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Hypolipidemic Properties of Ethanol Extract of *Cyperus rotundus* Rhizome

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GNO, SEA and COU conceptualized and designed the work, wrote the protocol and interpreted the data. Authors GNO, SEA, UVN and ACE anchored the laboratory work, gathered the initial data and performed preliminary data analysis. Author GNO handled the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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Original research Article

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ABSTRACT

This study investigated the effect of ethanol extract of *Cyperus rotundus* rhizome on hyperlipidemia induced with carbimazole and cholesterol in male wister rats. Acute toxicity analysis with the *Cyperus rotundus* rhizome extract produced no letality even at higher doses. Hyperlipidemia was induced using 400 mg/kg cholesterol and 2 mg/kg carbimazole. The lipemic control group was administered cholesterol and carbimazole but not the normal control group. Cholesterol and carbimazole administration caused a significant ($p = 0.05$) increase in the Total Cholesterol, Triglyceride (TG), Low Density Lipoprotein (LDL), non-High Density Lipoprotein (non-HDL) Cholesterol and LDL/HDL ratio and a significant ($p = .05$) decrease in the levels of HDL cholesterol in the lipemic control when compared to the normal control. Treatment with ethanol extract of *Cyperus rotundus* at 250 mg/kg, 500 mg/kg and the standard hypolipidemic drug (simvastatin) at 5mg/kg significantly ($p = 0.05$) reduced total cholesterol, TG, LDL, LDL/HDL ratio, total non-HDL Cholesterol and also significantly ($p=.05$) increased the level of HDL cholesterol when compared to

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the non-treatment group (the lipemic control group). Results of the present study indicate that *Cyperus rotundus* rhizome contains principles that have hypolipidemic potentials and which compare effectively with standard clinically used therapeutic Hypolipidemic agent, simvastatin.

Keywords: Cholesterol; carbimazole; Cyperus rotundus rhizome; hypolipidemia; simvastatin.

1. INTRODUCTION

Coronary heart disease (CHD) has been described as the most common cause of mortality and morbidity worldwide [1]. It is responsible for 17.1 million fatalities each year and the fatalities are expected to reach 20million in 2020 [2,3]. In Nigeria, CHD continues to be a major public health problem and available statistics indicate a high prevalence of risk factors of CHD (hypertension and hypercholesterolemia) among Nigerian adults [4]. Studies have shown an overall prevalence rate of 62.5% for hypercholesterolemia in adult Nigerians who present systemic hypertension [5]. According to WHO reports in 2008, the prevalence of hypertension in Nigeria was estimated at 42.8%, raised cholesterol was estimated at 16.1% while the cardiovascular diseases (CVDs) accounted for an estimated 12% of all deaths in Nigeria [6].

Hyperlipidemia is a term used to describe elevated plasma levels of lipids (triglycerides and cholesterol) and lipoproteins. Concentration of plasma total cholesterol and low density lipoprotein (LDL) cholesterol are highly correlated with the prevalence of CHD while a high plasma high density lipoprotein (HDL) cholesterol concentration is a powerful protective factor against CHD [7].

Currently available cardioprotective/ hypolipidemic drugs are not only very expensive but have been associated with a number of side effects like kidney and liver impairment, gall stone formation, rhabdomylosis (destruction of skeletal muscles) and gastrointestinal disturbances [8].

Herbs or medicinal plants have high density of important nutrients such as minerals, vitamins, phytochemicals and natural antioxidants which have a wide range of pharmacological properties that can prevent or ameliorate the effect of chronic diseases such as cancer, arteriosclerosis, diabetes etc [9,10]. These medicinal plants can serve as alternatives to conventional drugs especially as they are presumed safe [11]. The phytochemicals - phenols, steroids, alkaloids, glycosides and diterpenoids have shown potential in the prevention and management of cardiovascular disorders [12-14].

These phytochemicals have been shown to be present in the rhizomes of a medicinal plant, *Cyperus rotundus* Linn (family-cyperaceae) [15-17]. *Cyperus rotundus* L. is found in the temperate, tropical and subtropical zones of the world and the rhizomes of this plant have been reported to possess various medicinal properties like anthelmintic, antipyretic, antidepressant, anti-rheumatic, antispasmodic and anti-fungal [1,18]. In view of its phytochemical composition and its several therapeutic properties, the ethanol extract of *C. rotundus* rhizomes was investigated for its effect on hyperlipidemia. In other words, its hypolipidemic (lipid lowering) activities were investigated and these hypolipidemic activities were compared to the standard clinically used therapeutic hypolipidemic drug, simvastatin.

