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Effect of Ethanol leaf extract of *Chromolaena odorata* on lipid profile of streptozotocin induced diabetic wistar albino rats.

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ABSTRACT

Poor control of diabetes mellitus can result to impairment in lipid profile culminating to dyslipidemia, coronary artery disease and stroke. Measurement of triglyceride (TAG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) are recommended in cardiovascular screening. Herbal and natural products have been used in folk medicine for centuries throughout the world. The aim of this research was to determine the effect of ethanol leaf extract *of Chromolaena odorata* on lipid profile of streptozotocin-induced diabetic wistar albino rats. All the chemicals and reagents used in this research were of analytical grade. A total of 48 rats were randomly divided into 6 groups (n=8): diabetic rats in group 1 were not treated, rather received only 0.5ml normal saline; 0.5mg glibenclamide was given to diabetic rats in group 2; non-diabetic rats in group 3 received 0.5ml normal saline only, diabetic rats that were treated with 250 mg/kg, 350mg/kg and 450mg/kg b.w of ethanol leaf extract of *Chromolaena odorata*, were labeled groups 4, 5 and 6, respectively. At the end of the 21 days study period, the rats were fasted overnight and blood samples were collected via cardiac puncture. Lipid profile was assayed using standard biochemical methods. Injection of streptozotocin led to a significant ($p<0.05$) decline in HDL-C while the levels of TAG, TC, and LDL-C increased significantly. Remarkably, treatment with 250 mg/kg, 350mg/kg and 450mg/kg b.w of ethanol leaf extract of *Chromolaena odorata* led to reversal of the altered lipid profile. However, there were no significant differences ($p>0.05$) when the *Chromolaena odorata* extract-treated groups were compared to group 2 rats (treated with glibenclamide), a known standard antidiabetic drug. In conclusion, results from this research indicated that the ethanol leaf extract of *Chromolaena odorata* possess hypo-cholesterolaemic and hypo-triacylglycerolaemic effects as the extract decreased the LDL-cholesterol and increased the HDL-cholesterol levels.

Keywords: *Chromolaena odorata,* cholesterol, streptozotocin, Diabetes mellitus, Lipid profile, dyslipidemia.

INTRODUCTION

Diabetes mellitus (DM) is one of the global life-threatening metabolic disorders. It is characterized by hyperglycemia as a result of impaired insulin secretion, action or both [1]. Insulin resistance causes the emergence of serious complications like hypertension, dyslipidemia, and atherosclerosis [2]. The prevalence and burden of diabetes mellitus is very high globally. Based on insulin deficiency, DM can be classified into three viz: Types 1, 2 and gestational.

Type 1, also called Insulin Dependent Diabetes Mellitus (IDDM) or juvenile onset diabetes, accounts for 5–10% of the patients. It results from cellular-mediated autoimmune destruction of the pancreatic cells. It affects people of all ages but usually occurs in children or young adults and follows a hereditary pattern and it is common in people of African and Asian descent [3]. Noninsulin Dependent Diabetes Mellitus (NIDDM) also referred to as adult onset diabetes, accounts for 90–95% of all DM. Metabolic syndromes like obesity, insulin resistance, and dyslipidemia contribute to type 2 diabetes [4]. Type 2 DM is the most common form of diabetes and is the fourth leading cause of death in developed countries with increased risk of coronary heart disease and stroke [5]. Gestational Diabetes Mellitus (GDM) is hyperglycemia commonly diagnosed during pregnancy. GDM is a risk factor for type 2 diabetes in mothers [6]. There has been several medical breakthroughs in the management of DM including, stimulation of insulin secretion like Exenatide, sulfonylureas and Liraglutide; insulin injections; dipeptidyl peptidase-4 (DPP-4) inhibitors like Sitagliptin, metformin [7-9]. The hallmark of management of DM is achievement of complete glycemic regulation, possibly through assessment of present glycemic status [10]. Poor control of DM can result to impairment in lipid profile culminating to dyslipidemia, coronary artery disease and stroke [11]. Measurement of triglyceride (TAG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) are recommended in cardiovascular screening [12].

