RESEARCH ARTICLE

Ameliorative effect of crude and solvent partitioned fractions of *Gymnema sylvestre* in alloxan-induced hyperglycemic and dyslipidemic *Rattus norvegicus*

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ABSTRACT

The present study aims to evaluate the ameliorative effect of crude and solvent partitioned fractions of *Gymnema sylvestre* in alloxan-induced hyperglycemic and dyslipidemic rats. The extract and fractions of *Gymnema sylvestre* were administered to alloxan (120 mg/kg) induced diabetic rats at doses of 100, 300, and 600 mg/kg. Metformin (100 mg/kg) was used as the reference drug. All treatments were administered daily for 14 days through the oral route. Effects of the treatments on blood glucose levels, body weight, and serum lipid profiles were monitored. The results revealed that all the extracts and fractions were able to reduce the blood glucose levels and improved the body weight of diabetic rats, with more pronounced effects at the treatment of 300 mg/kg of crude methanol and ethyl acetate fraction. The blood glucose of diabetic untreated rats continued to increase up to the time of euthanization. There were significant reductions ($p<0.05$) in the levels of serum total cholesterol, triglyceride, and low-density lipoprotein cholesterol (LDL-C) of all the treated groups when compared to the diabetic untreated. However, a significant ($p<0.05$) decrease in the serum concentration of high-density lipoprotein- cholesterol (HDL- C) was observed in the diabetic untreated rats when compared to the HDL-C levels in the treated rats. The ameliorative effect of the crude extract and the partitioned fractions on the diabetic induced alterations in serum lipid profile occurs in a significant ($p<0.05$) dose-dependent manner. In conclusion, *G. sylvestre* extracts possess hypoglycaemic effects and attenuated diabetic induced dyslipidemia in rats

Keywords: *Gymnema sylvestre;* hypoglycemic; hypolipidemic; lipid profile; alloxan-induced dyslipidemia

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1.0 Introduction

Diabetes mellitus is a metabolic disorder characterized by high glucose level and alteration in metabolism of macromolecules (carbohydrates, fats and proteins) due to defects in the body's ability to produce or use insulin [1]. The alteration in the metabolism of macromolecules (carbohydrate, fat and protein) in diabetes is due to deficiency of insulin or its action on target cells which leads to hyperaminoacidemia hypercholesterolemia, abnormalities of lipoprotein metabolism and elevation in the activities of some enzymes [2].

In Nigeria, about 5 million people are living with diabetes, while more than 1.56 million cases of diabetes were recorded in 2015 [3]. Type 2 diabetes, also known as non-insulin dependent diabetes mellitus, is the most common form of diabetes and affects 2-6 % of adults in most western societies [4]. Due to limitation and adverse effects of available antidiabetic drugs, the disease is currently becoming difficult to manage [5]. With increasing incidence of

diabetes mellitus among rural population, there is a clear need for development of inexpensive agents that would be used for the management of diabetes [6]

Herbs are alternative medicines for treatment of various diseases due to their acceptability, effectiveness, affordability, assumed safety and low cost [7]. Traditional medicine existed from ancient times, and is estimated by World Health Organization to be in use by 80 % of the population in most of the developing countries [8]. About 75 % of Nigerians solve their health problems consulting traditional practitioners. Different medicinal plants have been used in most parts of the world as diabetic regime because of their hypoglycemic effects [9]. Reports have shown that more than 800 plants have been used as empirical treatment for diabetes. One tenth has been characterized as hypoglycemic plants with active components. Mechanisms such as the stimulating or regenerating effect on beta cells or extra pancreatic effects are proposed for the hypoglycemic action of these herbs [10]

Gymnema sylvestre is a vine-like plant that belongs to the family of Asclepiadaceae. The plant is also known as sugar destroyer and has been used in folkloric treatment of diabetes mellitus or managing its complications for many years [11]. The important active ingredient of *Gymnema sylvestre* is an organic acid called "gymnemic acid". The phytoconstituents responsible for sweet suppression activity includes triterpene-saponins known as gymnemic acids, gymnemasaponins, and a polypeptide, gurmarin[12].

The plant extract is used in dietary supplements since it reduces bodyweight, blood cholesterol, and triacylglycerol levels and holds great prospects in dietary as well as pharmacological applications [13,14]. Because *G. sylvestre* is used in the treatment of diabetes, there is therefore an urgent need to screen the plant for antidiabetic properties with the hope of discovering a lead compound that would aid pharmaceuticals in developing a new drug. The aim of this research is therefore to evaluate the Ameliorative effect of crude and solvent partitioned fractions of *Gymnema sylvestre* in alloxan-induced hyperglycemic and dyslipidemic rats

2.0 Materials and Methods

2.1 Sample Collection

The *Gymnema sylvestre* plant was collected randomly from a farm, two kilometres away from Bida town Niger State. The plant was identified and authenticated at National Research Institute for Chemical Technology, Zaria, where voucher number 1613 was deposited. The plant was rinsed under clean running water, and air dried at Department of Biochemistry laboratory for two weeks. The dried plant was pulverized into powder with mortar and pestle and then milled with an electric blender and stored in an air tight container until it is ready for use.

