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Hepatoprotective, Antioxidant and Hypolipidemic Potentials of *Mucuna pruriens* in a Diabetic Experimental Animal Model

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Authors' contributions

This work was carried out in collaboration among all authors. Author RCI designed the study and wrote the protocol. Authors HNO and DOD wrote the first draft of the manuscript. Author GCI performed the statistical analysis. Authors OEE, CCE and ICU helped with the analyses of the work. Author CPN managed the literature searches. Author GSA supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study investigates the effect of *Mucuna pruriens* leaves on the liver, antioxidant parameters and lipid profile of alloxan-induced diabetic rats.
Methodology: Thirty-five (35) Wistar albino rats were divided into five groups with seven (7) rats per group. The experiment was designed as follows: Group 1 – NC, Normal Control; Group 2 – DBC, Diabetic Control; Group 3 - DG, Diabetic glibenclamide (Positive control); Group 4 - DMPL

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(Diabetic *Mucuna pruriens* extract Low – 50 mg/kg); Group 5 - DMPH (Diabetic *Mucuna pruriens* extract High – 100 mg/kg). The rats were fed with normal feed and drinking water ad libitum. The diabetic control, diabetic glibenclamide and the extract groups (50 mg/kg and 100 mg/kg) were induced with diabetes by intraperitoneal injection of 120 mg/kg bodyweight of alloxan monohydrate and confirmation was done using a glucometer. The positive control was treated with glibenclamide (6 mg/65 kg/day). Blood samples were collected from seven (7) rats in each group through the retro-orbital plexus of the eye after 28 days of treatment, thereafter, the rats were sacrificed by cervical dislocation under light ether anesthesia, and biochemical parameters were determined using standard techniques (Randox kits).

Results: *Mucuna pruriens* leaf extract lowered blood glucose levels across the groups treated with the extract. The diabetic rat fed with *Mucuna pruriens* extracts showed a significant ($p<0.01$) reduction in hepatic ALT (Alanine Transaminase), AST (Aspartate Transaminase) and ALP (Alkaline Phosphatase) when compared to the diabetic control. Compared to the diabetic control, the extract groups showed a significant ($p<0.05$) reduction in serum Low-density lipoprotein (LDL) and total cholesterol with a significant ($p<0.05$) increase in HDL. The extract groups showed marked dose-dependent significant ($p<0.05$) increase in GPx over all other groups, and a significant ($p<0.05$) decrease in Malonaldehyde level when compared to the diabetic control group. There was no significant ($p>0.05$) difference in the levels of Catalase, Superoxide dismutase, and Glutathione across the groups. There is a significant ($p<0.05$) increase in the level of Vitamin E of extract groups when compared to the diabetic control and diabetic glibenclamide group with a significant increase ($p<0.05$) in Vit. C level of extract group (50 mg/kg) when compared to all other groups.

Conclusion: This result of the study suggests that *Mucuna pruriens* have a beneficial effect on the hepatocyte and some lipid profile parameters while also increasing antioxidant defense of the body and may be of value in the management of diabetes and its complications.

Keywords: Diabetes; Mucuna pruriens; hepatocytes; antioxidant; lipid profile.

1. INTRODUCTION

One of the most severe threats to humanity in the twenty-first century is Diabetes Mellitus [1]. The disease is a metabolic disorder that is characterized by an increase in blood glucose level, also known as hyperglycemia [2]. In this disease, there is an alteration in the metabolism of biomolecules - lipids, carbohydrates, and proteins [3]. The disorder is a result of an impairment in the actions of insulin secreted in the pancreas or some level of peripheral resistance to its underlying actions [4]. There are two common types of diabetes - Type I and Type II. The biochemical mechanism for the development of the type II disease is through damage to the beta cells of pancreatic islets, which result in an altered metabolism, causing disturbances in several organs like the liver and kidney [5]. This disease comes with complications such as dyslipidemia, hepatomegaly, liver enzyme abnormalities, weight loss, oxidative stress complications, and coma [6]. The management of this disease involves the use of modern medicine like Biguanides, Sulphonylurea, and Metformin. However, these drugs are costly and are associated with side effects [7]. A reliable pharmacological intervention is needed for effective management. Many plants have been discovered to possess anti-diabetic properties and are used as herbal drugs. Researches have shown that some of these herbal drugs have minimal adverse effects and prevention of secondary complications [8]. Among the many anti-diabetic plants used for diabetes mellitus, is *Mucuna pruriens*, also known as velvet beans, or Co-with in English [9].