2. MATERIALS AND METHODS

2.1 Collection and Extraction of Plant Materials

Plant Sample was collected from a farm at Egbeada housing estate in Owerri Municipal, Imo State, Nigeria. The identity of the plant was authenticated by Mr F.O. Iwueze of the Department of Forestry and Wildlife, Federal University of Technology, Owerri. It was thereafter deposited at the plant herbarium of Imo State University, Owerri with voucher number IMSU/025. The collected samples of rhizomes were sorted to remove extraneous materials, washed and dried to constant weight under a shade for one week and then ground into fine powder using a mill. About 300 g portion of the ground plant material was soaked in 800 ml of 80% ethanol and allowed to stand for 4 days. This was continuously shaken to ensure uniform extraction. The slurry sediment was removed by coarse filtration using a sieve, and afterwards the filtrate was filtered with Whatman No1 filter paper. The filtrate was evaporated at 49°C under reduced pressure using a rotary

evaporator, and then dried to a semi-solid form in a vacuum desiccator to obtain the ethanol extract of *C. rotundus* rhizome. The concentrated extract was then stored in a refrigerator at 4°C until required for analysis.

2.2 Acute Toxicity Study

The median lethal dose (LD_{50}) of *C. rotundus* extract was determined in rats using Lorke's method [19]. Male wistar rats fasted for 16 h were randomly divided into groups of three rats each. Three groups of three rats each were orally administered with the *C. rotundus* extract at doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight and were observed for 24 hours. The fourth group of three rats served as control and were given distilled water equivalent to volume of extract administered (2 ml/150 g bwt). In the second phase, four groups of one rat each were administered orally with *C. rotundus* extract at doses of 1500 mg/kg, 2250 mg/kg, 3500 mg/kg and 5000 mg/kg. The rats in both the 'test' and 'control' groups were then allowed free access to food and drinking water, and observed over a period of 72 hrs for behavioural changes and mortality. No observable behavioural change or mortality was noticed. Preliminary studies on the dose-dependent antihyperlipidemic properties of *Cyperus rotundus* rhizome extract showed maximum effect at 500 mg/kg. Based on this, we decided to use doses of 250 mg/kg and 500 mg/kg for the study.

2.3 Hypolipidemic Activity

Male Wistar rats weighing 60-90 g and 6 weeks old were bought from the animal house of Department of Verterinary Medicine, University of Nigeria, Nsukka. The animals were housed in metallic cages under hygienic conditions and natural light/dark cycle. This study was approved by the ethics committee of the Department of Biochemistry, Federal University of Technology, Owerri, Nigeria. All experiments were performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. After 7days acclimatization period, being fed with standard animal feed (Pelletized Vital Finisher) and portable water, 30 healthy rats were divided into 5 groups of 6 animals each for the determination of hypolipidemic effect of *C. rotundus*. Treatments were as follows:

Group I served as normal control (NC) and received food and water only *ad-libitum* throughout the treatment period.

Group II served as lipemic control (LC). They received food and water *ad libitum* and 2 mg/kgbwt of carbimazole, 400 mg/kgbwt of cholesterol in olive oil by intubation and also received 0.001% carbimazole in drinking water daily.

Groups III served as tests (250 mg/kgbwt *C. rotundus*) This group received food and water *ad libitum*, and 2 mg/kgbwt of carbimazole, 400 mg/kgbwt of cholesterol in olive oil, 250 mg/kgbwt *C. rotundus* extract by intubation, and also received 0.001% carbimazole in drinking water daily.

Group IV served as tests (500 mg/kgbwt *C. rotundus*) This group received food and water *ad libitum*, and 2 mg/kgbwt of carbimazole, 400 mg/kgbwt of cholesterol in olive oil, 500 mg/kgbwt *C.rotundus* extract by intubation, and also received 0.001% carbimazole in drinking water daily.

Group V served as reference Standard (5 mg/kgbwt Simvastatin) This group received food and water *ad libitum*, and 2 mg/kgbwt of carbimazole, 400 mg/kgbwt of cholesterol in olive oil, 5 mg/kgbwt simvastatin by intubation, and also received 0.001% carbimazole in drinking water daily.

At the end of twenty eight days of daily administration, animals were fasted overnight for 12hrs and after being lightly anaesthetized, were sacrificed by cervical dislocation and blood collected by cardiac puncture. Serum was separated by centrifugation at 600×*g* for 15 min and analyzed for various biochemical parameters. The enzymatic (cholesterol esterase/oxidase/peroxidase) method [20] was used for determination of total cholesterol (TC). The glycerol phosphate oxidase/peroxidase methods [21,22] were used in the determination of triglycerides (TG). The determination of Low Density Lipoprotein (LDL)-Cholesterol was carried-out as described [23]. The serum HDL-cholesterol concentration was determined using the phosphotungstate/Mg-cholesterol oxidase and peroxidase method [24]. The entire experiment lasted for a period of 6 months.