Medicinal plants are natural resources used widely in management of various diseases by rural communities, development of modern drugs and other pharmacological products [13]. Recently, many plant species have shown promising effects against diabetes mellitus [14-18]. More so, hypo-cholesterolaemic and hypo-triacylglycerolaemic effects of medicinal plants are well documented [19-25]. *Chromolaena odorata* is one of the medicinal plants with acclaimed benefits in the treatment and management of diseases. It belongs to the family *Asteraceae*. It originated from America but has spread to other regions including Nigeria [26]. *Chromolaena odorata* been reported to possess anti-inflammatory and hepatoprotective activities. Some rural dwellers in South-eastern Nigeria use the leaves for wound dressing, skin infection and to stop bleeding [27]. *Chromolaena odorata* have some phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds, which a responsible for their physiological action in man [27]. Despite the medical advancement in the management of DM, patients still face many health challenges emanating from use of antidiabetic drugs coupled with high cost of such drugs. The persistent increase in global morbidity and mortality rates of diabetes mellitus patients is high. There is therefore a dire need for the discovery of antidiabetic protocols that are readily available, less toxic and cost-effective. Therefore, the aim of this research was to determine the effect of ethanol leaf extract of *Chromolaena odorata* on lipid profile of streptozotocin-induced diabetic albino rats since individuals with diabetes have increased risk of lipid profile dysregulation and its associated health complications.

MATERIALS

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade and standard. Commercial test kits were sourced from products of Randox, UK.

Procurement of Biological Materials

Fresh leaves of *Chromolaena odorata* were collected from Abakaliki in Ebonyi State, Nigeria. This was authenticated by Nwankwo Onyebuchi, a plant Taxonomist in Ebonyi State University, Abakaliki, Nigeria. Forty-Eight (48) albino wistar rats were procured from the Department of Zoology, University of Nigeria, Nsukka, Nigeria. Rats were kept for two weeks prior to start of experiment, for

acclimatization. They were maintained at room temperature, and allowed unlimited access to water and growers mash. They were weighed prior to commencement of experiment and daily till the end of the experiment.

METHODS

Preparation of Plant Material

Matured fresh leaves of *Chromolaena odorata* were harvested, washed and air dried under room temperature for three weeks. Thereafter, it was milled to powder form using grinding machine.

Extraction of Plant Material

Exactly 500g of powdered leaves of *Chromolaena odorata* were soaked in 1500ml of ethanol with intermittent shaking for 72h at room temperature. The resulting solution was filtered using Whatman No.1 filter paper. Thereafter, filtrates were concentrated using rotary evaporator and a slurry extract was obtained. The extract was used for the experiment.

Experimental Design

A total of 48 Wistar albino rats were used and randomly distributed into 6 groups (n= 8). At the end of the 21 days study period, the animals fasted overnight and blood samples were collected after chloroform anaesthesia via cardiac puncture, into plain and lithium heparin bottles. The samples were centrifuged at 5000r/min for 10mins to obtain plasma and serum. The plasma and serum supernatants were separated into sterile plain bottles and were used for biochemical assays.

Animal grouping

Group 1: (Positive control) Rats in this group were induced with diabetes without treatment and received 0.5ml of normal saline daily.

Group 2: (Standard control) Rats in this group were induced with diabetes and treated with 0.5mg/kg body weight of glibenclamide daily.

Group 3: (Negative control) Rats in this group were not induced with diabetes and were not treated but received 0.5 ml of normal saline daily.

Group 4: Diabetic rats treated with 250 mg/kg body weight of the crude ethanol leaf extract of *Chromolaena odorata* daily.

Group 5: Diabetic rats treated with 350 mg/kg body weight of the crude ethanol leaf extract of *Chromolaena odorata* daily.

Group 6: Diabetic rats treated with 450 mg/kg body weight of the crude ethanol leaf extract of *Chromolaena odorata* daily.

Induction of Diabetes

The baseline blood glucose levels were assayed prior to induction of diabetes. After overnight fasting, diabetes was induced to rats by intraperitoneal injection of streptozotocin with a single dose of 70mg/kg body weight. STZ was dissolved in 0.1M citrate buffer at pH of 4.5. The Accu-Check one-touch blood glucose monitoring meter and test strips were used to determine glucose level. After three days, rats with blood glucose level greater than 250mg/dl that exhibit hyperglycemia were selected for the experiment.

Determination of Lipid profile of STZ-induced Diabetic Albino Rats

Levels of TAG, LDL-C, HDL-C, and Total cholesterol were determined using the enzymatic spectrophotometric method by Trinder [28].

