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Effect of Ethanolic Extract of *Azadirachta Indica* (Neem) Gum on Fasting Lipid Profile on Female Streptozotocin Induced Diabetes Rats

Aiki, G.*[1], Dallatu, M. K.[1], Abubakar, I. A.[2], Suleiman, N.[3], Wali, U. [1], Ngaski, A. A.[1], Bunza, J. M.[1] and Abubakar U.[4]
Department of Chemical Pathology School of Medical Laboratory Science Usmanu Danfodiyo University Sokoto [1], Department of Veterinary Parasitology and Entomology Faculty of Veterinary Medicine Usmanu Danfodiyo University Sokoto [2], Department of Veterinary Physiology and Biochemistry Faculty of Veterinary Medicine Usmanu Danfodiyo University Sokoto [3], Department of Histopathology School of Medical Laboratory Science Usmanu Danfodiyo University Sokoto [4].
Author for Corresponding*: +234-703-509-4902 / gamboaiki78@gmail.com
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Abstract
Diabetes is a metabolic disorder with prolong hyperglycemia ranking top tenth global causes of death that is linked to malfunction of pancreatic beta cell, macrovascular and microvascular complications characterized by elevated blood lipid levels (hyperlipidemia) leading to Lipotoxicity and susceptibility to dyslipidemia that is a risk factor for cardiovascular disease and atherosclerosis. This study assessed the effect of ethanolic extract of *Azadirichta indica gum* on fasting lipid profile in streptozotocin induced diabetes albino rats. Thirty-six female albino rats were randomly assigned into six (6) groups, Group I was a positive control. Groups II and III were streptozotocin- induced diabetic administered 2.0 ml each of 100 mg/kg and 200 mg/kg body weight of the ethanolic extract of *A. indica* gum respectively. Groups IV was a negative control while V and VI were non-diabetic rats administered 2.0 mL each of 100 mg/kg and 200 mg/kg body weight of the ethanolic extract of *A. indica*. The results showed a decrease in total cholesterol, Triglyceride and, Serum LDL- cholesterol, concentration (mmol/L) and an increase in HDL-Cholesterol (mmol/L), that was statistical significant ($p< 0.05$) after analysis using One Way independent ANOVA in JMP software version 11, and Dunnett's Post hoc test for comparison back to control The outcome of this study indicates that *A. indica gum* possesses potential in mitigating hyperlipidemia and preventing lipotoxicity that is related to complications of diabetes. This improvement *suggests its* consideration in the management of cardiovascular complications associated with diabetes. Study can also be done on dose optimization of *A. indica* gum for maximum therapeutic benefit while minimizing potential side effects coupled with further clinical studies following positive outcome observed in the animal models.

Keywords: *A indica,* Streptozotocin, Diabetes, lipid profile and hyperlipidemia

Introduction

Diabetes is raised as the tenth leading global causes of death (Rhys *et al.*, 2020) encompasses a cluster of metabolic disorders that is associated with the malfunctioning of pancreatic beta cells. This dysfunction results in the persistently elevated high blood glucose level over an extended period. It manifested either when the pancreas produce sufficient insulin (Type 1 diabetes mellitus) or when the body struggles to effectively utilize the produced insulin (Type 2 diabetes mellitus) or a combination of both *(ADA, 2014).* Beta (β) cells located in pancreatic islets play a crucial role in the synthesis and secretion of insulin and amylin hormones, contributing to the regulation of blood glucose levels. The destruction of Beta (β) cells is associated to glucose toxicity, lipotoxicity (*Chen et al., 2017;* Risheng *et al.,* 2018*)*. Diabetes, as outline by Vincent *et al.* (2010) is characterized by elevated blood lipid levels (hyperlipidemia) leading to lipotoxicity and susceptibility to dyslipidemia that is a risk factor for cardiovascular disease and atherosclerosis with an imbalance between the oxidizing species and endogenous antioxidants. (Chen *et al*., 2009; Krakauer,2015; Conti *et al*., 2016). There are

various traditional and synthetic medications like metformin, glucagon like peptide -1 agonist for type two diabetes (T2DM) and insulin for type two diabetes (T1DM), current in use but high cost and side effect have been associated to some of them especially metformin that in linked to a significant rise in lactic acid concentration leading to lactic acidosis and increased risk or mortality (Hung *et al*., 2015; Bell *et al.,* 2017). Natural remedies have been investigated and among the various methods and pharmacotherapies being developed, the use of *A. indica* extract has steadily been given great interest and attention because of its several medicinal applications and widespread accessibility (Joshi *et al.*, 2010; Al-Akeel *et al*., 2017). However, there is lack of information regarding the use of *A. indica* gum in diabetes management especially in tropical area of Asia and Africa where the trees are in abundance. *A. indica* (neem) gum have been proven to possess alkaloids, flavonoids, saponins, and tannins, which have the potential to interfere with the activity of the α-glucosidase enzyme thereby helping to lower blood glucose levels. D-glucoside, Quercetin-3-O- L-rhamnoside that exhibit antilipidemic activity (Mirghani *et al*., 2018; Fardhani and Graciella, 2023). The aim of this study was to investigate the effect of ethanol extract of *A. indica* (Neem) gum on lipid profile in streptozotocin induced diabetic albino rats model.