2.4 Data Analysis

Each sample was analysed in triplicate and data is expressed as mean±SD. Data was analysed using the statistical software "Analyze-it" for Microsoft excel and was analysed for statistical difference by one-way analysis of variance (ANOVA). The differences were considered significant at (p = .05).

3. RESULTS AND DISCUSSION

Elevated lipid levels, especially total cholesterol, triglycerides and decreased HDL cholesterol, are proven risk factors of Coronary Heart Disease [25]. In the present study, hyperlipidemia was induced by cholesterol and carbimazole. Cholesterol and carbimazole administration induced a significant (p =. 05) increase in the level of total cholesterol, triglyceride and LDL cholesterol when compared to the normal base line group (see lipemic control group, LC and normal control group, NC in Figs.1,2,4) and a significant (p = .05) decrease in the levels of HDL cholesterol (Fig. 3).

Administration of *C. rotundus* extract at 250 mg/kg and 500 mg/kg as well as the hypolipidemic drug, simvastatin at 5 mg/kg caused a significant (p = .05) decrease in serum total cholesterol and LDL cholesterol (Figs. 1,4). Triglyceride was also significantly reduced at 500mg/kg extract and simvastatin at 5mg/kg but the decrease at 250 mg/kg extract was not significant (Fig. 2). These results are consistent with the findings of previous studies in which alcoholic extract of *Cyperus rotundus* rhizome exhibited significant (p = .05) reduction in cholesterol, triglyceride and LDL-Cholesterol in high fat diet induced hyperlipidemia in rats [1] and in isoproterenol induced hyperlipidemia in rabbits [18].

In this study the level of HDL cholesterol was significantly (p = .05) increased in the treatment groups (Fig 3). Statistically significant increase in HDL cholesterol in the treatment groups had been observed in a previous study on the antilipidemic potential of methanol extract of *Cyperus rotundus* rhizome in isoproterenol induced hyperlipidemia in rabbits [18]. Increase in HDL-cholesterol is said to reduce atherogenic risk by virtue of increased reverse cholesterol transport from peripheral organs to the liver [26]. The significant decrease (p =.05) in LDL cholesterol (Fig. 4), LDL/HDL ratio and in total Non-HDL cholesterol (Figs. 5, 6) suggests abeneficial modulatory influence oncholesterol metabolism by the extract. Elevated levels of serum LDL cholesterol play a crucial role in arteriosclerosis, a progressive multifactorial disease of the arterial wall considered the most frequent cause of cardiovascular disease [17]. The significant increase in the level of HDL cholesterol and the significant decrease in the level of LDL cholesterol in the treatment groups in this study indicate that the ethanolic extract of *Cyperus rotundus* rhizome may be used to reduce the risk factors for cardiovascular diseases.

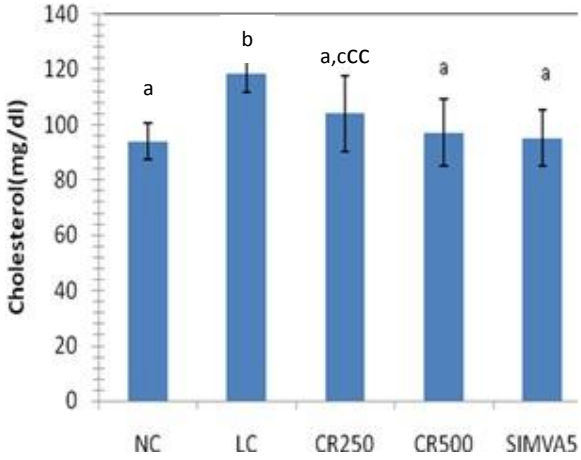

Fig. 1. Effect of *Cyperus rotundus* ethanol extract on total Cholesterol concentration of dyslipidemic male Wistar rats. Bars with different alphabets are statistically significant p = .05

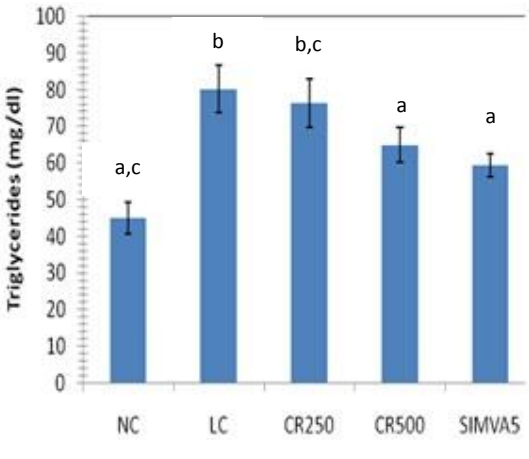

Fig. 2. Effect of *Cyperus rotundus* ethanol extract on Triglyceride concentration of dyslipidemic male Wistar rats. Bars with different alphabets are statistically significant p = .05