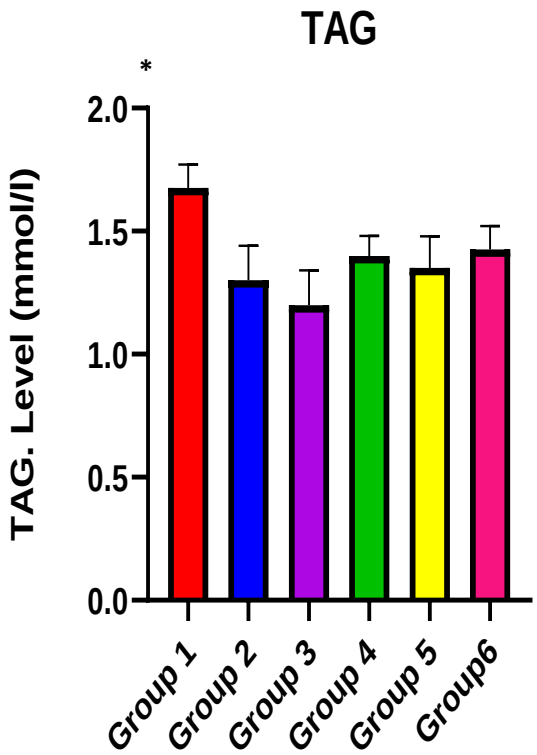
Statistical Analysis

Results were expressed as mean ± standard deviation (SD). Mean values were properly determined and compared using one-way analysis of variance (ANOVA) followed by Turkey's post hoc test; significance was accepted at $p<0.05$. All statistical analysis was carried out using Graph Pad Prism version 5.00 for Windows.

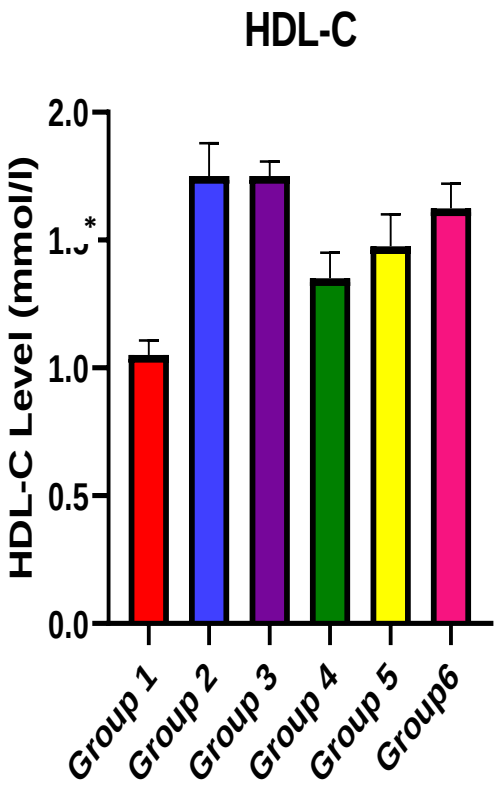
RESULTS

Effect of Ethanol leaf-extract of *Chromolaena odorata* on Lipid profile of STZ-induced Diabetic Albino Rats

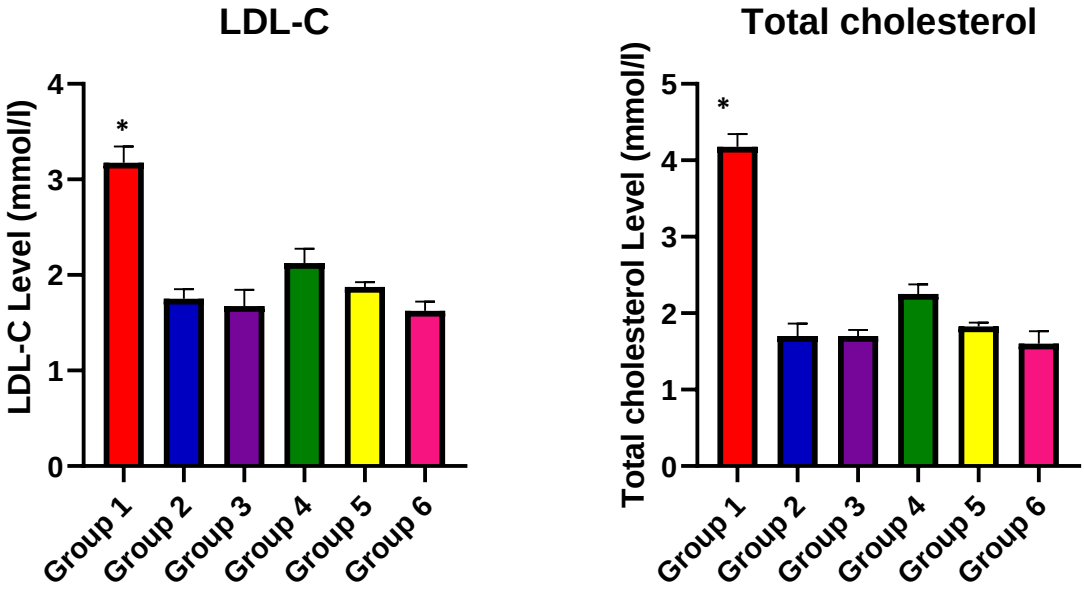
Injection of streptozotocin led to a significant ($p<0.05$) decline in HDL-C while the levels of TAG, TC, and LDL-C increased significantly. Remarkably, treatment with 250 mg/kg, 350mg/kg and 450mg/kg b.w of ethanol leaf extract of *Chromolaena odorata* led to reversal of the altered lipid profile. However, there were no significant differences ($p>0.05$) in TAG, TC and LDL-C levels when the *Chromolaena odorata* extract-treated groups were compared to group 2 rats (treated with glibenclamide), a known standard antidiabetic drug (Figure 1: A, C and D). Further, HDL-C levels varied significantly ($p<0.05$) among the treated groups, with the group treated with 450mg/kg b.w of ethanol leaf extract of *Chromolaena odorata* producing effect that was similar to the group 2 (treated with glibenclamide, a standard drug) (Figure1B).



