

Evaluation of Effect of Ethanol Extracts of *Moringa oleifera* and *Cajanus cajan* Leaves on Lipid Profiles in Alloxan Induced Diabetic Albino Rats.

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Abstract: The study was designed to evaluate the effect of ethanol extract of *Moringa oleifera* and *Cajanus cajan* leaves on lipid profiles in alloxan induced diabetic albino rats. Diabetes was induced in three groups of rats, one group was not treated while two groups were treated orally with *M. oleifera* and *C. cajan* ethanol leaf extracts at 200,400 and 800 mg/kg body weight once for seven days respectively. One group was not induced and received feed and distilled water only. The lipid profiles were determined from blood plasma by spectrophotometric method. Twenty four albino rats were grouped into A, B, C and D. Group C and D were further subdivided into C₁, C₂, C₃, D₁, D₂ and D₃ respectively. Diabetes was induced in all the groups, except group A (positive control). Group B (negative control) was induced and was not treated while Group C and D were treated with ethanol extract of *M. oleifera* and *C. cajan* leaves which were given orally to the rats once daily for seven days at varying concentration of 200,400 and 800 mg/kg body weight. The result of the study showed that there were significant (P<0.05) reductions in the mean body weights. The result revealed that a significant (P<0.05) decrease in the total cholesterol level in albino rats treated with ethanol extracts of *M. oleifera* and *C. cajan* leaves except in 200 mg/kg body weight where no significant (P>0.05) decrease was observed. A significant (P<0.05) increase was also observed in HDL-C level in albino rats treated with ethanol extracts of *M. oleifera* and *C. cajan* leaves except in 200 mg/kg body weight where a significant (P<0.05) decrease was recorded with *C. cajan*. The result also revealed that there was significant (p<0.05) decrease in the LDL-C level in albino rats treated with *C. cajan* extracts except in 800 mg/kg body weight where no significant (P>0.05) decrease was observed while a significant (p<0.05) increase was observed in LDL-C level in rats when *Moringa oleifera* ethanol leaf extract was administered. The result also showed a significant (p<0.05) increase in triglyceride level in albino rats treated with ethanol extracts of *M. oleifera* leaf while no significant (p<0.05) increase was observed in triglyceride level in the group treated with *C. cajan* leaf extract. The result indicates that *Moringa oleifera* and *Cajanus cajan* leaves may be useful in the management of cardiovascular disease.

Key words: *Moringa oleifera* • *Cajanus cajan* • Leaves • Lipid Profiles • Alloxan and Diabetic Albino Rats

INTRODUCTION

Diabetes mellitus is an endocrine metabolic disorder characterized by hyperglycemia, altered lipids (hyper or hypo-lipidemic), altered carbohydrates, altered proteins metabolism and it increase the risk of cardiovascular disease complication [1]. The two forms of diabetes, type 1 and 2, differ in their basic mechanisms of development and in physiologic characterization such as association with obesity, age and insulin.

Lipid profile is a blood test that measures total cholesterol, high density lipoproteins (HDLS), low-density lipoproteins (LDLS), low-density lipoproteins (LDLs) and triglycerides, to assess the risk of cardiovascular disease [2]. Cholesterol is the major sterol in animal tissues and its amphipatic nature enables it to occur in cell membranes (Nelson and Cox, 2000). It is abundant in the brain, liver, adrenal glands and nervous system. Cholesterol and triacylglycerol are transported in the body in combination with protein to form lipoproteins (HDL and LDL) [2].

Throughout history, plants have been used by human beings for medicinal purposes and even in modern times have formed the basis of many pharmaceuticals in use. Under traditional purposes, herbal medicines and medical plants like *Moringa oleifera* and *Cajanus cajan* have been used for the treatment of diabetes which in turns treat abnormal levels Lipoproteins as an alternative medicines [3].

Moringa oleifera belongs to the family of moringaceae, a fast growing drought resistant tree native of sub-Himalayan tracts of Northern India but now distributed worldwide in the tropics and sub tropics [4]. *Moringa oleifera* has Horseradish tree, Drumstick tree and Ben oil tree, as its English names. Its local names in Nigeria include: "Gawara" in Fulani, "Zogau" in Hausa, "Ikweoyibo" in Igbo and "Ewe ile" in Yoruba. The *Moringa* tree grows mainly in semi-arid, tropical and subtropical areas. Currently it is widely grown in Africa, central and South America, Sri-lanka, India, Mexico, Malaysia and the Philippines. It has a diverse uses. The tree's leaf and seed pod are widely consumed as food. The barks leaf and root have medicinal properties and have been used for countries in folk medicine for a variety of health promoting purposes [5].