Materials and Methods

Study Area

The research was conducted at the Animal House within the Faculty of Pharmaceutical Sciences at Usmanu Danfodiyo University Sokoto Nigeria, situated at metropolitan city of Sokoto.

Collection and *Gum* Identification of Neem Tree

A. indica gum was *randomly c*ollected from the tree trunk within 8 Division Army Barrack Dange-Shuni Local Government of Sokoto Nigeria. *Following, collection the tree specimen was identified* and authenticated at the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto with a Voucher number of PCG/UDUS/MALI /0001

Extraction of *A. indica* Gum

Approximately 100g of dried and grinded, *A. indica* was mixed in of 1000mL ethanol and left to undergo extraction for 48 hours. The mixture was then filtered using Whitman Number 1 filter paper to separate the filtrate from the residue. The process was repeated multiple times until the extraction solvents became colorless. The ethanol in the extract was evaporated at 50°C to yield dry extract of the specimen, as outlined by Nur *et al*. (2013).

Ethical Consideration

The handling of the animals was adhered to the ethical guideline including both institution's protocols and internationally accepted practices for the use and care of laboratory animals as well as the International Association for the study of pain (IASP) guideline (Zimmerman, 1983; Aniagu *et al*., 2005) and the ethical approval from the ethical committee of Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto.

Experimental Design

Thirty-six female albino rats (28 weeks) old were purchased from the Animal House of the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto. The rats were housed, fed and allowed to acclimatize at preset room temperature of 26 ^{0}C using an air conditioner for two weeks before the commencement of the experiment. Diabetes was induced in 21 animals through intraperitoneal using freshly prepared Streptozotocin (70 mg/kg body weight) dissolve in normal saline (0.859 %), while the remaining 15 animals remained uninduced (Adeyemi *et al.,*2014; Asghar and Sajad, 2023).

The rats were randomly allocated into six (6). Group I was diabetic induced rats that were administered 2.0 Ml of distilled water only as the positive control. Groups II and III were also diabetic induced rats that were administered 2.0 ml each of 100 mg/kg and 200 mg/kg body weight of ethanol extract of *A. indica* gum respectively. Group IV was non-diabetic induced rats administered 2.0 ml of distilled water only as negative control) while groups V and VI were non- diabetic induced rats also that were administered 2.0ml each of 100 mg/kg and 200 mg/kg body weight of ethanol extract of *A. indica gum* respectively.

Blood Sample Collection

Five milliliters (5 ml) of blood sample were collected at the end of the study following 12 hours overnight fasting. The blood were obtained through cardiac puncture under chloroform inhalation anesthesia. The serum was harvested after centrifuging at 3000rpm for five minutes using Biofuge 200. Subsequently the serum was dispensed into plain containers, separated into aliquots, and stored in a refrigerator at 4 °C for Subsequent use.

Laboratory Analysis

Total cholesterol Determination: Total cholesterol was enzymatically measured in serum by Trinder, 1969 using Randox kit (Randox Laboratories, UK) through a series of linked reactions. This reaction involves the hydrolysis of cholesteryl esters and the oxidation of the 3-OH group of cholesterol producing (H_2O_2). In the method 10µL of the sample, standard and distilled water were added to test, standard and blank respectively. Subsequently 1ml of cholesterol reagent was added into each test tube. The contents were thoroughly mixed and incubated at 37^{0}C for 5 minutes after which the absorbance was measured against the reagent blank using a SP 2000 spectrophotometer at 546nm. The values were expressed in mmol/L.