2.2 Experimental Animals

Healthy Swiss albino rats (*Rattus norvegicus*) were obtained from Department of Biochemistry, Benue state University. They were housed under standard laboratory conditions (temperature 27$\pm$ 2 ^{o}C, 70 % relative humidity, 12 hrs day light/night cycle) with access to commercial feed (grower's marsh) and water to feed *ad libitum*. Animals were kept in compliance with internationally accepted principles for humane handling and use of laboratory animals in the Canadian council on Animal Care Guidelines and Protocol Review and National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, 1985).

2.3 Sample Extraction

The methanol crude extraction, and n-Hexane and ethylacetate fractionations were conducted as described in our previous studies [15,16]. Briefly, six hundred grams (600 g) of powdered *Gymnema sylvestre* plant sample was extracted exhaustively extracted with 2.5 liters of methanol. The resulting extract was concentrated in rotary evaporator and air dried. The crude extract (30 g) was partitioned between n-hexane (1500 mL) and ethyl acetate (600 mL) using a separating funnel to yield n-hexane (1.03%) and ethyl acetate (2.4%) fractions which were concentrated using a rotary evaporator. The concentrated extract and fractions were stored in a refrigerator at 4 °C.

2.4 Experimental Design

Diabetes was induced in overnight fasted rats by intraperitoneal administration of a single dose of 110 mg/kg bodyweight of a freshly prepared alloxan monohydrate. Diabetes was confirmed after 72 hours of induction with fasting blood glucose level ≥180mg/dl[17]. Thirty-six rats that were able to meet the standard of diabetic conditions were assigned into twelve groups of three rats each. Groups 1-9 were treated with 100, 300 and 600 mg/kg each of crude extract, n-hexane, and ethylacetate fractions of *G. sylvestre*. Groups 10 and 11 serve as the standard control (100 mg/kg metformin) and diabetic control (5 mL/kg normal saline) respectively while the group 12 serve as the positive control (nomoglycemic). Rats in their respective groups were administered extracts orally once daily for fourteen days.

2.5 Measurement of bodyweight

Rats in their respective groups were weighed using a weighing balance every five days of the period of treatment to monitor any weight loss or gain as a result of extracts administration.

2.6 Measuring of fasting blood glucose

Fasting blood glucose was measured in mg/dl using Accu check glucometer through the drop of blood sample obtained by orbital puncture of the tail vein after overnight (12-15 hr) fast of rats on day 0, 1^{st}, 5^{th}, 10^{th}, 15^{th}.

2.7 Collection of Blood Sample

On the fifteenth day, following an overnight fast, they were anaesthesized under chloroform vapour and euthanized. The blood samples were collected by carotid puncture into sample bottles. The blood samples were left for fifteen minutes to clot and then centrifuged at 3000 rpm for 15 minutes to get the serum [18]. The sera were stored in the refrigerator at -20 °C for subsequent analysis.

2.8 Analysis of serum lipid profiles

Serum concentrations of lipid profile including total cholesterol, triglycerides and high-density lipoprotein (HDL)-cholesterol were assayed by enzymatic colorimetric methods using commercially available kits (Agape Diagnostic Kit, Switzerland GmbH) according to the manufacturer's instructions. VLDL-cholesterol was estimated as TG/5, and LDL-cholesterol was calculated using Friedewald formula[19] as follows:

LDL (mg/dl)=TC−(HDL+VLDL)LDL (mg/dl)=TC−(HDL+VLDL)

2.9 **Statistical Analysis**

Values were analyzed using statistical package for social science (SPSS) version 16 and presented as means ± standard error of the mean. Comparisons between different groups were carried out by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The level of significance was set at ($p < 0.05$).

3.0 Results

3.1 Hypoglycemic effects of *G. sylvestre* extracts in alloxan induced diabetic rats

Results in figures 1-3 shows the effects of crude methanol, ethylacetate and n-hexane fractions of *G. sylvestre* plant on the fasting blood glucose concentration of diabetic rats. All the extracts were able to reduce the blood glucose of diabetic rats with much effect seen in 300 mg/kg bodyweight of crude methanol and ethylacetate fraction. The blood glucose of diabetic untreated rats continued to increase up to the time of euthanization.