Mucuna pruriens belongs to the Fabaceae species. It is prevalent in India as a medicinal plant, which was used in the earliest period. It is also native to tropical regions like Africa and West Indices [10]. Every part of this plant such as seeds, flowers, and leaves has valuable medicinal properties in the traditional system of medicine [11]. The seed pods are covered with some orange hair-like needles and can cause severe irritation to the skin. Some of the phytochemicals in this plant are Saponin, alkaloids, Flavonoids, sterols [12]. Research had suggested that the plants have hypoglycaemic activity in normal and diabetic rats [13]. The present study explores the biochemical aspects of the potentials of *Mucuna pruriens* leaves and the medicinal properties on diabetic rats.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

Fresh leaves of *Mucuna pruriens* were collected from its natural habitat at Umuahia, Abia State, Nigeria and identified by a taxonomist, Dr. Garuba Omosun of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Abia State where a voucher specimen number VPP/2011/68 was deposited in the herbarium.

2.2 Preparation of Plant Material

The plant was extracted using the cold maceration. The leaves were washed, cut into small pieces, dried at room temperature, and pulverized into coarse of about 1 mm in diameter. 500 g of the plant material was macerated in 99% ethanol for 48 hours with intermittent shaking at 2 hours interval. The extract was then filtered using Whatman filter papers and concentrated in vacuum using a rotary evaporator at 40°C and 210 bar and a vacuum lyophilizer. The percent yield was calculated, and the *Mucuna pruriens* extract (MPE) was stored in a refrigerator at 4°C until the time of use [14].

2.3 Experimental Animals

Thirty-five (35) Mature Wistar albino rats, bred in the laboratory animal unit of the Faculty of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, were used for the experiments. Males and females were separated in different cages to avoid breeding. They were housed in an environment of normal ambient temperature, and the lighting period was 12 hours daily with a relative humidity of 40–60%. The rats were kept in stainless steel cages, supplied with clean drinking water and fed ad libitum with standard rat commercial pelleted feed (Vital feeds, Nigeria).

2.4 Induction of Diabetes

The Wistar albino rats (35) were randomly grouped into 5 groups of 7 rats per group. Animals in group 2-5 were induced with diabetes by intraperitoneal administration of alloxan monohydrate. The alloxan was dissolved in distilled water at a dose of 120 mg/kg body weight. After 72 hours of induction, diabetes was confirmed through testing of collected blood samples in glucometer (Accu-chek), procured from Liduna Pharmacy, Umudike. Animals with blood glucose levels above 240 mg/dl were selected for the study. The oral glucose tolerance test (OGTT) was performed toward the last week of the experiment after oral administration of glucose solution (2 g/kg BW), followed by blood glucose level measurement using glucometer at 0, 30, 60, 90, and 120 min.

2.5 Treatment of Animals

The thirty-five (35) Wistar albino rats were randomly grouped into 5 groups of 7 rats per group. The rats were then treated as follows:

NC	Received normal feed and water ad libitum.
DBC	Diabetic rats received normal feed and water ad libitum.
DG	Diabetic rats received normal feed and water ad libitum and treatment with (6 mg/65 kg/day) glibenclamide.
DMPL	Diabetic rats received normal feed and water ad libitum and treatment with 50 mg/kg extract.
DMPH	Diabetic rats received normal feed and water ad libitum with 100 mg/kg extract.

DBC, diabetic control; DBG, diabetic glibenclamide; DMPL, diabetic *Mucuna pruriens* low dose (50 mg/kg); DMPH, diabetic *Mucuna pruriens* high dose (100 mg/kg); NC, normal control.

Oral glucose tolerance test (OGTT) for all groups of animals was conducted in the last week of the experimental period through. Blood samples were collected from seven (7) rats in each group through the retro-orbital plexus of the eye of the rats after 28 days of treatment for biochemical analyses, after which the rats were sacrificed by cervical dislocation under light ether anesthesia.

2.6 Lipid Profile Assay

Total cholesterol was evaluated using the enzymatic colorimetric chod-pad test method [15] with the Randox test kit; Triglycerides was determined spectrophotometrically. High-density lipoproteins (HDL) were evaluated using the Randox test kit; low-density lipoprotein (LDL) was determined as the difference between total cholesterol and cholesterol content of the supernatant after precipitation of the LDL fraction. Very low-density cholesterol (VLDL) was calculated according to Wilson's method [16] as VLDL1⁄40.2TG (where TG is triglycerides).