HDL-Cholesterol Estimation: HDL-Cholesterol was estimated by Trinder (1969). The samples were first precipitated to removed chylomicrom and other lipoprotein fraction. The HDL-Cholesterol in the supernatant was determined by the addition of 300 µL of the sample, HDL standard and distilled water were dispense as, test, standard and blank respectively followed by the addition of 1mL of the working reagent. After thorough mixing the contents were incubated at 37^{0}C for 5 minutes and their absorbance was measured against reagent blank SP 2000 Spectrophotometrically at 546nm. The results were expressed in mmol/L

Triglyceride determination (TG): TG determination was carried out by Trinder (1969) enzymatically method were triglycerides undergoes hydrolysis to generate glycerol that is subsequently, oxidized in the presence of ATP and glycerokinase. The developed colour was quantitatively assessed at 546 nm using Sp 2000 spectrophotometer. Ten microliter (10 µL) of the sample, triglyceride standard and distilled water were added as test, standard and blank respectively. Then 1mL of the working reagent was added to each of the test tubes and then incubated at 37^{0}C for 5 minutes and their absorbance was read using Spectrophotometer at 546nm. Triglycerides concentration was determined using the expression bellow.

$$\text{Serum triglyceride conc. (mmol/L)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \text{ x Concentration of standard}$$

LDL-Cholesterol Determination: LDL-cholesterol is calculated the values of total cholesterol, triglycerides and HDL-cholesterol according to the relationship by Friedewald equation (1974) [LDL-chol] = [total chol] - [HDL-chol] - [TG]/5 and the values were presented in mmol/L.

Data analysis

Numerical data of fasting lipid profiles: total Cholesterol HDL-cholesterol, LDL- cholesterol, Triglyceride, in mmol/L were analyzed using One-way independent ANOVA in JMP soft ware version 11, and Dunnett's Post hoc test for comparism back to control was used to determine if there is any statistical differences between groups ($p < 0.05$).

RESULTS

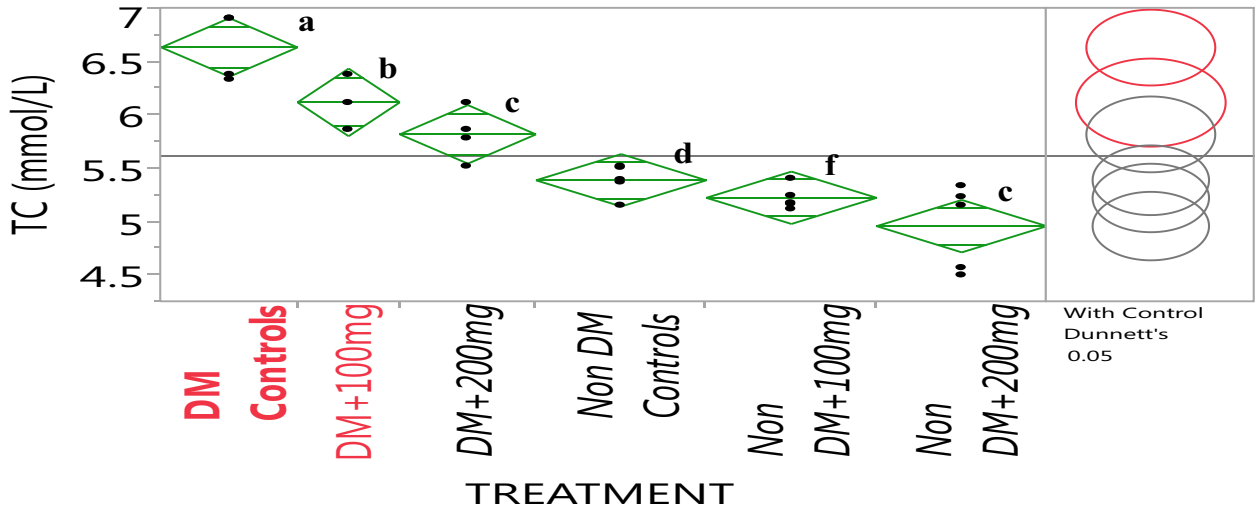

Figure 1: The comparison of mean Total cholesterol (TC) across groups of albino rats administered ethanol extract of A. *indica* gum with different alphabets on the diamond error bar as significantly different.