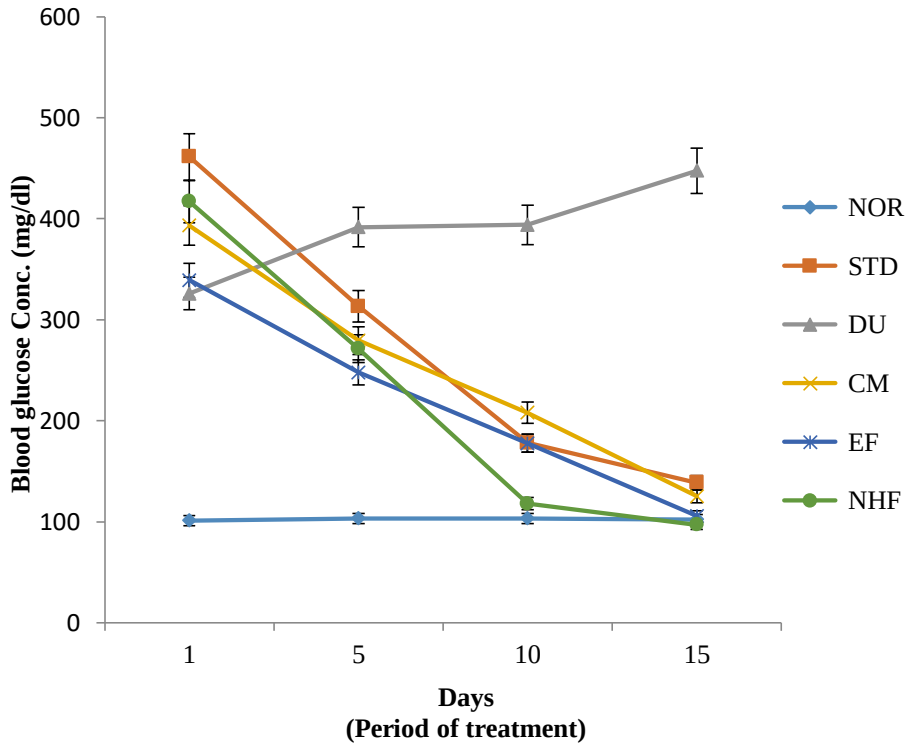

Figure 1: Effect of 100 mg/kg of crude methanol, and ethyl acetate and n-hexane fractions of *G. sylvestre* on blood glucose levels in alloxan-induced diabetic rats. NOR: Normoglycemic, STD: Standard drug (100 mg/kg metformin), DU: Diabetic untreated, CM: Crude methanol extract, EF: Ethyl acetate fraction, NHF: n-hexane fraction.

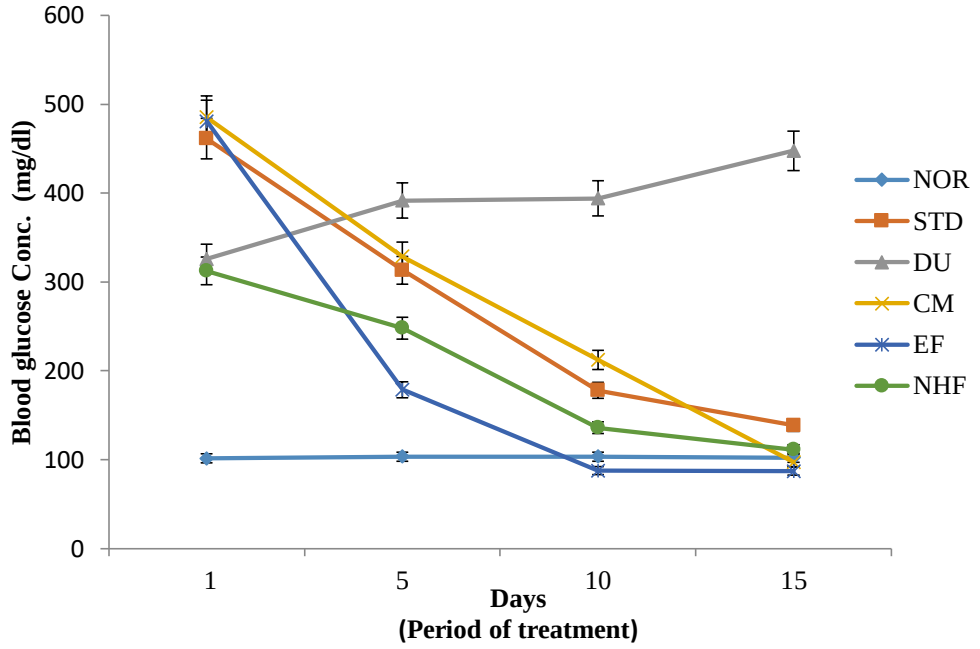

Figure 2: Effect of 300 mg/kg crude methanol, and ethyl acetate and n-hexane fractions of *G. sylvestre* on blood glucose levels in alloxan-induced diabetic rats. NOR: Normoglycemic, STD: Standard drug (100 mg/kg metformin), DU: Diabetic untreated, CM: Crude methanol extract, EF: Ethyl acetate fraction, NHF: n-hexane fraction.

