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Assessment of atherogenic indices and modification of lipid profiles by aqueous and methanol extracts of *Terminalia catappa* leaves in diabetic rats

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Abstract

Atherogenic indices are useful markers for prediction of cardiovascular diseases. This study investigates atherogenic indices in diabetic rats treated with different fractions of *Terminalia catappa* leaf extract. A total of Forty-two [42] male Wistar rats were randomly shared into 7 groups of 6 rats per group. Group 1 [control] received 5 ml/kg body weight of distilled water orally. Group 2 was treated with aqueous leaf extract of *T. catappa* at 130 mg/kg body weight orally while Group 3, diabetic untreated group orally received distilled water, 5ml/Kg body weight. Groups 4 and 5 were diabetic rats treated respectively with 130 mg/Kg body weight of aqueous leaf extract of *T. catappa* and subcutaneous administration of insulin, 0.75 U/Kg body weight. Group 6 received methanol fraction of *T. catappa* leaf extract; 130 mg/Kg body weight and group 7 was administered orally with 30 mg/Kg body weight of aspirin. Diabetes was induced with streptozotocin; 65 mg/Kg body weight. Results showed significant [$p < 0.05$] increase in TC, TG, LDL-c and VLDL-c in diabetic untreated group and significant reduction in HDL-c. These lipids were reduced and HDL-c increased significantly [$p < 0.05$] in aqueous and methanol extracts of *T. catappa* leaves treated groups. There was significant [$p< 0.05$] Fasting Blood Glucose reduction in both fractions. Therefore, modifications of lipid profile and reduction of atherogenic index in diabetic rats were similar in aqueous and methanol extracts of *T. catappa* leaves suggesting the potentials of preventing cardiovascular complications in diabetes mellitus.

Keywords: Atherogenic Index; *Terminalia catappa*; Insulin; Cardiovascular Complication; Diabetes Mellitus

1. Introduction

Cardiovascular complications are among the known leading causes of death in diabetes mellitus [1]. Both type 1 and type 2 diabetes constitute serious cardiovascular risks associated with attendant high morbidity and mortality [2, 3] in diabetic population. Clinically, a strong association between type 2 diabetes mellitus with macro and microvascular complications has been reported [4]. The microvascular complications include nephropathy, retinopathy and neuropathy while the macrovascular complications are peripheral artery disease [PAD], cerebrovascular disease and ischemic heart disease [IHD] [2]. It is commonly observed that diagnosis and subsequent attention on diabetic nephropathy, diabetic cardiomyopathy as well as peripheral arterial disease occur at the later stages of the disorders [4]. Sequel to this, there are increasing efforts to prevent or reduce these cardiovascular disorders which are targeted through different strategies such as life style changes, diet and exercise. Advocacy on consideration of diabetes patient as people with secondary cardiovascular disease prevention have been reported [5] but CVD risk differs with different diabetic patients. [6] which may not allow this advocacy to thrive. However, a common factor in cardiovascular disorders identified is dyslipidemia and it is an established cause of diabetes related cardiovascular complications [7]. A variety of approaches for management of cardiovascular risk have been employed and one of the approaches is regular determination of lipid profile in clinical laboratories [8]. Results obtained from this assessment provide information basically on quantitative abnormalities of lipid profile [9]. This component may be normal or subnormal but lacks direct provision of information on qualitative and kinetic components of lipid profile abnormalities [8] which implies

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predisposition to cardiovascular disease [8]. Early detection of cardiovascular disorder is key to timely intervention with appropriate drugs. This is possible through lipoprotein assay for assessment of qualitative and kinetic abnormalities of lipid profile specifically the small LDL particles [10]. This procedure is however expensive and not a common routine laboratory test [10]. Therefore, atherogenic cardiovascular risk scores calculation using regular quantitative lipid profile have been developed [8,11]. This calculation is known as cardiovascular or atherogenic indices. Among them is atherogenic index of plasma [AIP] involve a formula with mathematical ratio of quantitative values for TG and HDL to it base ten logarithms [12]. Atherogenic Index of Plasma is a reliable predictor of coronary artery disease and atherosclerosis [13,14]. This index determines the atherogenicity of plasma and small LDL particle which is the main culprit for development atherosclerosis in diabetes mellitus [15]. With the use of available cardiovascular indices [8], there is possible prognosis and timely prediction of atherogenic status of diabetic individual for management and early mitigation of atherosclerotic cardiovascular diseases [ASCD] [16]. Therefore, results of AIP provide necessary information on diabetic patient requiring immediate therapeutic attention [12,17]. According to American Diabetes Association Standards of Care on cardiovascular disease and risk management 2022 [18], drugs such as Sodium Glucose Co-Transporter 2 Inhibitors [SGLT2] and Glucagon like Peptide - 1 Agonist [GLP-IA] have been recommended for cardiovascular complications in diabetes mellitus [ADA]. Also, statins and anti-platelets drugs have been used in known cases of cardiovascular disorders secondary to diabetes but this does not provide prevention for asymptomatic individuals [18]. Moreover, use of low dose aspirin have been recommended and supported by professional societies such as for use in secondary cardiovascular disease in diabetes [19]. Unfortunately, required standard drugs are expensive and unavailable and aspirin use is controversial in diabetics with primary cardiovascular complications management [20]. The use of medicinal plant in management of diseases have been advocated by WHO [21] and this has instigated many research interests to develop alternative medicine to sustain human race in the face of disease and drug availability challenges. *Terminalia catappa* have been reported by many researchers and our team as useful antidiabetic [22], anti-inflammatory [23] and anti-oxidative [24] agents. This study seeks to investigate the atherogenic indices in diabetic rats treated with aqueous and methanol extracts of *Terminalia catappa* leaves in diabetic rats.

2. Materials and methods

2.1. Leaf Extract Preparation

The green leaves of *Terminalia catappa* were obtained from the plants within the University of Uyo Town campus. The leaves were presented to a botanist for authentication and registration at the Department of Botany and Ecological studies, University of Uyo and registration number obtained at the herbarium was UUPH 22[a].