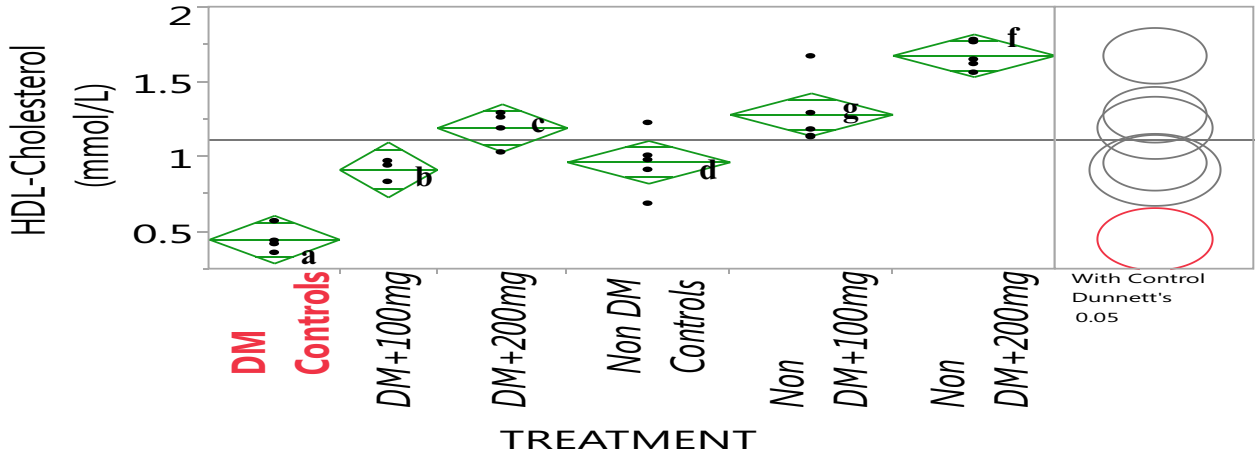

Figure 2: The comparison of mean high density lipoprotein (HDL) across groups of albino rats administered ethanol extract of A. *indica* gum with different alphabets on the diamond error bar as significantly different.

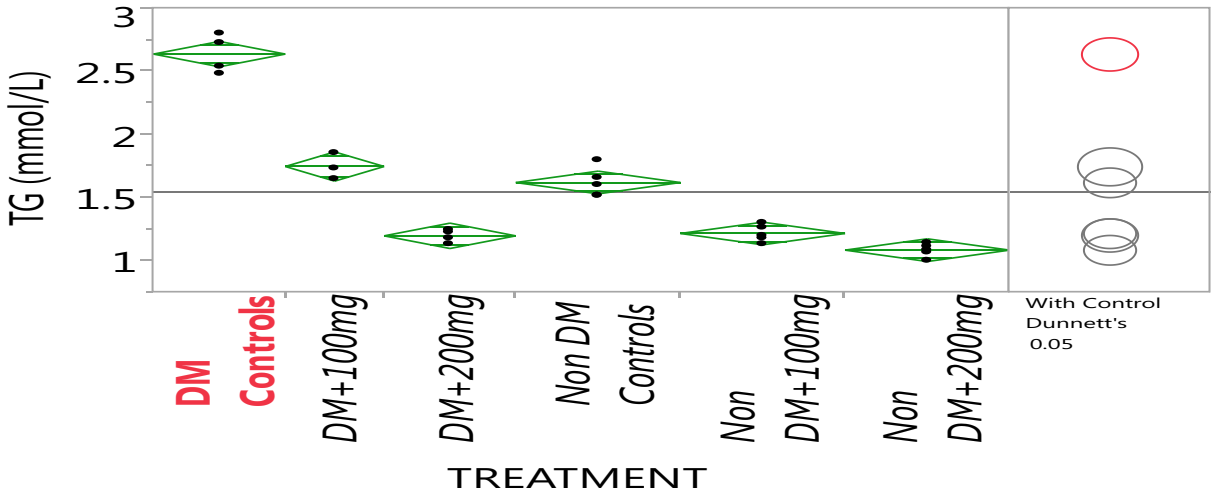

Figure 3: The comparison of mean triglyceride (TG) across groups of albino rats administered ethanol extract of *A. indica* gum with different alphabets on the diamond error bar as significantly different at ($p<0.05$) using one way Analysis of variance (ANOVA) .