NC = Normal control group, LC = Lipemic control group, CR250 = Group administered 250 mg/kgbwt Cyperus rotundus extract, CR500 = Group administered 500 mg/kgbwt Cyperus rotundus extract, SIMVA5 = Group administered 5 mg/kgbwt of reference standard drug (Simvastatin)

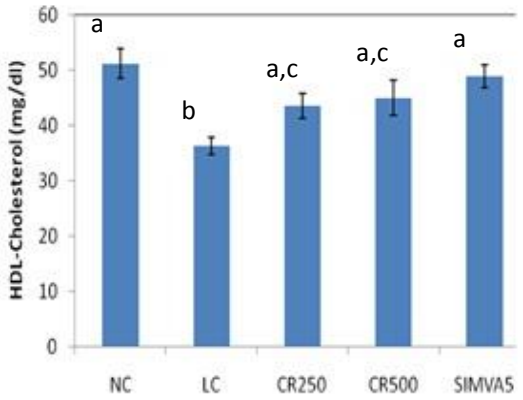

Fig. 3. Effect of *Cyperus rotundus* ethanol extract on HDL-Cholesterol concentration of dyslipidemic male Wistar rats. Bars with different alphabets are statistically significant p = .05

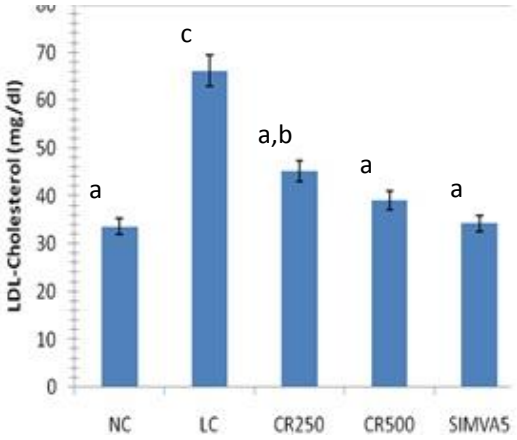

Fig. 4. Effect of *Cyperus rotundus* ethanol extract on LDL-Cholesterol concentration of dyslipidemic male Wistar rats. Bars with different alphabets are statistically significant p = .05

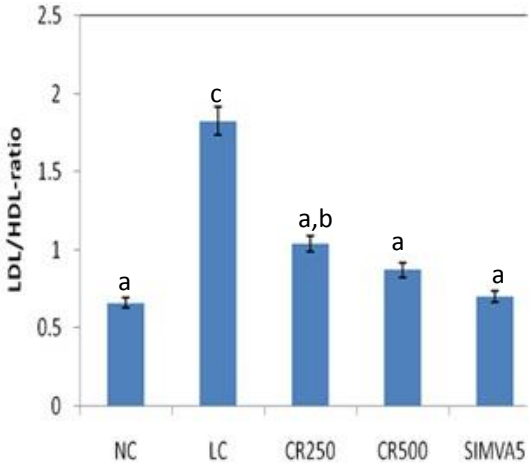

Fig. 5. Effect of *Cyperus rotundus* ethanol extract on LDL/HDL-Cholesterol-ratio of dyslipidemic male Wistar rats. Bars with different alphabets are statistically significant p = .05

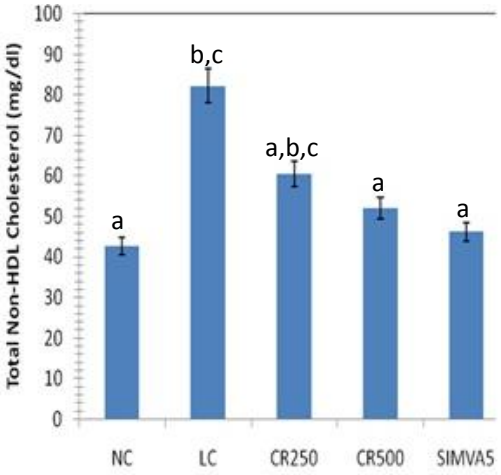

Fig. 6. Effect of *Cyperus rotundus* ethanol extract on Total Non- HDL-Cholesterol dyslipidemic male Wistar rats. Bars with different alphabets are statistically significant p = .05

In all the determinations, the extract was observed to be more effective at 500 mg/kg than at 250 mg/kg, although not statistically significant in most cases. In the same vein, although the standard hypolipidemic agent, simvastatin was observed to be slightly more effective `than the extract at 500 mg/kg, the difference was not satistically significant. This indicates that the ethanolic extract of *Cyperus rotundus* rhizome may compare favourably with the hypolipidemic drug, simvastatin.

4. CONCLUSION

The results of this study clearly demonstrate that the bioactive compounds present in *Cyperus rotundus* rhizome have hypolipidemic potentials and compare effectively with the standard clinically used therapeutic hypolipidemic agent, simvastatin.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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