2.1.1. Aqueous extract preparation

The leaves were washed and air-dried at room temperature overnight. The clean leaves were pulverized and 5000 g of the pulverized leaves were soaked in 5 litres of deionised water for 18 hours. The mixture was filtered using muslin cloth and evaporated to dryness using thermostatic water bath at 45 ^{0}C until a semi solid paste of 204.18 g of the extract was obtained after evaporation representing a percentage yield of 4.08 %. The extract was stored in refrigerator for use during the experiment.

2.1.2. Methanol extract preparation

The leaves were washed and air-dried at room temperature overnight. The clean leaves were pulverized and 1000 g of the pulverized leaves was macerated in 80 % [v/v] aqueous methanol. The mixture was filtered using muslin cloth and evaporated to dryness using thermostatic water bath at 45 ^{0}C until a semi solid paste of 204.18 g of the extract was obtained after evaporation representing a percentage yield of 20.42%. The extract was stored in refrigerator for use during the experiment.

2.2. Preparation of Experimental Animal

Adult male Wistar rats with average weight of 150 g were used for this study. The animals were procured from the animal house, Department of Physiology, Faculty of Basic Medical Sciences, University of Uyo. The animals were housed in wooden cages at the animal house, Faculty of Basic Medical Sciences. The animals were acclimatized for two weeks and maintained in a 24-hour dark and light cycle. The animals were fed with standard pellets [from Guinea Feeds, Plc Nigeria] and have access to water *ad libitum*.

2.3. Induction of diabetes

Diabetes was induced with streptozotocin [STZ, from Sigma-Aldrich] according to basic protocol [25,26]. Streptozotocin [STZ] was dissolved in citrate buffer (citric acid and sodium citrate, enzyme grade from; Fisher) with pH 4.5 prepared

just before administrations. The STZ was administered by intraperitoneal injection, 65 mg/Kg body weight [27]. The animals were provided with 10 % [w/v] sucrose [from Sigma] water for the first 24 hours to avoid severe hypoglycemia. The animals had free access to normal rat feed and water. Feed was withdrawn on previous night to measurement of fasting blood glucose and the animals were fasted for about 12 hours [26]. The animals were assessed for development of diabetes after 48 hours by obtaining blood sample from the tip of the tail. The blood sample was dropped into glucose strip to measure the glucose level using One Touch glucometer [One Touch Ultra, Life Scan Inc, U.S.A]. Blood glucose ≥200 mg/dL was considered diabetic [normal range of blood glucose in rat is 80 – 120 mg/dL] and were used for the experiment which lasted for 14 days.

2.4. Experimental Design

The experimental animals were randomly distributed into seven [7] groups of six [n=6] rats per group as follows:

- **Group 1:** Control group administered with only distilled water orally at a dose of 5 ml/kg body weight.
- **Group 2:** Normal rats with only aqueous extract of *Terminalia Catappa* at a dose of 130 mg/kg body weight administered orally.
- **Group 3:** Diabetic group administered with only distilled water orally at a dose of 5 ml/kg body weight.
- **Group 4:** Diabetic group treated with *Terminalia catappa* leaf extract at a dose of 130 mg/kg body weight by oral administration.
- **Group 5:** Diabetic group treated with oral administration of methanol fraction of *Terminalia catappa* leaf extract at 130 mg/kg body weight
- **Group 6:** Diabetic group treated with exogenous Insulin at a dose of 0.75 U/kg body weight by subcutaneous administration.
- **Group 7:** Diabetic group treated orally with aspirin at 30 mg/kg body weight.

2.5. Assessment of lipid profile

The total cholesterol, triglyceride and high-density lipoprotein was estimated using spectrophotometric method with commercial standard analysis kits [Biotech, China] following the manufacturer's procedures. 1.0 ml of reagent was prepared into sample tubes labelled blank, standard, control and samples and pre-warmed at 37 ^{0}C for at least 2 minutes. 10 µl of samples were added to respective tubes and mixed. The mixture was incubated for 10minutes at 37 ^{0}C and the absorbance was read at 520 nm. Low density lipoprotein and very low-density lipoprotein were determined by using a mathematical formula according to Friedewald [28] as follows;

Low density Lipoprotein [LDL, mmol/L] = Total cholesterol [TC, mmol/L] – High density Lipoprotein – Triglyceride [TG, mmol/L]/2.2

Very low-density Lipoprotein [VLDL, mmol/L] = Triglyceride [TG, mmol/L]/2.2

2.6. Cardiovascular Risk Assessment

Cardiovascular risk in the research was calculated using various indices in treated and untreated groups [29]. The indices are as listed below:

(a) **Atheroginic Index of Plasma [AIP]:** This was calculated using its mathematical formula [Dobiásová and Frohlich, 2001]; **AIP** = Log[TG/HDL-c]

(b) **Castelli's Risk Index-I [CRI-I]:** This is the Cardio Risk Ratio [CRR] calculated with its mathematical formula [30]; **CRI-I** = TC/HDL-c and

(c) **Castelli's Risk Index-II [CRI-II]:** this is the Atherosclerosis index [AI] calculated with its mathematical formulae [30];

CRI-II = LDL-c/HDL-c

Where: TG = Triglyceride, HDL-c = High density lipoprotein-cholesterol,
LDL-c = Low density lipoprotein -cholesterol and TC = Total cholesterol

2.7. Measurement of fasting blood glucose

Measurement of fasting blood glucose [FBG] was carried out in the animals after overnight fast of about 14 hours and blood samples were obtained by pricking the tip of the tail and blood glucose was determined using One glucometer [Life Scan, USA] according to Ben and Ekaidem [31].

2.8. Statistical Analysis

The research data collected was statistical analysed using GraphPad Prism 5.0 software. One-way analysis of variance [ANOVA] was conducted with post hoc Turkey test. The result presented was mean ±standard error of mean [SEM] and values with $p<0.05$ were considered significant.