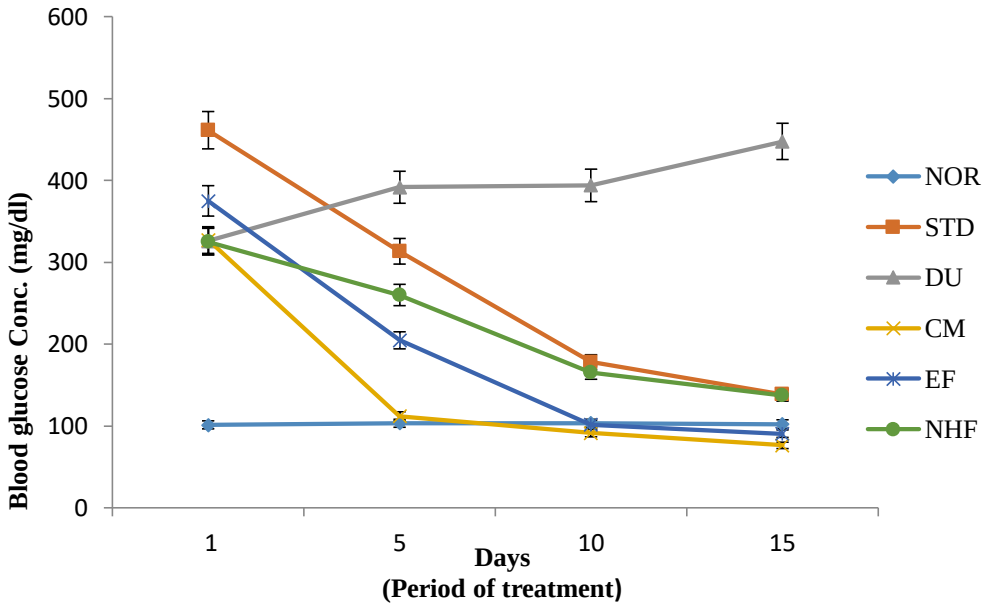

Figure 3: Effect of 600 mg/kg crude methanol, and ethyl acetate and n-hexane fractions of *G. sylvestre* on blood glucose levels in alloxan-induced diabetic rats. NOR: Normoglycemic, STD: Standard drug (100 mg/kg metformin), DU: Diabetic untreated, CM: Crude methanol extract, EF: Ethyl acetate fraction, NHF: n-hexane fraction.

3.2 Effects of *G. sylvestre* extracts on bodyweight of alloxan induced diabetic rats

Effect of crude methanol, ethyl acetate and n-hexane fractions of *G. sylvestre* on bodyweight of diabetic treated rats are shown in figures 4-6. All rats in respective groups demonstrated a weight reduction when compared with normoglycemic group that gained weight in the period of experiment.

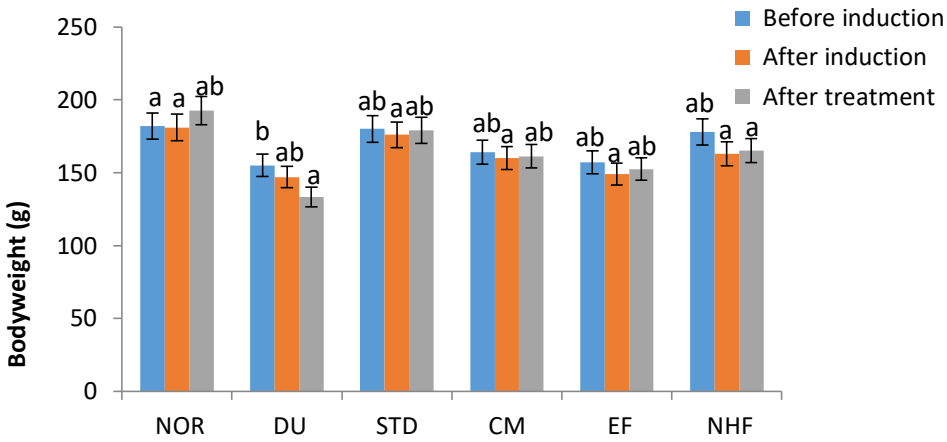

Figure 4: Effect of 100 mg/kg crude methanol, and ethyl acetate and n-hexane fractions of *G. sylvestre* on body weight of alloxan-induced diabetic rats. NOR: Normoglycemic, STD: Standard drug (100 mg/kg metformin), DU: Diabetic untreated, CM: Crude methanol extract, EF: Ethyl acetate fraction, NHF: n-hexane fraction.