Cajanus cajan belongs to the family fabaceae, a fast growing drought resistant. It is a perennial woody shrub which originated from Asia, from where it traveled to East Africa and by means of slave trade to the American continent [6]. *Cajanus cajan* has Congo pea, pigeon pea, red gram, yellow dahl, as its English name. Its local name in Nigeria includes "Otili" in Yoruba, "Fiofio" in Igbo, "Aduwa" in Hausa and "Olele" in Edo. It is an erect, branched, hairy shrub, 1-2 meters high with oblong leaves and yellowed flowers. Pods are hairy, -7 cm long, 1cm wide, containing two to seven seeds. In India, its leaves are used for rearing silk worms and green pods are used as a vegetable. Husk, green leaves are used as a fodder and also as a green manure [7].

Amongst its many medicinal uses, *C. cajan* is indicated in the relief of pain in traditional Chinese medicine and as a sedative and also explored recently for the treatment of ischemic necrosis of the caput femora, bedsore and wound healing [8]. It has also been used widely for many years for treating diabetes, sore, skin irritation, hepatitis, hyperlipidemic, jaundice, dysentery, measles and stabilizing menstrual period [8]. This study, therefore comparatively evaluate the effect of ethanol extracts of *Moringa oleifera* and *Cajanus cajan* leaves on lipid profiles in alloxan induced diabetic albino rats.

MATERIALS AND METHODS

Materials

Biological Materials: *Moringa oleifera* leaves, *Cajanus cajan* leaves and albino rats were the biological materials used for the study.

Collection of Plant Materials: Fresh leaves of *Cajanus cajan* and *Moringa oleifera* were collected from Agharaoza village in Izzi L.G.A, Ebonyi State and was Authenticated and identified by Prof. Onyekwelu, S.S of the applied Biology Department, Ebonyi State University, Abakaliki, Nigeria.

Preparation of *Moringa oleifera* and *Cajanus cajan*

Leaves Extracts: Fresh leaves of *Moringa oleifera* and *Cajanus cajan* were collected and air-dried under ambient temperature. The dried leaves were ground into powdered form using blender. 200g of grinded *Moringa* and *Cajanus cajan* leaves were soaked in ethanol and were allowed to stand for 24hrs. The mixtures were filtered using clean sieve cloth and the filtrates evaporated forming substance pastry in nature. The extract was kept in a dried clean container and stored in a refrigerator.

Experimental Animals: Albino rats weighing between 125-200g of opposite sex were used for study. They were purchased from animal house at Nnamdi Azikiwe University (UNIZIK), Anambra State, Nigeria and they were kept in animal house at Ebonyi State University, Abkaliki, Nigeria in the Department of Biochemistry. They were feed with clean water and poultry growers mash and were acclimatized for seven days.

Experimental Design: A total of thirty two (32) albino rats were used for the study. Sixteen albino rats each for the extract of *Moringa oleifera* and *Cajanus cajan* leaves respectively. They were distributed randomly into four groups designed as group A, B, C and D. Group A is called the positive control which is given water and rats feed only with 4 rats. Group B with also four (4) rats is called the negative control which diabetes was induced without treatment.. Group C and D were further divided into six (6) group C1, C2, C3, D1, D2 and D3 with 4 rats each representing the doses of 200, 400, 800 mg/kg body weight of *Moringa oleifera* and *Cajanus cajan* ethanol leaves extracts respectively. They were administered orally throughout the period of the experiment.

Methods

Collection of Blood Sample: At the end of the treatment with the ethanol extracts of *Moringa oleifera* and *Cajanus cajan* leaves, the albino rats were starved for overnight and blood were collected using capillary tube via ocular puncture.

Lipid Profile Determination: Twenty-four hours after the last dose administration, whole blood (4ml) was collected from each of the albino rats through the femoral artery into a test tube. The blood was later spun in a centrifuge at 5000revolution for 10minutes. Serum was separated from the clot with Pasteur pipette into another clean EDTA bottle and stored at -20°C for the measurement of the biochemical parameters

Total cholesterol (T-C), triglycerides (TG), low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) levels were determined using the methods described by Roschlaw *et al.* [9], Quinmca Aplicanda [10] and Friedelward *et al.* [11].