3. Results

3.1. Total Cholesterol [TC] level

The results of Total cholesterol are represented in figure 1. The serum total cholesterol level was 2.47±0.04 mmol/L in the control group, 2.17±0.06 mmol/L in control+extract group and 2.67±0.08 mmol/L in diabetic group. The diabetic group showed a significantly [$p < 0.05$] raised TC level compared with control group. The TC level was reduced significantly [$p < 0.05$] in the diabetic+extract group to a mean value of 2.13±0.06 mmol/L compared with the diabetic untreated group but this value was still higher than the control group value. In diabetic insulin treated group, there was also a significant [$p < 0.05$] reduction to mean value of 2.30±0.04 mmol/L when compared with diabetic untreated group but not with control group. In the diabetic+methanol extract and diabetic+aspirin treated groups, the values were also reduced respectively to 2.13±0.06 mmol/L and 2.27±0.06 mmol/L which were significant [$p<0.05$] when compared with control and diabetic group.

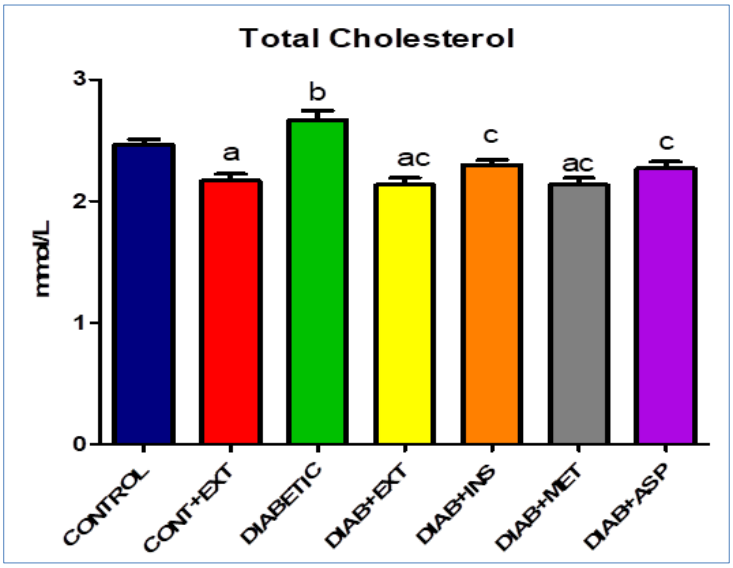

Figure 1 Total cholesterol levels in nondiabetic and diabetic groups. Values are in mean ± SEM, $p<0.05$. a= test vs control, b= test vs control+extract, c= test vs diabetic group.

3.2. Triglyceride [TG] Level

The results of Triglyceride [TG] are represented in figure 2. The value of triglyceride in the control group was 0.82±0.03 mmol/L, the control+extract was 0.74±0.01 mmol/L while diabetic group was 1.44±0.04 mmol/L. the TG of the diabetic group was significantly [$p< 0.05$] higher than control group. The diabetic group treated with *T. catappa* aqueous leaf extract and insulin showed significant [$p < 0.05$] reduction to a mean value of 0.71±0.01 mmol/L and 0.84±0.02 mmol/L respectively when compared with diabetic group but was however significantly [$p<0.05$] higher than the control group value. In diabetic group treated with methanol extract the value was 0.83±0.09 mmol/L and was not different from the control. But the aspirin treated diabetic group had 1.17±0.07 mmol/L and was significantly higher than the control group but significantly [$p<0.05$] lower than diabetic group.

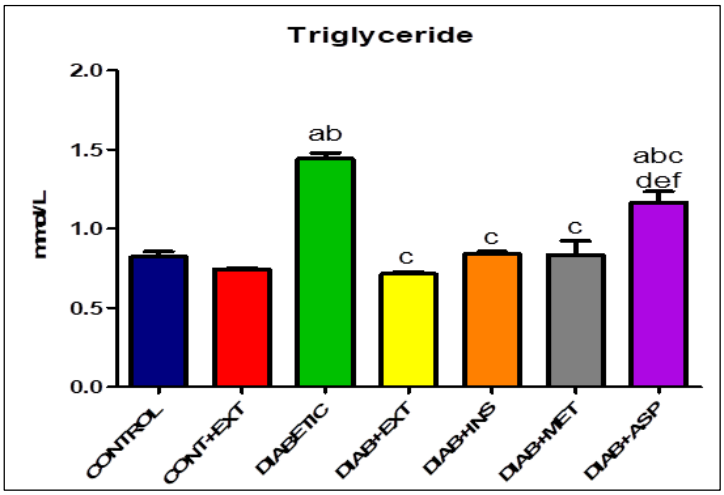

Figure 2 Triglyceride levels in non-diabetic and diabetic groups. Values are in mean ± SEM, p<0.05. a= test vs control, b= test vs control+extract, c= test vs diabetic group, d= test vs diabetic+extract, e= test vs diabetic+insulin, f= test vs diabetic+methanol extract.

3.3. High Density Lipoprotein Cholesterol [HDL-c] Level

In figure 3, the results of high-density lipoprotein [HDL-c] are represented. The serum levels of HDL for control, control+extract and diabetic groups were 1.54±0.08 mmol/L, 1.26±0.02 mmol/L and 1.13±0.08 mmol/L respectively. Comparing the results, the HDL-c of diabetic untreated group was significantly [$p < 0.05$] higher than the control group while the control+extract group showed no significant change on the HDL-c. The HDL-c in diabetic group treated with aqueous extract was 1.35±0.01 mmol/L and insulin treated group was 1.32±0.03 mmol/L. The observed reductions were significant [$p < 0.05$] when compared with the diabetic group but not the control group. Methanol extract treated group had increased HDL-c of 1.38±0.03 mmol/L which was higher than diabetic group but not up to the level of control group. Also, the value of HDL-c in diabetic+aspirin treated group was 1.41±0.01 mmol/L and was equally higher than diabetic group yet lower than control although this was not statistically significant.