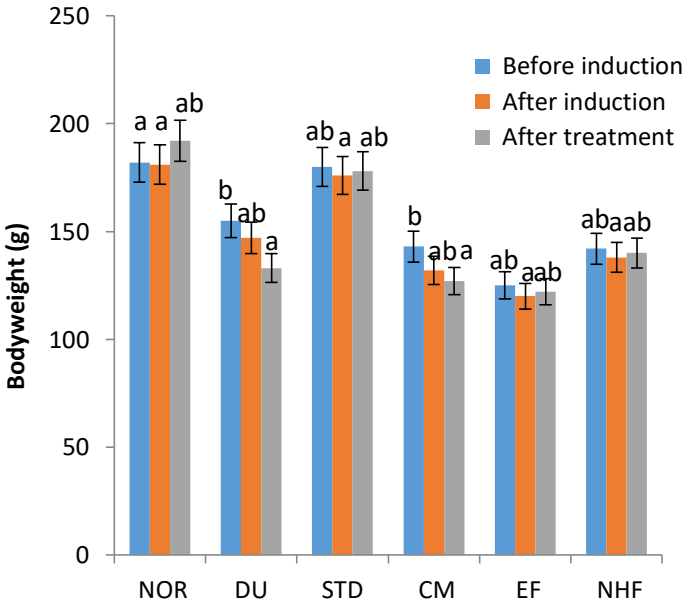

Figure 5: Effect of 300 mg/kg of crude methanol, and ethyl acetate and n-hexane fractions of *G. sylvestre* on body weight of alloxan-induced diabetic rats. NOR: Normoglycemic, STD: Standard drug (100 mg/kg metformin), DU: Diabetic untreated, CM: Crude methanol extract, EF: Ethyl acetate fraction, NHF: n-hexane fraction.

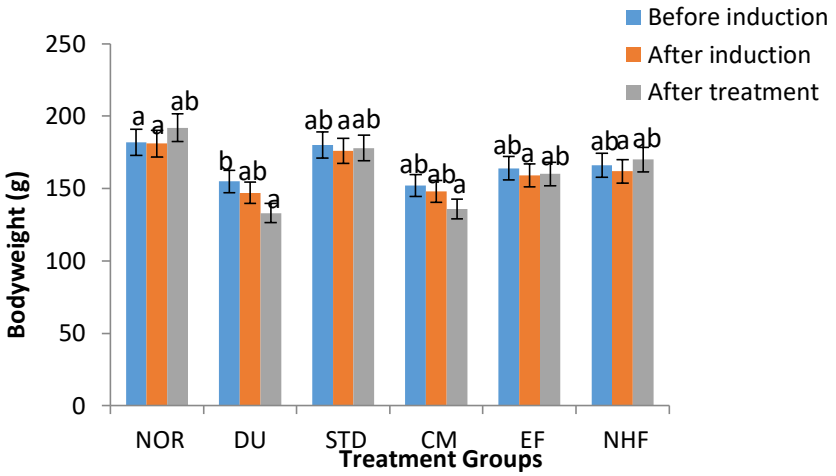

Figure 6: Effect of 600 mg/kg crude methanol, and ethyl acetate and n-hexane fractions of *G. sylvestre* on body weight of alloxan-induced diabetic rats. NOR: Normoglycemic, STD: Standard drug (100 mg/kg metformin), DU: Diabetic untreated, CM: Crude methanol extract, EF: Ethyl acetate fraction, NHF: n-hexane fraction.

3.3 Effects of *G. sylvestre* extracts on lipid profile of alloxan induced diabetic rats

Effects of crude methanol, ethylacetate and n-hexane fractions of *G. sylvestre* on concentration of total cholesterol, triglyceride, high density lipoprotein- cholesterol (HDL- C) and low density lipoprotein – cholesterol (LDL-C) in diabetic rats is presented in figure 7-10 respectively. The result shows significant reduction ($p<0.05$) in total cholesterol, triglyceride, and low density lipoprotein – cholesterol (LDL-C) of all the treated groups when compared to diabetic untreated. However, a significant ($p<0.05$) decrease in the serum concentrations of high density lipoprotein- cholesterol (HDL- C) were observed in the diabetic untreated rats when compared to the HDL-C levels in the groups of rats treated with the crude methanol, ethylacetate and n-hexane fractions of *G. sylvestre.* The ameliorative effect of the crude methanol, ethylacetate and n-hexane fractions of *G. sylvestre* on the diabetic induced alterations in serum lipid profile occurs in a significant ($p<0.05$) dose dependent manner