Statistical Analysis: Results were expressed as mean ± standard deviation. The significant difference between the controls and treated groups were determined using one-way analysis of variance (ANOVA) at p<0.05.

RESULTS

Percentage Yield: The percentage yield of ethanol extract of leaves of *M. oleifera* and *C. cajan* were 25 and 6.25% respectively (Table 1). This means that ethanol may be a good solvent for some of the active ingredients.

Result of the Weight of Rats and Extract Doses During 7days of Treatment: The result showed the variation on the body weight of albino rats during seven days of treatment with *M. oleifera* and *C. cajan* leaves (Figure1). The result showed a significant (p<0.05) increase in the weight of rats in the positive control and the treated groups while a significant (p<0.05) decrease was observed in the body weight of rats in the negative control group.

Result of Lipid Profiles in Albino Rats Treated with Ethanol Extracts of *M. Oleifera* and *C. Cajan* Leaves: The treatment of alloxan induced diabetes in albino rats with ethanol extract of *Cajanus cajan* and *M. oleifera* leaves at 200, 400 and 800 mg/kg body weight of the extracts showed that there was significant (P<0.05) decrease in the total cholesterol level in albino rats treated with ethanol extracts of *M. oleifera* and *C. cajan* leaves except in 200 mg/kg body weight where no significant

Table 1: Percentage yield of extracts of *Moringa olerifera* and *Cajanus cajan* leaves use of ethanol.

Solvent Used	Plants Used	Mass of leaves before extraction(g)	Mass of leaves after extraction(g)	Volume Solvent Used (ml)	Mass of extracts (g)	Percentage yield
Ethanol	<i>M. oleifera</i>	200	150	600	50	25
	<i>C. cajan</i>	200	187.5	1600	12.5	6.25

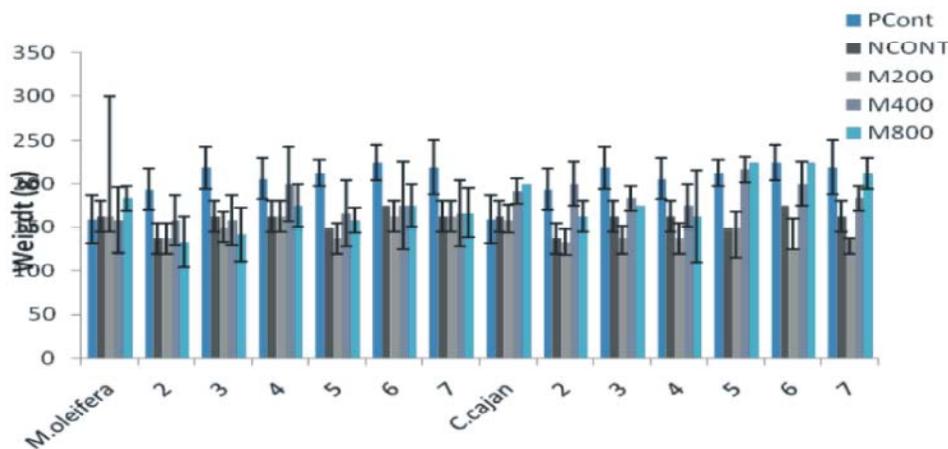


Fig. 1: Weight of Albino Rats during the seven (7) Days of Treatment with Ethanol Extracts of *M. oleifera* and *C. cajan* leaves.

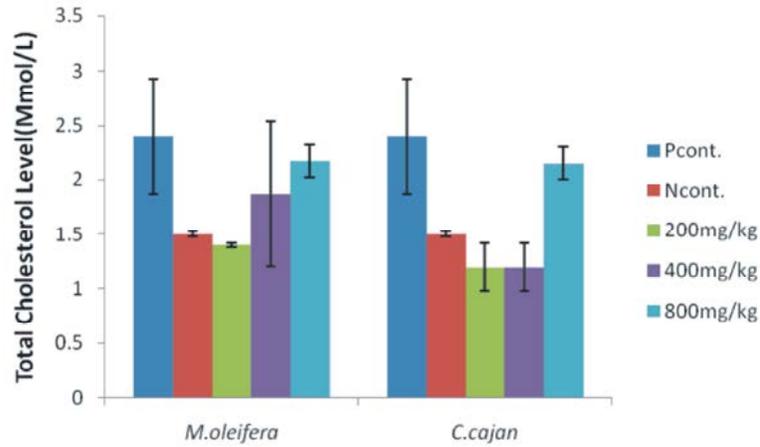


Fig. 2: Total Cholesterol level in albino Rats Treated with Ethanol Extracts of *M. oleifera* and *C. cajan* leaves. Data are shown as Mean \pm Standard Deviation (n=4). Significant difference are shown at P<0.05.