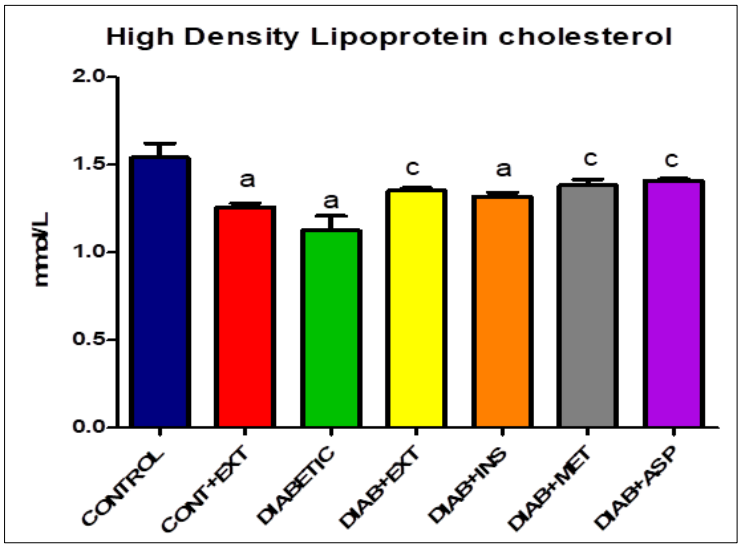

Figure 3 High density lipoprotein cholesterol levels in nondiabetic and diabetic groups. Values are in mean ± SEM, p<0.05. a= test vs control, c= test vs diabetic group.

3.4. Low Density Lipoprotein Cholesterol [LDL-C] Level

The results of Low-density lipoprotein level as represented in figure 4 was 1.71±0.09 mmol/L in the control group, 1.24±0.07 mmol/L in control+extract group and 1.78±0.14 mmol/L in diabetic group. But in diabetic group treated with aqueous extract, the value significantly [p<0.05] reduced to 1.12±0.07 mmol/L compared to the control and diabetic group. Diabetic+insulin group also showed significant [$p < 0.05$] reduction to mean value of 1.36±0.02 mmol/L. The

methanol extract treated group value was 1.13±0.06 mmol/L significantly [p<0.05] lower than control and diabetic groups while the aspirin treated diabetic group with 1.39±0.06 mmol/L of LDL-c was only significantly [p<0.05] lower than the diabetic group.

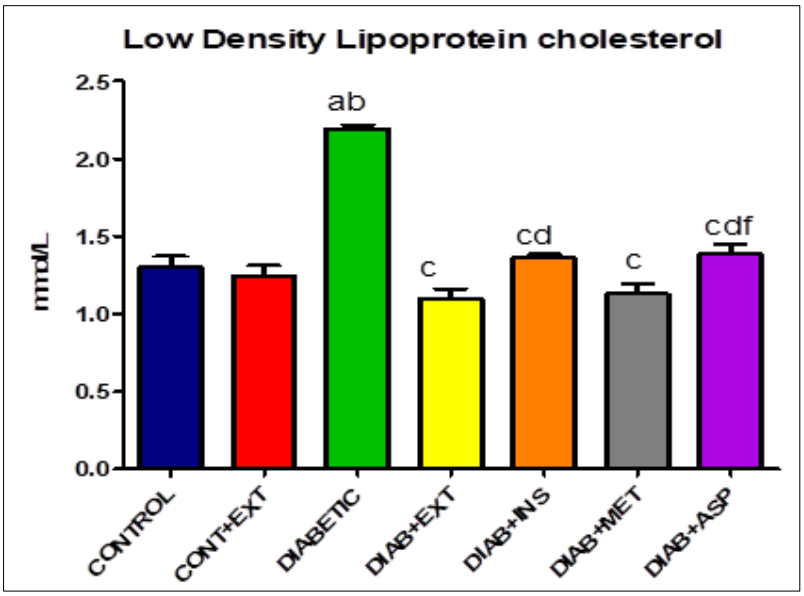

Figure 4 Low density lipoprotein cholesterol levels in nondiabetic and diabetic groups. Values are in mean ± SEM, p<0.05. a= test vs control, b= test vs control+extract, c= test vs diabetic group, d= test vs diabetic+extract, f= test vs diabetic+methanol extract.

3.5. Very Low-Density Lipoprotein Cholesterol [VLDL-C] Level

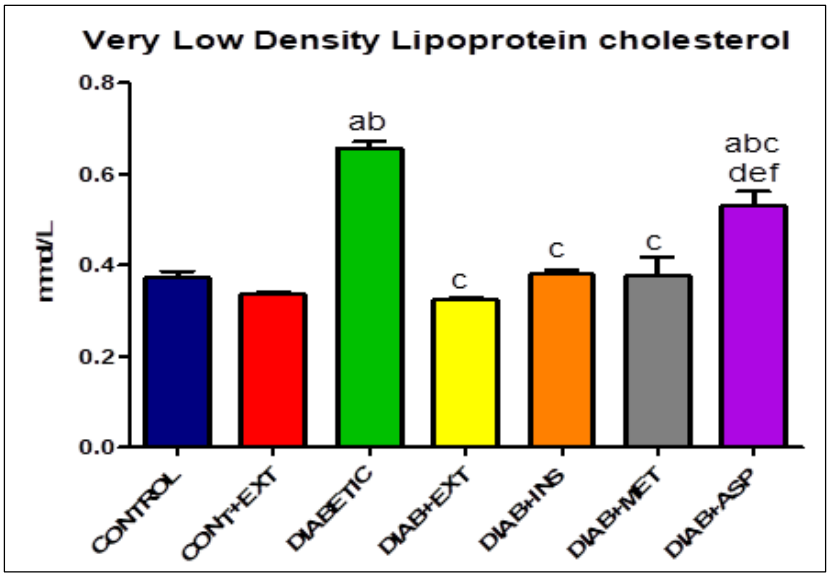

Figure 5 Very low-density lipoprotein cholesterol levels in nondiabetic and diabetic groups. Values are in mean ± SEM, p<0.05. a= test vs control, b= test vs control+extract, c= test vs diabetic group, d= test vs diabetic+extract, e= test vs diabetic+insulin, f= test vs diabetic+methanol extract.