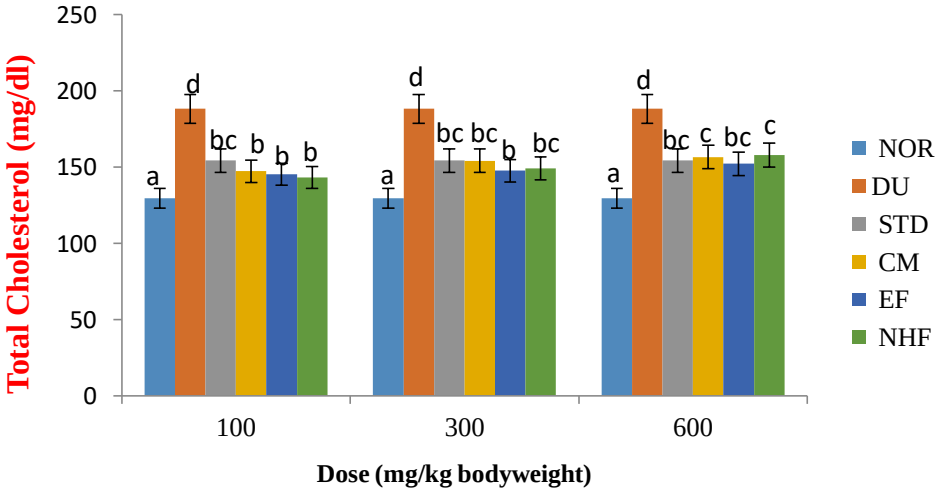

Figure 7: Effect of crude methanol, and ethyl acetate and n-hexane fractions of *G. sylvestre* on total cholesterol levels in serum of alloxan-induced diabetic rats. NOR: Normoglycemic, STD: Standard drug (100 mg/kg metformin), DU: Diabetic untreated, CM: Crude methanol extract, EF: Ethyl acetate fraction, NHF: n-hexane fraction. Means with different superscripts differ significantly ($p<0.05$)

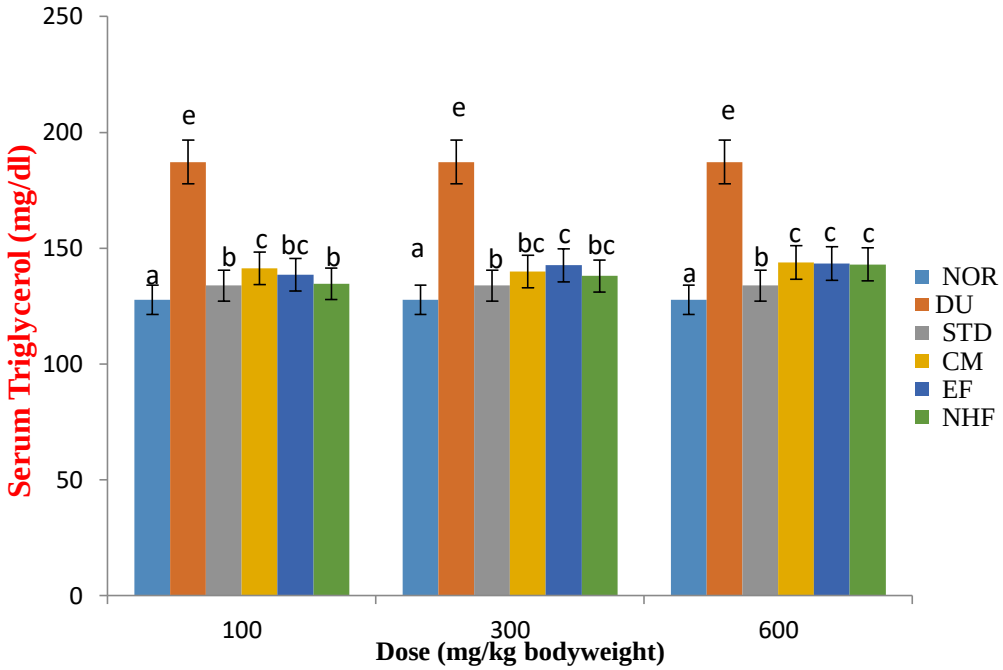

Figure 8: Effect of crude methanol, and ethyl acetate and n-hexane fractions of *G. sylvestre* on triglyceride levels in serum of alloxan-induced diabetic rats. NOR: Normoglycemic, STD: Standard drug (100 mg/kg metformin), DU: Diabetic untreated, CM: Crude methanol extract, EF: Ethyl acetate fraction, NHF: n-hexane fraction. Means with different superscripts differ significantly ($p<0.05$)