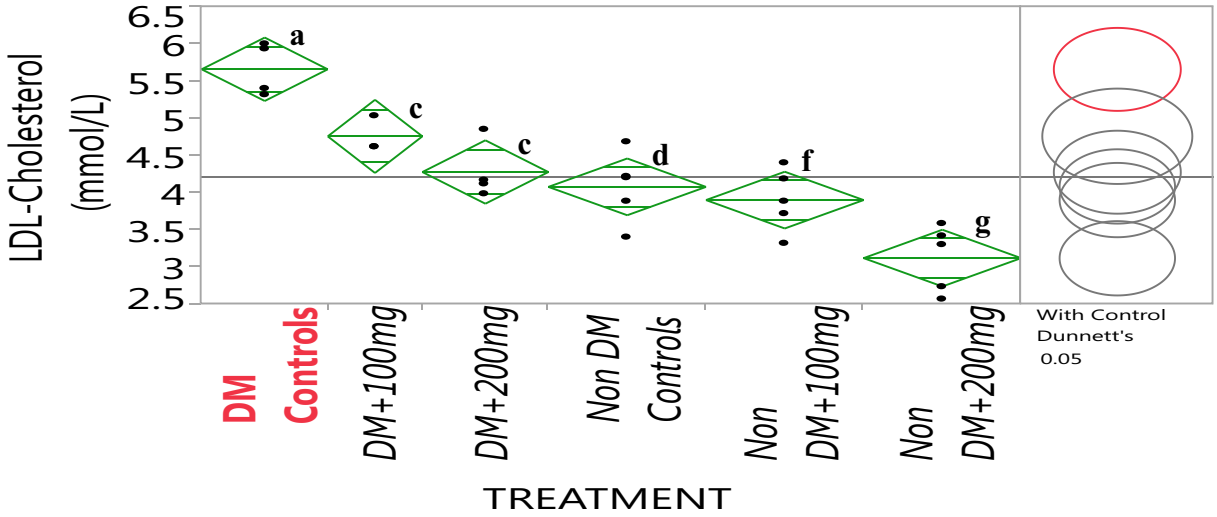

Figure 4: The comparison of mean low Density Lipoproteins (LDL) across groups of albino rats administered ethanol extract of *A. indica* gum with different alphabets on the diamond error bar as significantly different.

Discussion

The measurement of serum, total cholesterol (TC) high density lipoprotein cholesterol, (HDL) triglyceride (TG) and high-density lipoprotein cholesterol (LDL) serves as reference method for the determination of lipid profile. The effect of *A indica* gum on fasting TC, TG LDL, HDL in this study illustrate negative (reduction) effect on serum fasting TC (Fig 1), TG (Fig 3.), LDL-Cholesterol (Fig 4) that could be linked to the presence of D-glucoside, Quercetin-3-O- L-rhamnoside which is presumed either wholly or partly to be responsible for antihyperlipidemic activity which is also presence in both *A. indica* leaf and gum (Chattopadhyay, 1999).

The results also elicit a positive (increase) effect on HDL-Cholesterol in the STZ induced DM and non STZ induced DM groups treated with *A indica* gum. The increase in HDL-Cholesterol may be attributed to the reduction in TG level as it is well known that the particle size of HDLs correlates inversely with the concentration of plasma triglyceride and low level of HDL-C may actually promote development of diabetes and predict cardiovascular disease, according to Barter and Hugh (2002) and Drew *et al.* (2012). The increase in HDL-C levels may improve antioxidant activity by activating other HDL apo-lipoproteins, such as Apo-E and Apo-J, to act also as antioxidant activity and inhibiting T cell-mediated macrophage secretion of pro-inflammatory cytokines and chemokine in diabetic condition as well increases insulin-independent glucose uptake via Akt signaling pathway (Heywood *et al*., 2014).

There are no much research on the effect of *A indica* gum on lipid profile. A similar work by Chattopadhyay and Bandyopadhyay (2005) support the use *A. indica* extract (leaf) in the reduction of total cholesterol, LDL- and TG and total lipids of serum in streptozotocin-induced diabetic rats as observed in this study. This study agrees with a previous report by Lamis *et al.* (2019) in the reduction of total cholesterol (TC), TG, and LDL by Arabic gum which has similar composition as *A. indica.*

Diabetes, as outlined by Vincent *et al.* (2010) and Risheng *et al. (*2018), is characterised by elevated blood lipid levels (hyperlipidemia) leading to Lipotoxicity. This is typically characterized by high TC, TG, LDL-cholesterol and low HDL-cholesterol resulting in increased risk for coronary heart disease (CHD) (Mooradian, 2009; Santos-Gallego and Rosenson, 2014). Individuals with Diabetes are more prone to dyslipidemia which could contribute to cardiovascular disorder (Bhambhani *et al*., 2017).

The results obtained in this study proved no substantial evidence of hyperlipidemia and susceptibility to dyslipidemia *that can be* attributed to high presence of antioxidant natural compounds with large hydroxyl group that can scavenge free radical in *A. indica* (Malviya *et al*., 2017).

This result also justify taking *A. indica* gum in the reduction of the risk for cardiovascular disorders which is seen in diabetes condition as ascertained by *Bhambhani et al.* (2017), Furthermore, the utilization of *A. indica* gum in diabetes management may offer advantages including cost effectiveness and ready availability and potentially reduced financial burden associated to diabetes care. The natural and affordability nature of *A. indica* gum makes it a promising resource for improving lipid profile levels there by contributing to effective diabetes management.

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