The results of very low-density lipoproteins are represented in figure 5. The serum level of very low-density lipoprotein was 0.37±0.01 mmol/L in the control group, 0.34±0.01 mmol/L in control+extract group and 0.66±0.01 mmol/L in diabetic group. The diabetic+extract and diabetic+insulin groups showed significant [p<0.05] reductions to mean values of 0.32±0.01 mmol/L and 0.38±0.01 mmol/L respectively compared to the diabetic group. These were higher than the control group value. Diabetic group treated with methanol extract had VLDL-c value of 0.38±0.04 mmol/L significantly [p<0.05] reduced compared with diabetic group but no significant difference with control. The diabetic group treated

with aspirin on the other hand showed a significant [p<0.05] rise to a mean value of 0.53±0.03 mmol/L which was higher than both control and diabetic group.

3.6. Atherogenic Index of plasma [AIP]

In figure 6, result of the atherogenic index of plasma showed that control group had -0.27±0.01, control+extract group had -0.23±0.01 and diabetic group had 0.11±0.04. In the diabetic treated groups; aqueous extract group had -0.28±0.01, insulin group was -0.20±0.01, methanol extract group was -0.23±0.03 and aspirin treated group was -0.09±0.03. Diabetic group value was significantly [p<0.05] higher than control group and every other groups. Aqueous and methanol extract treated groups were significantly [p<0.05] lower than the diabetic group. The aqueous result was more reduced than the methanol and insulin treated groups but were of no significant difference statistically from control group and other test groups. It is observed that aspirin treated group had least reduction which was however still significant [p<0.05] compared with control and diabetic groups

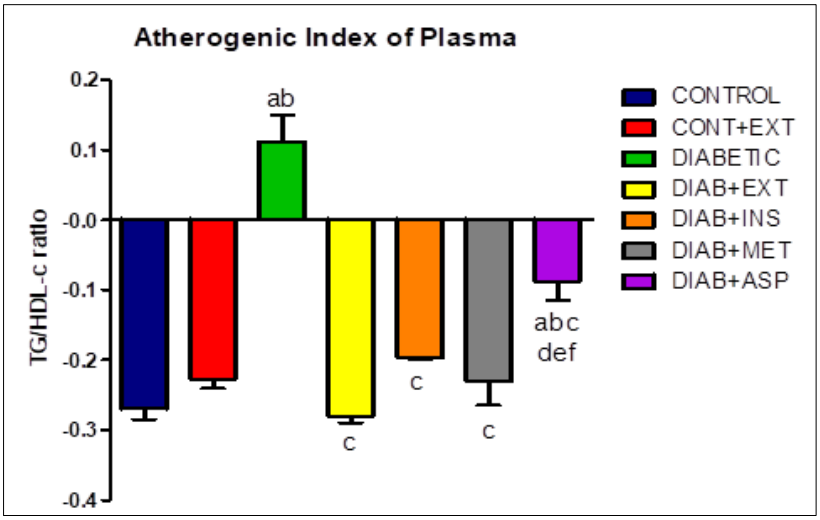

Figure 6 Atherogenic Index of Plasma in nondiabetic and diabetic groups. Values are in mean ± SEM, p<0.05. a= test vs control, b= test vs control+extract, c= test vs diabetic group, d= test vs diabetic+extract, e= test vs diabetic+insulin, f= test vs diabetic+methanol extract.

3.7. Castelli's Risk Index-I [CRI-I]

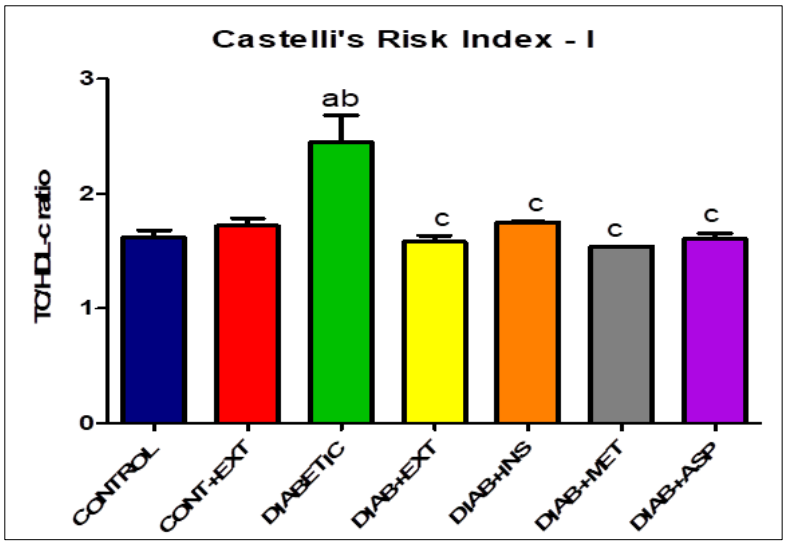

Figure 7 Castelli's Risk Index-I in nondiabetic and diabetic groups. Values are in mean ± SEM, p<0.05. a= test vs control, b= test vs control+extract, c= test vs diabetic group, d= test vs diabetic+extract, e= test vs diabetic+insulin, f= test vs diabetic+methanol extract.

The Castelli's risk index results are as presented in figure 7. The CRI-I of control and control+extract was 1.62±0.06 and 1.72±0.07 respectively. These two were not significantly different from each other. In the diabetic group, the value was raised significantly to 2.45±0.24 but was reduced to 1.58±0.06 in diabetic extract treated group. Similarly, the values were reduced to 1.75±0.01 in insulin treated group, 1.54±0.01 and 1.61±0.05 in methanol extract and aspirin treated groups respectively. Theses decrease were significant [p<0.05] when compared with diabetic group but not with control group.

3.8. Castelli's Risk Index-II [CRI-II]

In figure 8, the results of the Castelli's risk index-II is shown. The CRI-II was 1.11±0.03 for control group, 0.63±0.16 for control+extract group and 1.64±0.21 for diabetic group. The value for control+extract was reduced significantly [p<0.05] compared with control while diabetic group value was significantly [p<0.05] higher than both control and control+extract groups. The diabetic+extract group was 0.85±0.06, diabetic+insulin group was 1.03±0.01, diabetes+methanol extract group was 0.81±0.03 and diabetic+aspirin group was 0.99±0.05. These values were all significantly [p<0.05] lower than diabetic group but no different with the control group.