2.7 Liver Markers Enzymes

Aspartate aminotransferase (AST) was evaluated using Randox kits; Alanine aminotransferase (ALT) was measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenyl hydrazine using the method as described in Randox kits [17]; alkaline phosphatase (ALP), total protein in serum, and conjugated bilirubin were determined using laboratory procedures and kits.

2.8 Determination of Antioxidant Biomarkers

2.8.1 Malondialdehyde (MDA) estimation

1 ml of serum was heated at pH 3.0 --- 0.1 with 4 ml of saturated TBA reagent in a boiling water bath for 30 min. After the sample has cooled, MDA was estimated from the absorbance of the TBA-MDA complex in the cooled sample at 532 nm using a spectrophotometer [18].

2.8.2 Estimation of superoxide dismutase (SOD)

Adrenaline (10 mg) was dissolved in 17 ml of distilled water to make adrenaline solution. Serum (0.1 ml) was added to 0.9 ml of phosphate buffer (pH 7.8). 0.2 ml of the extract was taken in triplicate and 2.5 ml of buffer added inside a cuvette and 0.3 ml of adrenaline solution added, mixed well, and absorbance was read at 450 nm at 30 s interval for five times [19].

2.8.3 Determination of catalase

0.2 ml serum was incubated in 1.0 ml of phosphate buffer at 37°C for 60 s. 0.12 ml of Hydrogen peroxide (H_2O_2) was also added to the solution. Serum catalase activity was determined spectrophotometrically at 240 nm for 3 minutes at 25°C [20].

2.9 Data Analysis

The results are presented in mean ± SEM and analyzed using a one-way analysis of variance (ANOVA). The differences in means were tested using post hoc LSD, and values of $p<0.05$, and were considered statistically significant. SPSS version 22 was used for the analysis.

3. RESULTS

Results of the effect of *Mucuna pruriens* leaves on the liver, antioxidant parameters, and lipid profile of alloxan-induced diabetic rats are presented below.

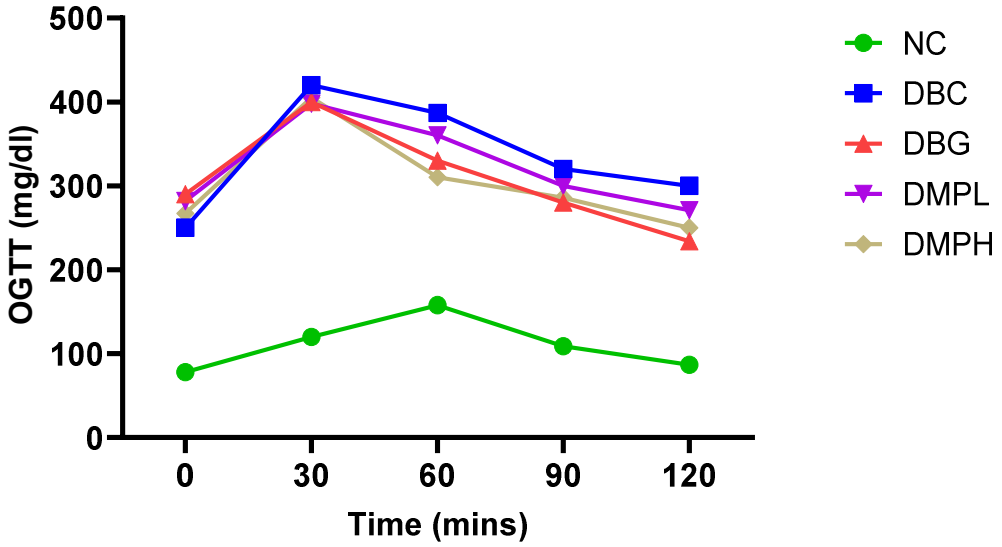

Fig. 1. Effect of ethanol extract of *Mucuna pruriens* leaves on oral glucose tolerance test (OGTT) of alloxan-induced diabetic rats

Oral glucose tolerance test (OGTT) for all groups of animals in the last week of the experimental period. Data are presented as the mean ± SD (n = 7). DBC, diabetic control; DBG, diabetic glibenclamide; DMPL, diabetic Mucuna pruriens low dose (50 mg/kg); DMPH, diabetic Mucuna pruriens high dose (100 mg/kg); NC, normal control