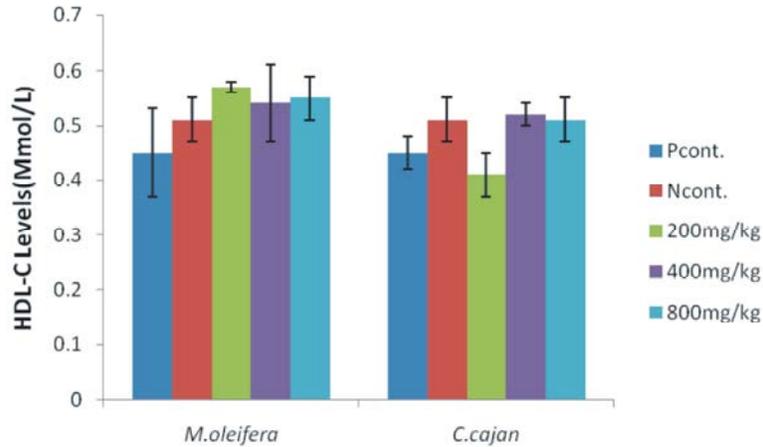


Fig. 3: HDL-C levels in Albino Rats Treated with Ethanol Extracts of *M.oleifera* and *Cajanus cajan* leaves. Data are shown as Mean \pm Standard Deviation (n=4). Significant difference are shown at P<0.05.

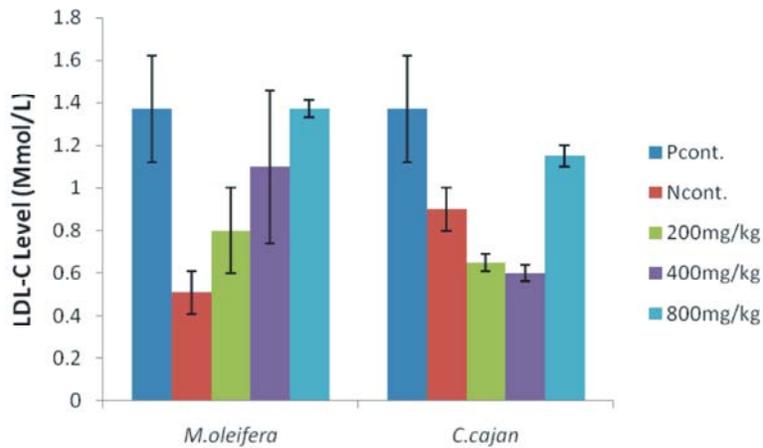


Fig. 4: LDL-C levels in Albino Rats Treated with Ethanol Extracts of *M.oleifera* and *C. cajan* leaves. Data are shown as Mean \pm Standard Deviation (n=4). Significant difference are shown at P<0.05.

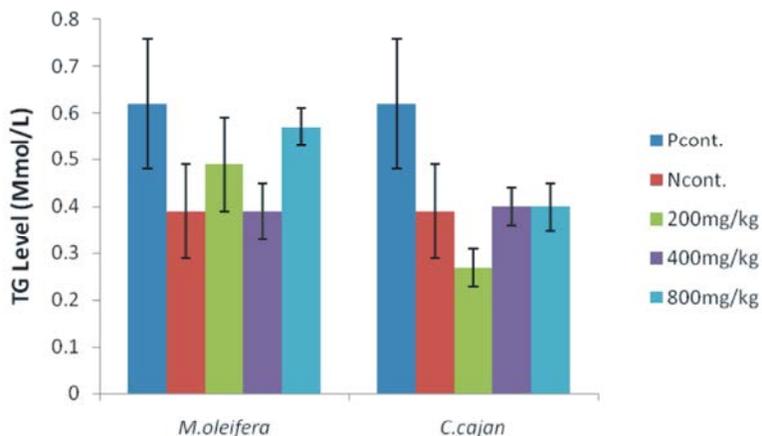


Fig. 5: TG level in Albino Rats Treated with Ethanol Extract of *M. oleifera* and *C. cajan* leaves.

Data are shown as Mean \pm Standard Deviation (n=4). Significant difference are shown at $P < 0.05$.

