.

Biochemical assays

Lipid profile analysis was carried out by estimating the Total Cholesterol (TC), Triglycerides (TG), High-Density Lipoprotein cholesterol (HDL-C) and Low-Density Lipoprotein cholesterol (LDL-C) as described by [10]. The HMG-CoA reductase activity was assayed colorimetrically using the method of [11] with some modifications, Lecithine Cholesterol Acyltransferase (LCAT) was evaluated by the method of [12].

Data analysis

The results were analysed using Software Package for Social Sciences (SPSS) version 20 and the results were expressed as mean±standard deviation (SD) and mean P values<0.05 were considered as significant.

RESULTS

Effect of *Emilia praetermissa* on the levels of lipids and lipoproteins

Tables 1,2 and 3 present the levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) in the plasma, liver and the heart in normal and CCl_4-induced rats. CCl_4-induced rats showed a significant increase in the levels of TC, TG, and LDL-C in in the tissues when compared to normal control rats. Pre-treatment with *Emilia praetermissa* to CCl_4-induced rats significantly minimized the alterations in these parameters when compared to CCl_4-alone induced rats.

Table 4 shows the levels of high-density lipoproteins (HDL), in the tissues of normal and CCl_4-induced rats. Rats treated with CCl_4 showed a significant decrease in HDL. Pre-treatment with *Emilia praetermissa* to CCl_4-induced rats showed subsequent increase in the levels of HDL cholesterol.

Effect of *Emilia praetermissa* on the levels of lipid metabolic enzymes

Table 5 and 6 show the activities of lecithin cholesterol acyl transferase (LCAT) and HMG CoA reductase in plasma, liver and heart of normal and CCl_4-induced rats. A Significant decrease of the activities of LCAT in CCl_4 untreated group when compared with the Normal control was noticed. *E. praetermissa* extract elevate the activities of LCAT significantly in the group pre-treated with 200 mg/kg of Extract in plasma, liver and heart of the experimental rats. The notable activities of LCAT in the plasma of group treated with highest does of the extract are also similar to a standard drug (100 mg/kg of Simvastatin) and the normal control group. A Significant increase in the activities of HMG-CoA reductase in CCl_4 untreated group when compared with the Normal control was observed. *E. praetermissa* inhibits the activities of HMG-CoA reductase significantly in the group pre-treated with 200 mg/kg Extract in plasma, liver and heart of the experimental rats, which is also similar to the effect of the standard HMG-CoA reductase inhibitor (100 mg/kg of Simvastatin) and the normal control group.

Table 1: Prophylactic effect of *Emilia preatemissa* aqueous extract on total cholesterol (mmol/dl) in albino rats tissues

Groups	Treatment	Plasma	Liver	Heart
1	Normal control	65.10 ± 6.82^{a}	144.41 ± 12.42^{a}	40.80 ± 3.51^{a}
2	Positive control (CCl_4)	125.13 ± 13.11^{b}	156.97 ± 13.50^{b}	46.47 ± 3.99^{a}
3	Standard drug (Simvastatin)	75.81 ± 7.94^{c}	31.92 ± 2.74^{c}	27.56 ± 2.37^{b}
4	Extract of 50 mg/kg	117.53 ± 12.32^{b}	93.57 ± 8.05^{d}	42.03 ± 3.61^{ab}
5	Extract of 100 mg/kg	94.89 ± 9.94^{d}	39.70 ± 3.41^{c}	38.22 ± 3.28^{ab}
6	Extract of 200 mg/kg	85.84 ± 8.99^{c}	37.36 ± 3.21^{c}	35.14 ± 3.02^{b}

Results are mean of 5 determinations±Standard Deviation (SD). Using Analysis of Variance (ANOVA) and Duncan Multiple Range Test. Means with different superscript are significantly different at ($P<0.05$).

Table 2: Prophylactic effect of *Emilia preatemissa* on Triglyceride (mmol/dl) in albino rats tissues

Groups	Treatment	Plasma	Liver	Heart
1	Normal control	100.72 ± 10.55^{a}	53.21 ± 4.57^{a}	57.68 ± 4.96^{a}
2	Positive control	141.37 ± 14.81^{b}	89.42 ± 7.69^{b}	85.43 ± 7.35^{b}
3	Standard drug (Simvastatin)	110.06 ± 11.53^{a}	61.04 ± 5.25^{c}	62.09 ± 5.34^{a}
4	Extract of 50 mg/kg	118.86 ± 12.45^{a}	81.99 ± 7.05^{b}	70.44 ± 6.06^{c}
5	Extract of 100 mg/kg	113.12 ± 11.85^{a}	74.36 ± 6.39^{ac}	67.65 ± 5.82^{a}
6	Extract of 200 mg/kg	107.49 ± 11.26^{a}	68.68 ± 5.91^{c}	66.98 ± 5.76^{a}

Results are mean of 5 determinations±Standard Deviation (SD). Using Analysis of Variance (ANOVA) and Duncan Multiple Range Test. Means with different superscripts are significantly different at ($P<0.05$).