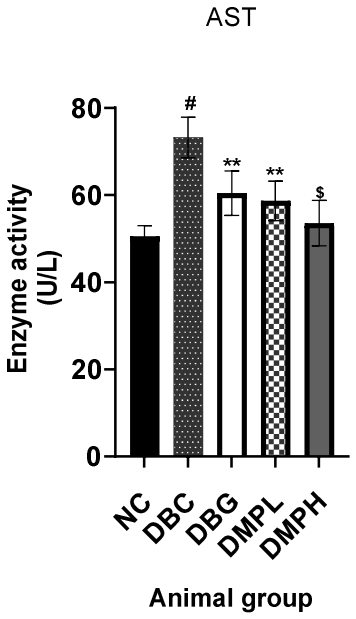

Fig. 2. Effect of ethanol extract of *Mucuna pruriens* leaves on aspartate transaminase activity of alloxan-induced diabetic rats

Effect of oral treatment of Mucuna pruriens or gilbenclamide on the serum aspartate transaminase activity in the normal and type 2 diabetes model of rats. Data are presented as the mean ± SD (n = 7). DBC, diabetic control; DBG, diabetic gilbenclamide; DMPL, diabetic Mucuna pruriens low dose (50 mg/kg); DMPH, diabetic Mucuna pruriens high dose (100 mg/kg); NC, normal control. Multiple comparisons were made using one way ANOVA. $^{\#}p < .01$ versus NC; $^{**}p < .01$ versus DBC; $^{\$}p < .01$ versus DBG groups

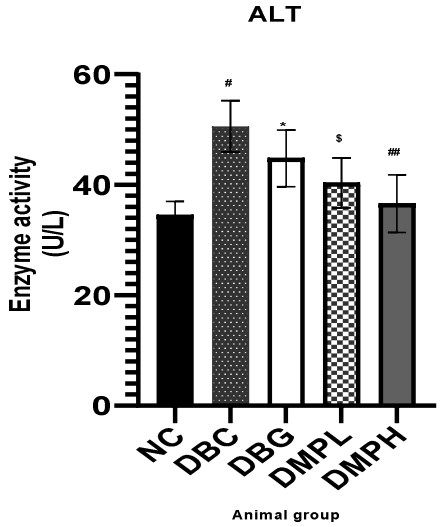

Fig. 3. Effect of ethanol extract of *Mucuna pruriens* leaves on alanine transaminase activity of alloxan-induced diabetic rats

Effect of oral treatment of Mucuna pruriens or gilbenclamide on the serum alanine transaminase activity in the normal and type 2 diabetes model of rats. Data are presented as the mean ± SD (n = 7). DBC, diabetic control; DBG, diabetic gilbenclamide; DMPL, diabetic Mucuna pruriens low dose (50 mg/kg); DMPH, diabetic Mucuna pruriens high dose (100 mg/kg); NC, normal control. Multiple comparisons were made using one-way ANOVA. $^{\#}p < .01$ versus NC; $^{*}p < .01$ versus DBC; $^{\#\#}p < .01$ versus DBG groups

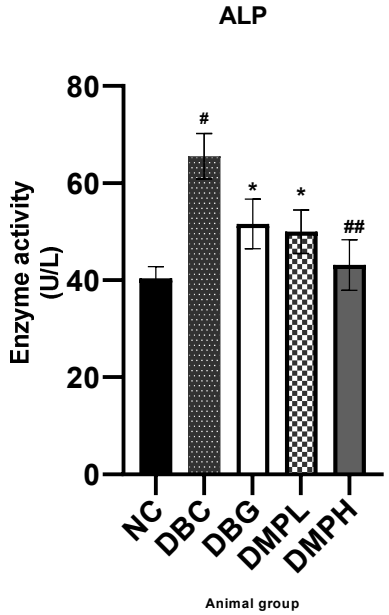

Fig. 4. Effect of ethanol extract of *Mucuna pruriens* leaves on alkaline phosphatase activity of alloxan-induced diabetic rats

Effect of oral treatment of Mucuna pruriens or gilbenclamide on the serum alkaline phosphatase activity in the normal and type 2 diabetes model of rats. Data are presented as the mean ± SD (n = 7). DBC, diabetic control; DBG, diabetic gilbenclamide; DMPL, diabetic Mucuna pruriens low dose (50 mg/kg); DMPH, diabetic mucuna pruriens high dose (100 mg/kg); NC, normal control. Multiple comparisons were made using one way ANOVA. $^{\#}p < .01$ versus NC; $^{*}p < .01$ versus DBC; $^{\#\#}p < .01$ versus DBC groups

Table 1. Effect of ethanol extract of *Mucuna pruriens* leaves on some lipid profile biomarkers of alloxan-induced diabetic rats