(A)



(B)



(C) **(D)**

Figure 1 (A-D): Effect of Ethanol Leaf-Extract of *Chromolaena odorata* on Lipid profile of STZ-induced Diabetic Albino Rats (n=6). **'*'** represents significant difference ($p<0.05$) of positive control from treated groups. There were no significant differences ($p>0.05$) among treated groups but all were significantly different from positive control (bars without **'*'**).

Group 1: induced and treated with 0.5ml normal saline (positive control); Group 2: induced and treated with 0.5mg/kg of glibenclamide; Group 3: No induction nor treatment (Negative control); Group 4: diabetic rats treated with 250mg/kg of ethanol leaf-extract of *Chromolaena odorata;* Group 5: diabetic rats treated with 350mg/kg of ethanol leaf-extract of *Chromolaena odorata*; Group 6: diabetic rats treated with 450mg/kg of ethanol leaf-extract of *Chromolaena odorata.*

DISCUSSION

Diabetes mellitus (DM) is one of the global life-threatening metabolic disorders whose key attribute is hyperglycemia as a result of impaired insulin secretion, action or both [1]. Poor control of Diabetes mellitus can result to impairment in lipid profile culminating to dyslipidemia, coronary artery disease and stroke [2]. Therefore, measurement of triglyceride (TAG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) are recommended in during treatment of DM. This study was planned to ascertain the effect of ethanol leaf extract of *Chromolaena odorata* on lipid profile of streptozotocin-induced diabetic albino rats since individuals with diabetes have increased risk of lipid profile dysregulation and its associated health complications. Previous studies have reported increase in TAG, LDL-C, TC, and decline in levels of HDL-C in diabetic rats [29-32]. In this study, there was a significant ($p<0.05$) decline in HDL-C in diabetic rats while the levels of TAG, TC, and LDL-C increased significantly. This result is in line with previous authors' [29-33]. Interestingly, treatment with 250 mg/kg, 350mg/kg and 450mg/kg b.w of ethanol leaf extract of *Chromolaena odorata* led to reversal of the altered lipid profile. Remarkably, there were no significant differences ($p>0.05$) in TAG, TC and LDL-C levels when the *Chromolaena odorata* extract-treated groups were compared to group 2 rats treated with glibenclamide, a known standard antidiabetic drug. This implies that the effect of treating with the extract was similar to that of glibenclamide (a standard drug).

A decline in HDL-C and rise in TAG, TC and LDL-C are suggestive of

hyperlipidemia and could predispose to cardiovascular disorders [29,34]. Some plants possess pharmacological potentials due to the phytochemicals present in them [35]. *Chromolaena odorata* have some phytochemicals such as alkaloids, tannins, flavonoids and other phenolic compounds, which are responsible for their physiological action in man [27].Therefore, the phytochemicals present in *Chromolaena odorata* could be responsible for the restoration of altered lipid profile in the diabetic rats. Evidence abound suggesting the lipid-lowering effects of medicinal plants [25, 32-34, 36, 37].TAGs are chemical compounds digested by the body to supply the energy needed for various biological activities [38]. They are also the most common form of fat in the body. Elevated TAGs levels can cause narrowing of arteries and building up of fatty plaques. These can increase risk of developing atherosclerosis, heart attack, stroke, fatty liver, pancreatitis and other arterial diseases [12]. HDL-C is termed "good cholesterol". This is because it scavenges and removes harmful bad cholesterol (LDL-C) out of the arterial walls and send them back to the liver for conversion into bile and subsequent excretion. Therefore, HDL-C reduces the risk of cardiovascular diseases [38]. LDL-C circulates in the blood and move cholesterol around the body where cholesterol is needed. During this movement, LDL-C can deposit fat in the arterial walls and cause narrowing of the arterial walls. Therefore, LDL-C is often called “bad cholesterol” and can heighten the risk of cardiovascular disease [39].

CONCLUSION

In conclusion, results from this research indicated that the ethanol leaf extract of *Chromolaena odorata* possess hypo cholesterolaemic and hypo triacylglycerolaemic effects as the extract decreased the raised TAGs, LDL-C and TC levels in diabetic rats and also increased the lowered HDL-cholesterol levels in samerats.

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