($P > 0.05$) decrease was observed (Figure 2). A significant ($P < 0.05$) increase was also observed in HDL-C level in albino rats treated with ethanol extracts of *M. oleifera* and *C. cajan* leaves except in 200 mg/kg body weight where a significant ($P < 0.05$) decrease was recorded with *C. cajan* (Figure 3). The result also revealed that there was significant ($p < 0.05$) decrease in the LDL-C level in albino rats treated with *C. cajan* extracts except in 800 mg/kg body weight where no significant ($P > 0.05$) decrease was observed while a significant ($p < 0.05$) increase was observed in LDL-C level in rats when *Moringa oleifera* ethanol leaf extract was administered (Figure 4). The result also showed a significant ($p < 0.05$) increase in triglyceride level in albino rats treated with ethanol extracts of *M. oleifera* leaf while no significant ($p < 0.05$) increase was observed in triglyceride level in the group treated with *C. cajan* leaf extract (Figure 5).

DISCUSSION

In this study the 25% yield of ethanol leaf extract of *M. oleifera* and 6.25% yield of ethanol leaf extract of *C. cajan* showed that ethanol is a better solvent for some of the active ingredients in *Moringa oleifera*. It can be seen from the result that weight of the positive control and that of the treated animals increased while those of the negative control decreased (Figure 1). This confirmed that alloxan reduced the body weight of the albino rats while the extracts of *C. cajan* and *M. oleifera* reversed the effect. Nwanjo [13], reported a significant ($p < 0.05$) decrease in the body weight of a diabetic rats. Aja *et al.* [14], also reported significant ($P < 0.05$) reductions in the mean body weight of albino rats administered ethanol

extract of *Cajanus cajan* leaf. This appears to be consistent with the previous report of world health organization, that diabetes mellitus is often characterized by rapid and significant weight loss leading to fatigue which is not easily reversed [13].

Treatment of alloxan induced diabetes in albino rats with ethanol leaf extracts of *M. oleifera* and *C. cajan* significant ($p < 0.05$) decrease the total cholesterol and LDL-C level in albino rats while a significant ($p < 0.05$) increase was observed in HDL-C level (Figures 2, 3 and 4). The TG level in albino rats treated with ethanol extracts of *C. cajan* and *M. oleifera* leaves showed significant ($P < 0.05$) decrease except in 800mg/kg body weight in *M. oleifera* where a significant ($p < 0.05$) decrease was observed (Figure 5). According to Luo *et al.* [15], Mayilvaganan *et al.* [16] and Ogbunugafor *et al.* [17] reported that various doses of ethanol extract of *Cajanus cajan* showed a significant ($p < 0.05$) increase in the level of high density lipoprotein cholesterol (HDL - C) and significant ($p < 0.05$) decrease in total cholesterol (TC), low density lipoprotein cholesterol (LDL - C) and triacylglycerol after four weeks of treatment in albino rats. The results which correspond to the reports of Luo *et al.* [15], Mayilvaganan *et al.* [16] and Ogbunugafor *et al.* [17] also revealed a significant ($P < 0.05$) decrease in the level of triacylglycerol and thus infer the importance of the extract in weight and cardiovascular risk management. Ofor *et al.* [18] earlier reported that total cholesterol, triacylglycerol and LDL-cholesterol levels were significantly ($p < 0.05$) reduced by ethanol leaf-extracts of *Vernonia amygdalina* and *Azadirachta indica* while HDL-cholesterol levels were increased significantly. Aja *et al.* [14] reported no significant ($P > 0.05$) increase in

total cholesterol (TC), high density lipoprotein - cholesterol(HDL - C) and low density lipoprotein - cholesterol (LDL - C) levels in rats administered ethanol extract of *Cajanus cajan* leaves.

High blood cholesterol concentration is one of the important risk factors [19]. Thus, the reduction in serum total cholesterol concentration produced by the extract is beneficial and may reduce the risk of cardiovascular disease because agents that have been reported to function. Elevated HDL-C is very significant in human health because it improves the transportation of cholesterol from plasmas to the liver for biotransformation and excretion, thereby preventing althema formation and blood vessel occlusion [13]. The result equally revealed that, there was significant increase in LDL-C level in albino rats treated with *M. oleifera* and this result was not in correlation with the report of Aja *et al.* [14].

CONCLUSION

The results obtained from this study revealed that *M. oleifera* and *C. cajan* leaves have potentials to lower cholesterol and LDL-C and increase TG and HDL-C in diabetic rats and therefore could be useful for diabetics patients.

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