Animal groups	TAG	HDL	LDL	T.CHOL
NC	1.38±0.11^{a}	1.65±0.12^{c}	2.73±0.10^{b}	4.50±0.12^{b}
DBC	1.52±0.12^{b}	1.28±0.08^{a}	3.12±0.08^{d}	5.95±0.17^{e}
DG	1.35±0.05^{a}	1.72±0.09^{d}	2.67±0.09^{a}	4.46±0.08^{a}
DMPL	1.65±0.05^{c}	1.65±0.10^{c}	2.73±0.10^{b}	4.95±0.11^{d}
DMPH	1.52±0.09^{b}	1.54±0.19b	2.83±0.19^{c}	4.71±0.15^{c}

Data are represented as means ± SD (n=7). Multiple comparisons were made using one-way ANOVA. Values with different superscript are significantly different at P<0.05. NC; Normal Control; DC, Diabetic Control; DG, Diabetic glibenclamide (Positive control), DMPL (Diabetic Mucuna pruriens extract Low – 50 mg/kg); DMPH (Diabetic Mucuna pruriens extract High – 100 mg/kg). TAG, Triacylglycerol; HDL, High-density lipoprotein; LDL, Low-density lipoprotein

Table 2. Effect of ethanol extract of *Mucuna pruriens* leaves on the redox status of alloxan-induced diabetic rats

Animal groups	SOD	CAT	MDA	GPx	GSH
NC	1.09±0.03^{a}	2.46±0.06^{a}	5.13±0.27^{b}	24.19±1.05^{b}	5.35±0.07^{c}
DBC	1.10±0.01^{a}	4.84±0.12^{c}	9.88±0.19^{d}	15.60±0.55^{a}	3.95±0.18^{a}
DG	1.12±0.02^{a}	3.59±0.06^{b}	7.30±0.14^{c}	29.80±1.05^{b}	4.79±0.05^{b}
DMPL	1.17±0.07^{b}	2.44±0.10^{a}	4.90±0.15^{a}	54.79±1.23^{c}	4.70±0.47^{b}
DMPH	1.13±0.02^{a}	2.62±0.05^{a}	4.60±0.26^{a}	70.29±1.72^{d}	5.21±0.19^{c}

Values with different superscript are significantly different at P<0.05. NC; Normal Control; DC, Diabetic Control; DG, Diabetic glibenclamide (Positive control), DMPL (Diabetic Mucuna pruriens extract Low – 50 mg/kg); DMPH (Diabetic Mucuna pruriens extract High – 100 mg/kg). SOD, Superoxide dismutase; CAT, Catalase; MDA, Malondialdehyde; GPx, Glutathione peroxidase; GSH, Glutathione

Table 3. Effect of ethanol extract of *Mucuna pruriens* leaves on some selected vitamins in an alloxan-induced diabetic rats

Animal Groups	VIT.C	VIT.E
NC	1.54±0.12[a]	1.85±0.04[b]
DBC	1.65±0.06[b]	1.84±0.20[b]
DG	1.59±0.11[a]	1.78±0.11[a]
DMPL	1.72±0.01[c]	1.99±0.13[c]
DMPH	1.63±0.02[b]	1.93±0.13[c]

Values with different superscript are significantly different at P<0.05. NC; Normal Control; DC, Diabetic Control; DG, Diabetic glibenclamide (Positive control), DMPL (Diabetic Mucuna pruriens extract Low – 50 mg/kg); DMPH (Diabetic Mucuna pruriens extract High – 100 mg/kg)

4. DISCUSSION

Synthetic drugs offer excellent therapy for diseases, but their side effects and high cost remain a challenge to its global adoption [21]. Providing alternative therapy by using medicinal plants remains the best route to manage these health problems. Thousands of plants have been studied in the last ten years, and several compounds have been extracted and isolated, and a shred of evidence has shown the immense therapeutic potential of plants [22]. Besides drugs used for the treatment of diabetes, several species of plants have shown hypoglycemic activity [23]. Some pharmacological studies show that the ethanol extract of the *Mucuna pruriens* seeds are a rich source of dietary fibers and antioxidants, and contains anti-diabetic components like saponins, squalenes, D-chiro-inositol and oligocyclitols [24].