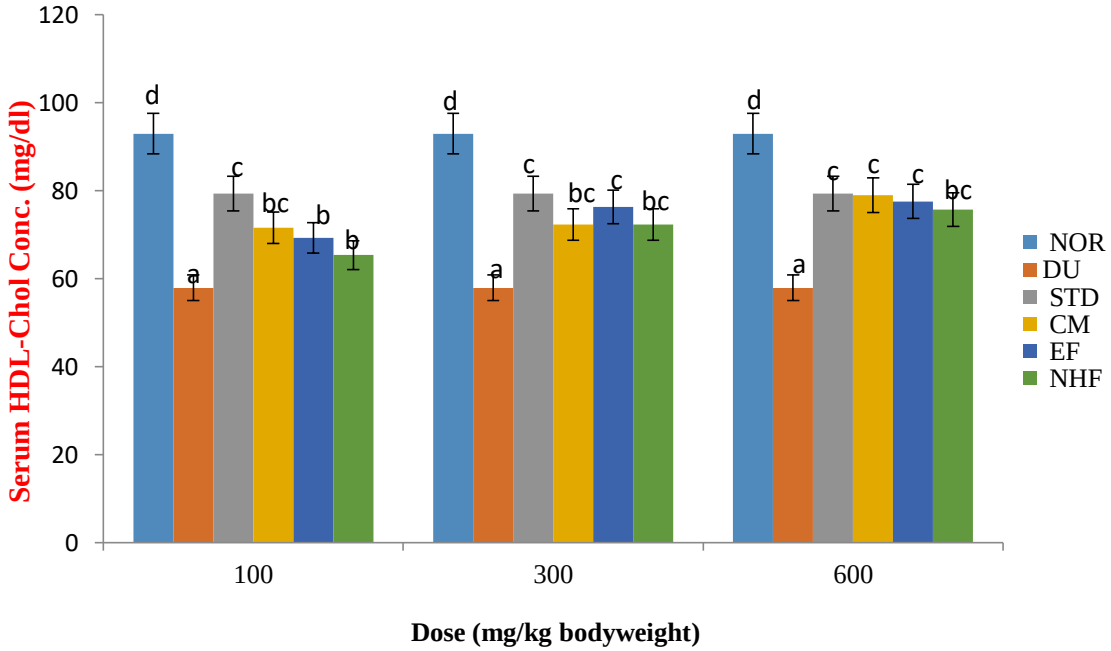

Figure 9: Effect of crude methanol, and ethyl acetate and n-hexane fractions of *G. sylvestre* on high density lipoprotein – cholesterol (HDL-C) levels in serum of alloxan-induced diabetic rats. NOR: Normoglycemic, STD: Standard drug (100 mg/kg metformin), DU: Diabetic untreated, CM: Crude methanol extract, EF: Ethyl acetate fraction, NHF: n-hexane fraction. Means with different superscripts differ significantly ($p<0.05$)

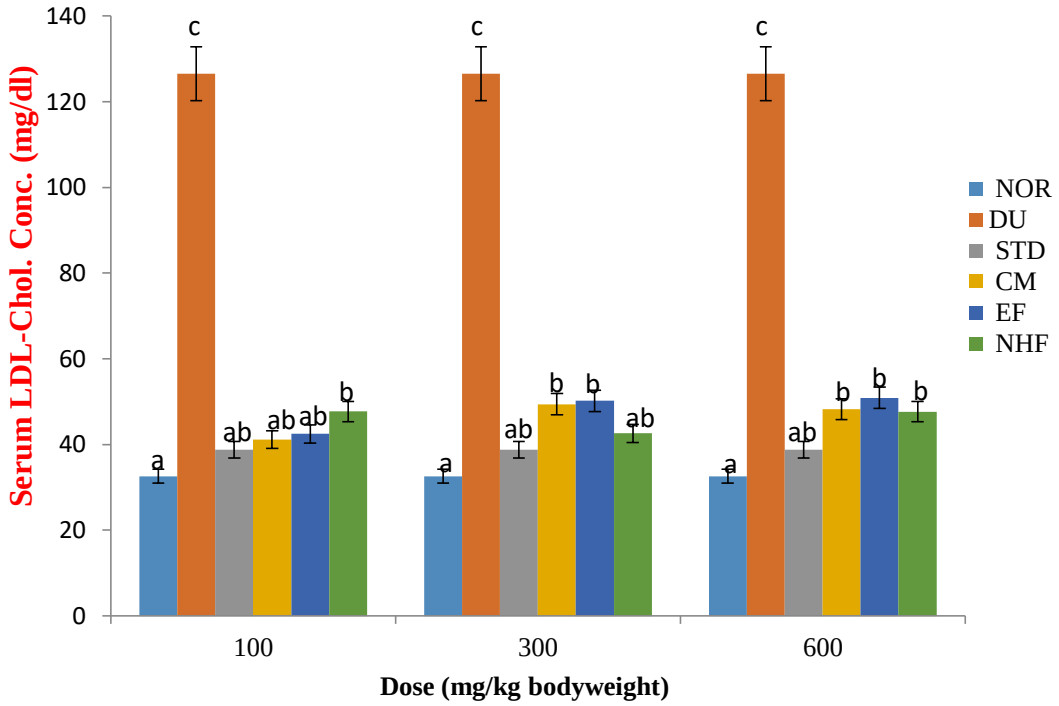

Figure 10: Effect of crude methanol, and ethyl acetate and n-hexane fractions of *G. sylvestre* on low density lipoprotein – cholesterol (LDL-C) levels in serum of alloxan-induced diabetic rats. NOR: Normoglycemic, STD: Standard drug (100 mg/kg metformin), DU: Diabetic untreated, CM: Crude methanol extract, EF: Ethyl acetate fraction, NHF: n-hexane fraction. Means with different superscripts differ significantly ($p<0.05$)

4.0 Discussion

Recently, much attention has been focused on using natural products as alternative therapy for treatment of different aliments including diabetes mellitus [20]. Effect of methanol extracts, ethylacetate and n-hexane fractions of *G. sylvestre* in reducing glucose level and ameliorating the metabolic abnormalities accompanied with alloxan induced diabetes in Swiss albino rats was examined in this research.