Table 3: Prophylactic effect of *Emilia preatemissa* on low density lipoprotein cholesterol (mmol/dl) in albino rats tissues

Groups	Treatment	Plasma	Liver	Heart
1	Normal control	54.14±5.67[a]	66.97±5.76[a]	28.90±6.89[a]
2	Positive control	98.36±10.31[b]	99.53±8.56[b]	56.03±13.37[b]
3	Standard drug (Simvastatin)	65.91±6.90[c]	79.78±6.86[c]	37.08±8.85[c]
4	Extract of 50 mg/kg	80.04±8.39[b]	90.2±7.76[b]	37.51±8.95[c]
5	Extract of 100 mg/kg	74.92±7.85[c]	84.23±7.24[c]	33.43±7.97[ac]
6	Extract of 200 mg/kg	55.58±5.82[a]	77.09±6.63[ac]	29.25±6.98[a]

Results are mean of 5 determinations±Standard Deviation (SD). Using Analysis of Variance (ANOVA) and Duncan Multiple Range Test. Means with different superscripts are significantly different at (P<0.05).

Table 4: Prophylactic effect of *Emilia preatemissa* on high density lipoprotein cholesterol (mmol/dl) in albino rats tissues

Groups	Treatment	Plasma	Liver	Heart
1	Normal control	112.35±16.26[a]	62.61±5.38[a]	49.11±4.22[a]
2	Positive control	64.19±9.29[b]	30.35±2.61[b]	30.51±2.62[b]
3	Standard drug (Simvastatin)	119.52±17.30[a]	59.93±5.15[a]	43.80±3.76[a]
4	Extract of 50 mg/kg	72.38±10.48[b]	33.12±2.85[b]	34.96±3.00[b]
5	Extract of 100 mg/kg	92.43±13.38[a]	39.28±3.38[b]	42.73±3.67[a]
6	Extract of 200 mg/kg	104.27±15.09[a]	50.27±4.32[a]	51.38±4.42[a]

Results are mean of 5 determinations±Standard Deviation (SD). Using Analysis of Variance (ANOVA) and Duncan Multiple Range Test. Means with different superscripts are significantly different at (P<0.05).

Table 5: Prophylactic effect of *Emilia preatemissa* on LCAT (Unit/mg protein) in albino rats tissues

Groups	Treatment	Plasma	Liver	Heart
1	Normal control	10.86±1.13[a]	15.38±1.32[a]	5.98±0.51[a]
2	Positive control	7.70±0.80[b]	9.34±0.80[b]	3.84±0.33[b]
3	Standard drug (Simvastatin)	9.85±1.03[a]	13.29±1.14[a]	6.26±0.53[a]
4	Extract of 50 mg/kg	7.85±0.82[ab]	10.43±0.89[b]	4.21±0.36[b]
5	Extract of 100 mg/kg	8.38±0.87[a]	11.23±0.96[a]	4.56±0.39[a]
6	Extract of 200 mg/kg	9.26±0.97[a]	13.27±1.14[a]	5.52±0.47[a]

Results are mean of 5 determinations±Standard Deviation (SD). Using Analysis of Variance (ANOVA) and Duncan Multiple Range Test. Means with different superscripts are significantly different at (P<0.05).

Table 6: Prophylactic effect of *Emilia preatemissa* on HMG-CoA reductase (Unit/mg protein) in albino rats tissues

Groups	Treatment	Plasma	Liver	Heart
1	Normal control	107.68±11.28[a]	62.55±5.38[a]	45.94±3.95[a]
2	Positive control	132.45±13.88[b]	87.00±7.48[b]	57.00±4.90[b]
3	Standard drug (Simvastatin)	98.35±10.30[c]	57.01±4.90[a]	42.69±3.67[a]
4	Extract of 50 mg/kg	117.95±12.36[b]	68.11±5.86[a]	55.92±4.81[b]
5	Extract of 100 mg/kg	108.48±11.37[a]	59.77±5.14[a]	51.46±4.42[ab]
6	Extract of 200 mg/kg	101.80±10.67[ab]	54.79±4.71[a]	44.71±3.84[a]

Results are mean of 5 determinations±Standard Deviation (SD). Using Analysis of Variance (ANOVA) and Duncan Multiple Range Test. Means with different superscript are significantly different at (P<0.05).

DISCUSSION

Hyperlipidemia has been the basic risk factor involved in the development of cardiovascular diseases (CVD); therefore, it becomes very important to explore the use of medicinal plants using scientific means to counter the menace of the life-threatening health condition. Elevated Total cholesterol level is a recognized and well-established risk factor for developing atherosclerosis and other cardiovascular diseases [13, 14]. Therefore, a reduction in total cholesterol level reduces the risk of cardiovascular diseases. An elevated plasma triglyceride level is both an independent and synergistic risk factor for cardiovascular diseases [15]. Oxidative modification of LDL is one of the key steps in the development of atherosclerosis. The LDL-cholesterol lowering could result from an increased LDL metabolism and/or a reduced LDL-synthesis[16]. Decrease in LDL-C has been considered to reduce risk of coronary heart disease [17], while the elevated level of LDL cholesterol and decreased HDL cholesterol is a risk factor for cardiovascular disease [13] and often accompany obesity [18]. Clinical data revealed that increase in HDL cholesterol concentration decreases cardiovascular risk [19].