Blood glucose level is one of the critical parameters for the evaluation of the first signs of diabetes and an increased blood sugar level is an indication of diabetes. This study showed that *Mucuna pruriens* extract reduced the increased blood sugar level (Fig. 1) in a dose-dependent fashion and at increasing time frame. The standard drug glibenclamide showed more hypoglycemic activity than *Mucuna pruriens,* and the glucose level was lower at 100 mg/kg than at 50 mg/kg dose (Fig. 1), which shows an increased dose effect. This suggests that a long-term and increase in dose administration of the *Mucuna pruriens* extract is highly effective in diabetes management. This observation agrees with the hypotheses of Majekudonmi [25], where 12 weeks of increased extract treatment proved valuable in diabetes management.

There is hyperlipidemia in diabetic patients, as a result of an increased hepatic biosynthesis of VLDL, and since the activities of lipoprotein lipase are dependent on insulin: glucagon ratio, the VLDL that is synthesized have a low clearance from the bloodstream [26]. In the present study, there was a significant ($p<0.05$) reduction in serum LDL (Low-density lipoprotein) and total cholesterol for extract groups as compared to the diabetic control. However, non-significant ($p>0.05$) decrease occurred for triglyceride levels when compared to the diabetic extract group (100 mg/kg) (Table 1). The reduced LDL and total cholesterol are in agreement with the observations made by Enechi [27]. This observed mechanism may be due to the presence of squalene, which increases biliary cholesterol excretion, leading to a decreased level of serum cholesterol [28]. The *Mucuna pruriens* extract promoted an increase in HDL cholesterol when compared to the diabetic control group and could be useful in ameliorating diabetes and some of its complications.

Alloxan-induced diabetes usually comes with structural changes in the liver, including the distortions of the hepatic lobule, necrosis, and lymphatic infiltrations in the parenchyma [29]. These changes indicate the metabolic disturbances generated by alloxan through oxidative stress. The diabetic rat fed with *Mucuna pruriens* extracts (50 mg/kg and 100 mg/kg) showed a significant ($p<0.01$) reduction in hepatic ALT, AST, and ALP when compared to the negative control. The increased levels of these enzymes can be attributed to the liver damage induced by diabetes. The reduced levels of these plasma enzymes suggest that *Mucuna pruriens* extract possess antioxidant activities that can impact greatly on the liver against alloxan-induced hepatotoxicity in a similar manner to the standard drug (glibenclamide). This agrees with Chukwudi [30], who ascribed the decrease in the liver enzymes activities to the antioxidant property of the *Mucuna pruriens*. The antioxidant property of the extract might be due to the presence of antioxidants, which include

flavonoids, saponins and glycosides, which prevent the oxidative stress generated during the liver metabolic process [31].

Decreases in the levels of catalase, Glutathione, Glutathione peroxidase (Gpx) and superoxide dismutase (SOD) activities and increase in the level of malondialdehyde (MDA) signifies increased oxidative stress and reduced antioxidant activities in the system which reduces the capability of the body to get rid of free radicals [32]. Compared to all other groups, the extract groups showed a significant increase ($p<0.05$) in levels of Gpx and a significant reduction ($p<0.05$) in MDA levels. GSH levels of extract groups was significantly higher than the diabetic control group while SOD was fairly the same. However, catalase level was highest in the diabetic control group (Table 2). This observation agrees with a report by DivyaP [33] where extract treatment of *Mucuna pruriens* showed signigicant antioxidant activity in an experimental animal model. This suggests that *Mucuna pruriens* have a beneficial effect in increasing antioxidant defense of the body and may be of value in the reduction of oxidative stress.

There is a significant ($p<0.05$) increase in the level of Vitamin E of extract groups when compared to the diabetic control and diabetic glibenclamide group (Table 3). Not only that, there is a significant increase ($p<0.05$) in Vit. C level of diabetic extract (50 mg/kg) when compared to all other groups. Previous studies have shown that Vitamins C and E are important constituents of medicinal plants and are also biological antioxidants that function as detoxifying agents, immunopotentiators, and immune activators in [34]. This increase can contribute greatly to the lowering of lipid peroxidation in the diabetic rat.

5. CONCLUSION

This study showed that *Mucuna pruriens* extract has great potentials in ameliorating hepatic and lipidemic complications that may arise due to diabetes and also possess antioxidant properties that can improve the integrity of cellular organs. Thus, we suggest that the medicinal plant be considered for incorporation in the management of diabetes and its complications. However, further analyses are recommended so as to characterize and identify the phytochemicals eliciting these effects.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The study was done in accordance with the policies of the Animal Care and Use Committee of the Department Biochemistry, Michael Okpara University of Agriculture, Umudike. The institution's ethical committee approved the experimental protocol for the use of laboratory animals.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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