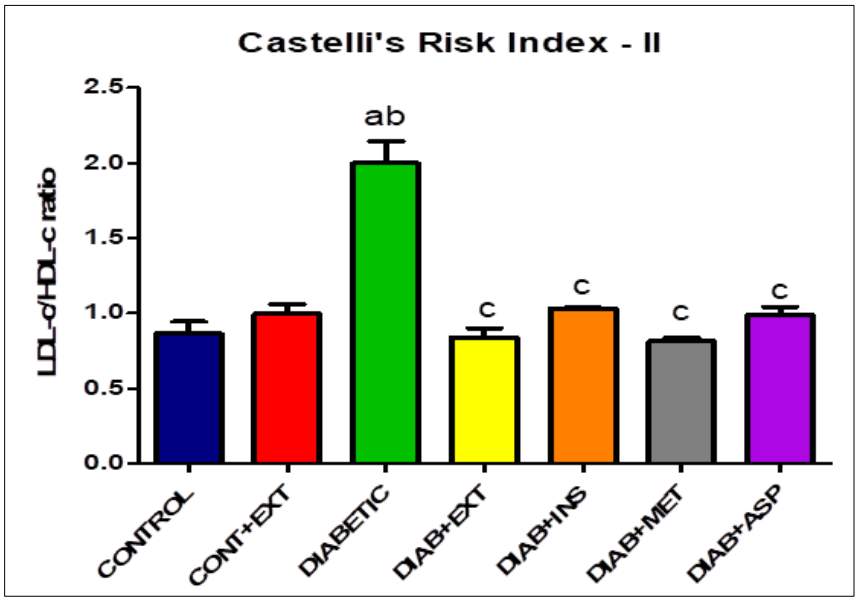

Figure 8 Castelli's Risk Index in nondiabetic and diabetic groups. Values are in mean ± SEM, p<0.05. a= test vs control, b= test vs control+extract, c= test vs diabetic group.

3.9. Blood glucose level

Blood glucose levels on day 1, 4, 7 and 14 are represented in figure 9. On day 1, the blood glucose levels [figure 9a] in all the groups were 86.17±6.1 mg/dL, 84.67±4.7 mg/dL, 71.67±2.4 mg/dL, 80.50±6.3 mg/dL, 80.33±1.3 mg/dL, 88.17±1.5 mg/dL and 92.83±1.5 mg/dL for control, control+extract, diabetic, diabetic+extract, diabetic+insulin, diabetic+methanol and diabetic+aspirin groups respectively. There were no significant changes in the group except in aspirin treated group which was significantly [p<0.05] higher than diabetic blood glucose level.

Blood glucose on day 4 [figure 9b] were obtained as 98.0±2.7 mg/dL, 92.17±2.8 mg/dL, 492.0±31.9 mg/dL, 395.3±38.3 mg/dL, 435.2±44.9 mg/dL, 535.8±24.0 mg/dL and 295.0±8.2 mg/dL for control, control+extract, diabetic, diabetic+extract, diabetic+insulin, diabetic+methanol and diabetic+aspirin groups respectively. There was no significant change between control+extract compared with control group. However, the glucose levels in all diabetes induced groups were significantly [p<0.05] higher than the control and control+extract groups.

The day 7 blood glucose levels [figure 9c] were 80.67±6.0 mg/dL for control group and 81.17±2.6 mg/dL for control+extract group. The diabetic group glucose value was 262.5±9.7 mg/dL and was significantly [p<0.05] higher than the control group. In diabetic aqueous extract treated group the glucose level was 140.5±4.9 mg/dL and was significantly lower than the diabetic group glucose level but was still higher significantly [p<0.05] compared with control group. The insulin treated group glucose level was 153.5±4.7 mg/dL and was also significantly [p<0.05] reduced compared with diabetic and control groups. The methanol extract and aspirin treated groups glucose values were 302.8±5.5 mg/dL and 87.67±1.7 mg/dL respectively. The methanol extract and aspirin treated group also showed

significant [p<0.05] reduction in glucose levels compared with diabetic group. But the glucose level of methanol extract treated group was higher than the control group significantly [p<0.05] while the aspirin treated group was not significantly different compared with control.

On day 14, the blood glucose level [figure 9d] for control group was 83.33±3.8 mg/dL and control+extract group was 87.67±2.9 mg/dL. The diabetic group with glucose level of 377.3±29.2 mg/dL was significantly [p<0.05] higher than control group while the extract treated diabetic group with mean value of 253.3+12.2 mg/dL was observed to reduced significantly [p<0.05] when compared with diabetic group. The insulin treated diabetic group glucose value of 87.0±5.0 mg/dL was significantly [p<0.05] reduced compared with diabetic and diabetic aqueous extract treated groups. Moreover, the methanol extract treated diabetic group showed significant [p<0.05] reduction of glucose level to 134.3±3.4 mg/dL when compared with diabetic group and diabetic extract treated groups. The diabetic group treated with aspirin also showed significant [p<0.05] reduction in the glucose level compared with diabetic group but was significantly [p<0.05] higher than control group value.

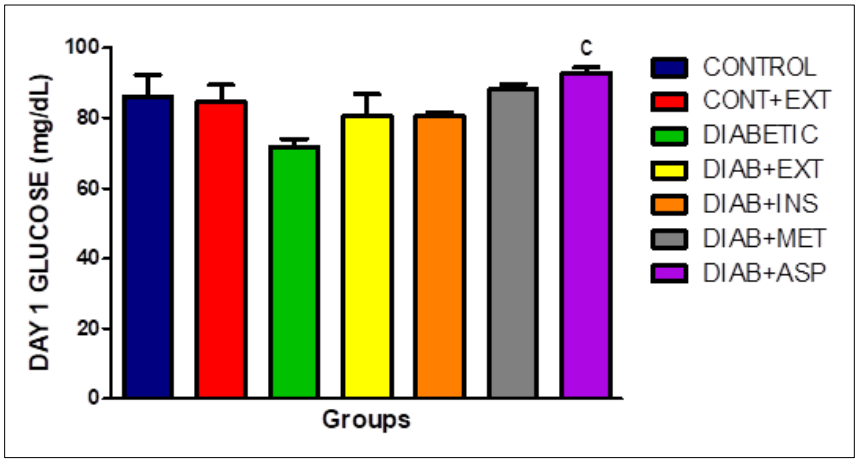

Figure 9a Blood glucose levels in nondiabetic and diabetic groups. Values are in mean ± SEM, p<0.05. c= test vs diabetic group.