In this study, pre-treatment with aqueous extracts of *Emilia praetermissa* significantly decrease the concentrations of Total Cholesterol (TC), Triglyceride (TG) and Low-Density Lipoprotein Cholesterol when compared with CCl_4 untreated group and the standard drug (100 mg/kg of simvastatin) pre-treated group. The significant increase caused by CCl_4 administration was found to be reduced in the treated groups in a dose-dependent manner but the most significant was noticed in the group treated with highest dose (200 mg/kg) of the extract as the significant reduction were almost similar to both the normal control and the standard drug. However, the High-Density Lipoprotein cholesterol significantly increased in a dose-dependent manner in the groups pre-treated with extracts when compared with CCl_4 untreated group. The administrations of the 200 mg/kg extract were similar to the elevated level of HDL in groups treated with 100 mg/kg of simvastatin. These results were similar to previous research by [13]. Therefore, a reduction in total

cholesterol level reduces the risk of cardiovascular diseases. Thus, the significantly lower total cholesterol levels produced by the aqueous extract of *Emilia praetermissa*, revealed the ability of the extract to protect against cardiovascular diseases and could be used in its management.

The major metabolic pathway for reducing cholesterol is via conversion to bile acids or preventing the cholesterol synthesis by inhibiting the HMG CoA reductase enzyme. This result shows a significant reduction of HMG CoA reductase activities in the pre-treated animals. This inhibition is dose-dependent between the groups. *E. praetermissa* inhibits most the activities of HMG-CoA reductase significantly in the group pre-treated with 200 mg/kg Extract in plasma, liver and heart of the experimental rats. The notable inhibition by the highest does of the extract is also similar to a standard HMG-CoA reductase inhibitor (100 mg/kg of Simvastatin) and the normal control group. The administration of lower dose of extract (50 mg/kg) slightly inhibits the enzymes activities but is not statistically significant. These results confirmed that the administration of *E. praetermissa* inhibits the activities of HMG-COA reductase significantly. Clinical trials have shown that the use of HMG-CoA reductase inhibitors in patients with coronary risk improved endothelial function through reduction of oxidative stress and/or up-regulation of NO activities [20].

Lecithin Cholesterol Acyltransferase (LCAT) is an enzyme that is responsible for transesterification of cholesterol and its activities were reported to be inhibited by CCl_4 that resulted to hyperlipidemia. LCAT is one of the major modulators of plasma high-density lipoprotein cholesterol (HDL-C) and plays a central role in the reverse cholesterol transport (RCT) process [21]. LCAT is a plasma enzyme that circulates mostly in association with the high-density lipoproteins (HDL) and is responsible for the synthesis of cholesterol esters present in human plasma. Cholesterol esterification catalysed by LCAT also reduces the amount of unesterified cholesterol in plasma. The activities of LCAT was significantly reduced in the CCl_4 Untreated group as compared to Normal control, groups treated with standard drugs and groups pre-treated with plant extracts of different doses. *E. praetermissa* extract elevate the activities of LCAT significantly in the group pre-treated and treated with 200 mg/kg of the extract in plasma, liver and heart of the experimental rats. The notable activities of LCAT in the plasma of group treated with highest does of the extract were also similar to a standard drug (100 mg/kg of Simvastatin) and the normal control group. This demonstrates the increase of esterification of cholesterol which lead to the synthesis of HDL-C. LCAT plays a key role in the incorporation of free cholesterol into HDL and its transfer back to VLDL and LDL, which are later returned in liver cells [21]. Obesity is a chronic problem that has to be addressed in various ways. According to the World Health Organization (WHO), "Obesity is characterized by abnormal or excessive accumulation of body fat that can be harmful to the health." [22] Nearly all the lipid profile biochemical assay analysed indicate a significant possible protective mechanism of the *Emilia praetermissa* extract against the hyperlipidemia and other lipid related disorder.

CONCLUSION

The results from this study have shown that pre-treatment with aqueous extract of *Emilia praetermissa* leaves for three weeks significantly reduced the plasma total cholesterol concentration with its LDL-fraction. Therefore, it may be regarded as a useful therapy for hyperlipidemia lowering of circulating cholesterol. Also, the inhibition of HMG-CoA reductase by the aqueous extract of *Emilia praetermissa* leaves shows that it exhibits a cardioprotective effect could help prevent coronary atherosclerosis.

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AUTHORS CONTRIBUTIONS

All authors scientifically contributed in the preparation and execution of this manuscript.

CONFLICT OF INTERESTS

The authors declare that we have no conflict of interest.

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