Elevation of glucose level recorded in the alloxan treated rats was attributed to low secretion of insulin by pancreatic beta cells. Alloxan, an analogue of glucose is urea derivative that destroys and reduces beta cells of the pancreas through inhibition of glucokinase enzyme or formation of reactive oxygen species that damage the beta cells of the pancreatic islets. These results are consistent with previous findings recorded by Ankur and Shahjad, [21], who confirmed that high glucose concentration causes the development of insulin resistance in peripheral tissues owing to impairment of both insulin secretion and insulin sensitivity. The biochemical bases for insulin resistance induced by hyperglycemia may be attributed to modifications in structure of insulin receptors and the glucose transport system, resulting in impaired signal transmission [22]

Though, the crude methanol, ethylacetate and n-hexane fractions of *G. sylvestre* plants were able to reduce the blood glucose level of the diabetic rats at 100, 300 and 600 mg/kg, the 300 mg/kg of ethylacetate fraction of *G. sylvestre* showed highest (82 %) reduction in the blood glucose. The blood glucose lowering effect of this plant may be attributed to the

presence of flavonoids, phenol, alkaloids, saponins and tannins that are associated with hypoglycemic activity [15]. It was also reported by Kanetkar *et al*. [23] that *G. sylvestre* leaves contain gymnemic acid (a saponnin) which are believed to block the absorption of glucose in the small intestine. The biochemical mechanism by which this plant exerts its hypoglycemic effects could be by increasing secretion of insulin, promotion of regeneration of islets cells, increasing in the activity of the enzyme involve in utilization of glucose by insulin dependent pathways or inhibition of glucose absorption from intestine [24].

In diabetic rats, reduction in bodyweight observed might be the result of degradation of structural proteins due to deficiency of carbohydrate for energy metabolism, dehydration due to excessive urination or increase in the breakdown of fats in the adipose tissues [25]. Administration of 100, 300, 600 mg/kg of methanol extract, n-hexane and ethylacetate factions of *G. sylvestre* plant gave a non-significant increase in the bodyweight of the diabetic treated rats when compared to normoglycemic group after treatment for fourteen days. This result is in line with the findings of Prabhakar and Doble, [26]

Abnormalities in lipid profile are common complications in diabetes mellitus and such abnormality are the risk factors for coronary heart diseases [27]. Deficiency of insulin leads to activation of lypolysis which causes increase in free fatty acids mobilization from adipose tissue [28]. Increase in total cholesterol, triacylglycerol, low density lipoprotein (LDL) and decrease in high density lipoprotein is observed hyperglycemic condition. [29]**.** In this study, serum total cholesterol, triacylglycerols and LDL-C levels was decreased with increase in HDL-C in all rats treated with extracts of *G. sylvestre*. Serum total cholesterol lowering property of *G. sylvestre* leaf extracts could be attributed to the presence of hypocholesterolemic compounds in *G. sylvestre* that may act as inhibitor for hepatic hydroxymethylglutaryl CoA (HMG CoA) reductase in liver, which take part in cholesterol synthesis [30] or increasing the fecal content by inhibiting the absorption of cholesterol from intestine [31].This reduction might also be attributed to the phytochemical constituents by reducing lipid peroxidation via scavenging free radicals [32].

The remarkable control of high serum triacylglycerol of diabetic rats treated with *G. sylvestre* could be due to inhibition of endogenous triacylglycerol synthesis in liver [33] or improvement in insulin level or the presence of active components in the plants that suppressed the activity of hormone sensitive lipase in adipose tissue or increased activity of hepatic lipase or lipoprotein lipase accountable for the hydrolysis of excess lipoprotein bound triacylglycerol into fatty acids [34]. Increased level of HDL-C in diabetic rats treated with the extracts could be due to the enhancement of lecithin: cholesterol acyltransferase (LCAT) which plays a key role in incorporating the free cholesterol in to HDL taking back to the liver [35]. Decrease in LDL-C in diabetic rats treated with *G. sylvestre* could be attributed to increased expression of low density lipoprotein receptor (LDLR), which enhance LDL particles uptake in liver from the circulation through the depletion of intracellular cholesterol [36]. These findings indicate that the extracts have the ability to reverse the abnormalities of lipids associated with diabetes. This is in line with the findings of Rachh *et al*. [37] in their research carried out on antihyperlipidemic activity of *Gymenma sylvestre* leaf extract on rats fed with high cholesterol diet.

5.0 Conclusion

All the extracts of *G. sylvestre* were able to lower the blood glucose of diabetic rats with 300 mg/kg bodyweight of ethylacetate fraction having a higher blood glucose reduction by 82 %. Diabetes induced dyslipidemia were also ameliorated.

Conflict of Interest: The author declared no conflict of interest exist

Author's contributions:

All authors participate in research design. Author ROR, SSM & HFR conducted the research work and wrote the manuscript. Author HLM supervised the work and revised the manuscript while authors HAM co-supervised and revised the work. All authors read and approved the final manuscript.

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