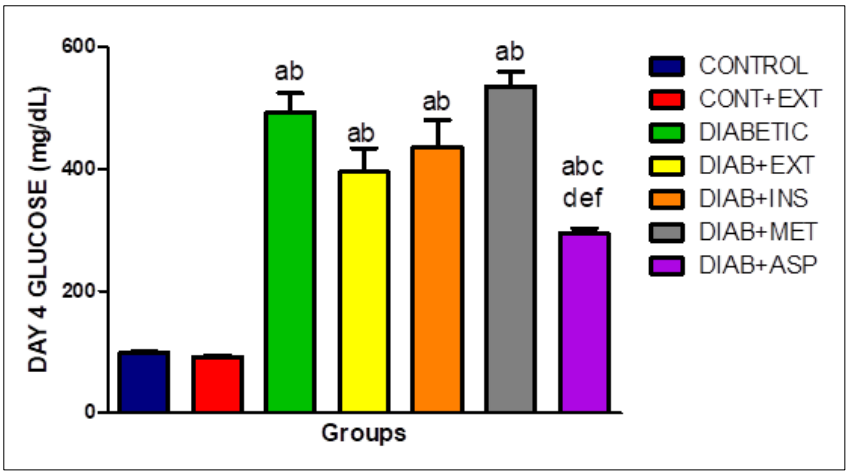

Figure 9b Blood glucose levels in nondiabetic and diabetic groups. Values are in mean ± SEM, p<0.05. a= test vs control, b= test vs control+extract, c= test vs diabetic group, d= test vs diabetic+extract, e= test vs diabetic+insulin, f= test vs diabetic+methanol extract.

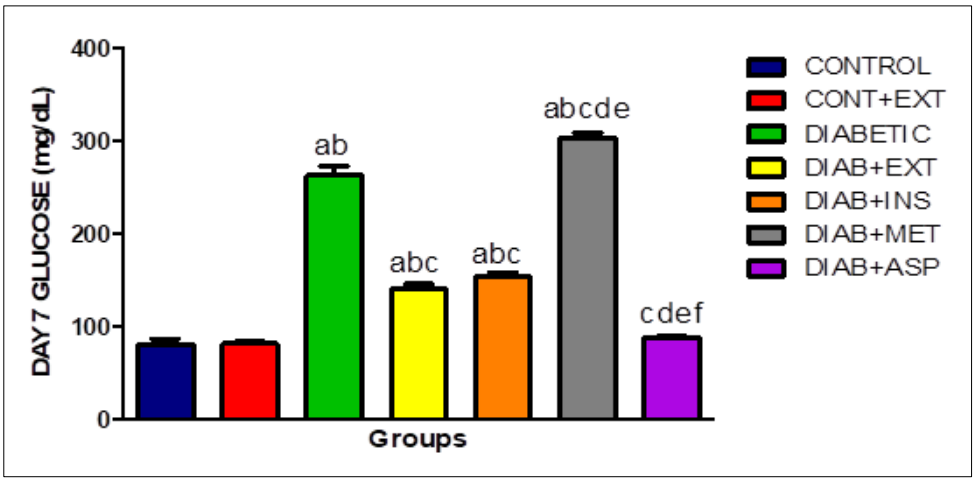

Figure 9c Blood glucose levels in nondiabetic and diabetic groups. Values are in mean ± SEM, p<0.05. a= test vs control, b= test vs control+extract, c= test vs diabetic group, d= test vs diabetic+extract, e= test vs diabetic+insulin, f= test vs diabetic+methanol extract.

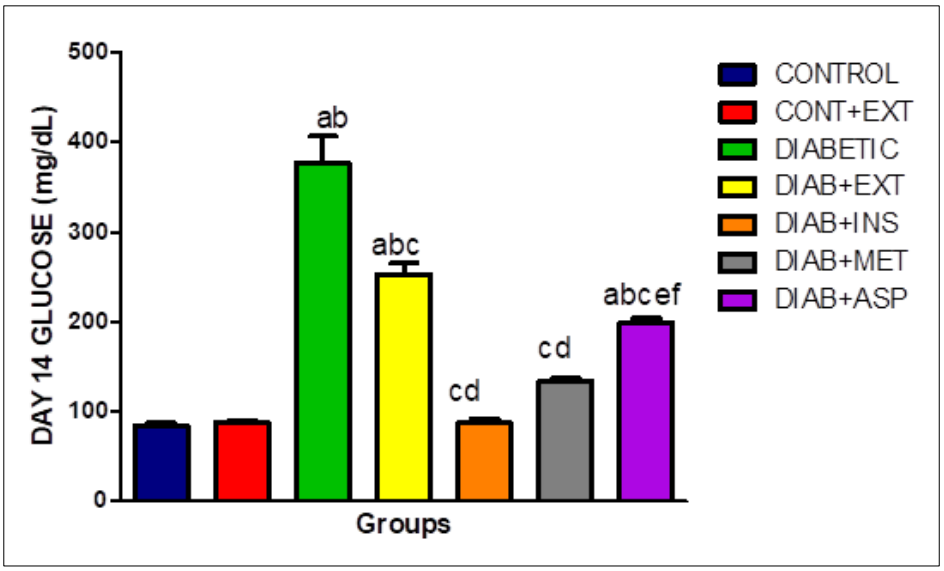

Figure 9d Blood glucose levels in nondiabetic and diabetic groups. Values are in mean ± SEM, p<0.05. a= test vs control, b= test vs control+extract, c= test vs diabetic group, d= test vs diabetic+extract, e= test vs diabetic+insulin, f= test vs diabetic+methanol extract.

4. Discussion

The use of cardiovascular indices for early prediction and timely management is required as a panacea for prevention of atherosclerotic cardiovascular diseases [ASCVD] in diabetes mellitus. Atherogenic Index of Plasma [AIP] and other cardiovascular indices are reliable for predictions of atherogenicity and commencement of relevant medical interventions. The result of lipid profile showed that total cholesterol [TC], triglyceride [TG], low density lipoprotein-cholesterol [LDL-c] and very low density lipoprotein- cholesterol [VLDL-c] were increased significantly while high density lipoprotein-cholesterol [HDL-c] significantly decreased in the diabetic group as compared with control group. Other research findings have corroborated these results [32, 33]. Elevated cholesterol level in blood is a generally established CVD risk [34-37]. And increased LDL-c in blood is associated with development of atherosclerotic plagues in vascular walls [38] which blocks or narrows the lumens of arteries supplying blood to the heart or brain [39]. These vessel can equally be blocked by floating clots released from ruptured plaques [40,41]. Blocking of arterial blood supply to the heart or brain are with attendant serious consequences. Increased LDL-c is also a follow up increase in TG and

VLDL-c which results from abnormal lipid metabolism triggered by various factors such as excess consumption of high fat diets, sedentary life style or metabolic disorders like diabetes mellitus [42].

It is establishe that hyperglycemia is implicated in diabetic dyslipidemia [43] and cardiovascular complications centered majorly on dyslipidemia [44]. This involves increased TC, TG, LDL-c and VLDL-c and reduced HDL-c [45] The absence of insulin action due to insulin resistance, non availability of insulin or dysfucntional adipose tissue results in reduced activity of hormones sensitive lipase [46]. This consequently leads to increased breakdown of intracellular triglycerides and release of free fatty acid into blood. There is a positive correlation between increased free fatty acid and increased hepatic triglycerides synthesis and this culminates into raised blood level of very low density lipoproteins. Remodelling of very low density lipoproteins and high density lipoprotein by various lipases results in increased renal clearance of HDL-c and resultant low HDL-c in blood.

However, it was observed that results from aqueous and methanol extracts treated diabetic groups significantly reduced concentrations of triglycerides, total cholesterol, low density lipoprotein cholesterol, very low-density lipoprotein and increased significantly the levels of high-density lipoprotein cholesterol compared to untreated diabetic group with no significant difference when compared with control group. This result was in agreement with the work of Vishnu et al. [47]

The aqueous and methanol extracts treated groups' results showed a reduction in blood glucose levels and this is attributed to the anti-hyperglycemic properties of the *T. catappa* leaves. Lipoproteins modifications occur in the presence of high blood glucose, thus the observed reductions in blood glucose levels could be considered as direct extracts effects on lipid profile modifications. But epidemiological studies have reported improved vascular function and reduced cardiovascular events of some hypoglycemic drugs by mechanisms other than their anti- hyperglycemic effects in type 2 diabetes [48]. In the same vain, the changes in lipid profile by aqueous and methanol extracts of *Terminalia catappa* leaves may activate mechanisms other than the anti-hyperglycemic function which is yet to be elucidated.

In this study the atherogenic index of plasma [AIP], Castelli's index –I [CRI-I] representing Cardio Risk Ratio [CRR] and Castelli's index-II [CRI-II] representing Atherosclerosis Index [AI] were significantly increased in diabetic untreated group. This result is consistent with the work of Rajesh et al [49] who reported similar changes in diabetic animals. The aqueous and methanol extracts treated groups showed significant reductions in the cardiovascular indices; AIP, CRI-I and CRI-II compared with diabetic group. Atherogenic Index of Plasma is used to assess plasma atherogenicity and small LDL particles [29] and predicts the ratio of atherogenic lipid molecules and non-atherogenic molecules. Such prediction is useful in the prognosis of atherosclerosis and coronary heart disease which are not achieved with routine lipid profile determination [13,14]. Moreover, the role LDL in development of atherosclerosis had been established and targeting it is the focus in CHD prevention. The observed reduction in AIP, CRI-I and CRI-II in extract treated groups signifies the influence of some phytochemical constituents on the different LDL components and improvement of its qualitative and kinetic abnormalities. Therefore, *Terminalia catappa* leaf extracts showed capacity of modifying the quality of lipid profile altered by hyperglycemia. The results of this study had shown that leaf extract of *Terminalia catappa* in aqueous or methanol fractions is beneficial in diabetes mellitus by improving lipid profiles and reducing diabetes associated atherogenic risk. This position is supported by the previous findings by Iheagwam et al [50] on the safety of *T. catappa* aqueous leave extract sub-acute administration on lipid profile in rats".

Varying patterns of changes were observed in insulin and aspirin treated diabetic groups but with great proportion similar to that of the extracts. The TC, TG, LDL-c and VLDL-c were significantly reduced and HDL-c increased in insulin treated diabetic group compared with diabetic untreated group. Also, in aspirin treated group, TC, TG LDL-c was significantly reduced but VLDL-c and HDL-c was significantly increased compared with diabetic untreated group but the reason for raised VLDL-c was not verified.

5. Conclusion

In summary, comparing the effects of the two extracts of *T. catappa* leaves [aqueous and methanol extracts], the changes in the lipid profile were similar in reduction of TC, TG, LDL-c, VLDL-c and improvement of HDL-c. It is also observed that atherogenicity was reduced in diabetic groups treated with both extracts in similar patterns across the cardiovascular indices. The effect on blood glucose was again observed to be the same except on day 7 of methanol extract treated group. The observed level of glucose on day 7 is not understood but may be attributed to some undetectable experimental errors as the 14^{th} day glucose level follows the trend with that of aqueous extract treated group. Therefore, modifications of lipid profile and reduction of atherogenic index in diabetic rats were similar in aqueous and methanol

extracts of *Terminalia catappa* leaves and both extracts may be useful in prevention of cardiovascular complications in diabetes mellitus.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The experimental protocol received full ethical approval from Faculty Animal Research Ethics committee - Faculty of Basic Medical Sciences (FAREC-FBMS) with approval number 021PY30417.

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