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Antidiabetic activity and phytochemical screening of *Acalypha wilkesiana* (Euphorbiaceae) Mull Arg. roots in alloxan-induced diabetic rats

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The aim of this study was to investigate the anti-diabetic effects and biochemical parameters of methanol root extract of *Acalypha wilkesiana* Mull Arg. (MEAW) in alloxan-induced diabetic rats. The effect of the extract (200 and 400 mg/kg, *p.o.*) on fasting blood glucose (FBG), total cholesterol (TC), triglycerides (TG), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) level and liver glycogen content were investigated in alloxan-induced diabetic rats after 14 days. An oral glucose tolerance test (OGTT) was also performed on the diabetic rats. Dose selection was made on the basis of acute oral toxicity study. Phytochemical analysis of the root extract was carried out following standard procedures. The most significant ($p<0.05$) reduction of FBG level of 74.06% was observed for 400 mg/kg in alloxan induced diabetic rats. A significant reduction ($p<0.05$) in serum TC and TG level of 50.43 and 58.05% respectively was also observed for the high dose of the extract. The SGOT and SGPT levels were significantly ($p<0.05$) reduced. The MEAW also showed improvement of body weight in diabetic rats. The animals showed no mortality at a dose of 5000 mg/kg while results of phytochemical analysis revealed the presence of mainly alkaloids, terpenoids, flavonoids, saponins, steroids and tannins. These results show that the root of *Acalypha wilkesiana* possesses antidiabetic, antihyperlipidemic and hepatoprotective effects.

Key words: *Acalypha wilkesiana,* cholesterol, fasting blood glucose, oral glucose tolerance test (OGTT), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), triglyceride.

INTRODUCTION

Today, medicinal plants are increasingly being used in most parts of the world as: Hypolipidemic (Yadav et al, 2008), contraceptive, abortifacients, emmenagogues or oxytocic (Ritchie, 2001), antihypertensive (Nworgu et al., 2008), treatment of skin diseases (Ajose, 2007), antimicrobial (Okwu and Uchegbu, 2009) and hypoglycemic (Osadebe et al., 2004; Ezugwu et al., 2005; Patel et al., 2008; Yadav et al., 2008; Odoh et al., 2013). Hypoglycemic agents from plants have been used in the management of diabetes mellitus.

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Diabetes mellitus (DM) is a multifactorial disorder which is characterized by hyperglycemia, lipoprotein abnormalities, raised metabolic rate, defect in reactive oxygen species scavenging enzymes and altered intermediary metabolism of major food substances (Scoppola et al., 2001). Diabetes is a major degenerative disease in the world today, affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders (Edem, 2009). There has been increasing demand for the use of plant products with antidiabetic activity. The high cost, low availability, uncertainty of use during pregnancy and undesirable side effects of synthetic drugs have been some of the factors leading to a strong preference for hypoglycemic drugs of plant origin, which are believed to be suitable for chronic treatments (Okigbo and Mmeka, 2006).

Acalypha wilkesiana belongs to the family Euphorbiaceae. The common names are copperleaf, Joseph's coat, fire dragon, beef steak plant and match-me-if-you-can (Christman, 2004). The Hausas of Northern Nigeria call it "Jiwene" and "Jinwinini", while the Yorubas of Southern Nigeria call it "aworoso" (Ikewuchi et al., 2010). Aqueous leaf extract of *A. wilkesiana* is traditionally used to treat neonatal jaundice in western part of Nigeria on short-term basis. The plant is popularly used for the treatment of malaria, dermatological disorders, gastrointestinal disorders (Akinde and Odeyemi, 1987). It is widely used in southern Nigeria as a remedy for the treatment of undefined skin infections in children (Alade and Irobi, 1992). The antihypertensive (Nworgu et al., 2011) and antimicrobial (Gotep et al., 2010) properties of the plant have been reported. The antihyperglycemic, antihyperlipidemic and ameliorative role on electrolytes disturbances of the leaf extract have been demonstrated in streptozotocin-induced diabetic mice (Al-Attar, 2010). The present study aims to screen plant phytochemically, further investigate the antidiabetic and biochemical parameters of the root extract of the plant in alloxan-induced diabetic rats and in oral glucose tolerance test models.

MATERIALS AND METHODS

Collection and preparation of plant material

The roots of *A. wilkesiana* were collected from Orba area in Nsukka District, Enugu State, Nigeria in November, 2012 and authenticated by Mr. A. O. Ozioko, a taxonomist with International Centre for Ethnomedicine and Drug Development (InterCEDD). A voucher herbarium specimen No.PCG/12/415 was preserved in the Department of Pharmacognosy and Environmental Medicines, University of Nigeria Nsukka, Nigeria. The collected roots were dried under shade and powdered to a coarse consistency in a grinder mill. The powder was passed through 40 # mesh particle size 0.422 mm. Exactly 500 g of this powder was packed into a soxhlet apparatus and extracted exhaustively with methanol to obtain the methanol extract. This was concentrated *en vacuo* and the methanol extract (MEAW, yield: 21.80% w/w) was preserved in a refrigerator and used for subsequent assays.

Animals

Adult albino Wistar rats (175 to 210 g) of both sexes were procured from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria and housed in the University's Animal House in the Department of Pharmacology and Toxicology. They were fed with standard rat pellet diet (Topfeeds PLC, Nigeria). The experimental protocols were in accordance with the guidelines of the Ethics Committee of the University of Nigeria (approved ref: NHREC/05/01/2008B). The care and handling of animals was in line with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (86/609/EEC) (EEC, 1986).

Chemicals

The chemicals used in the study were: alloxan monohydrate (Spectrochem Pvt. Ltd, Mumbai), glibenclamide (Aventis Pharma Ltd, Verna, Goa), dextrose (Emkay Labs, Mumbai), Tween 80 (S.D. Fine-Chem Ltd, Mumbai), and anesthetic ether (Ozone International, Mumbai). All other chemicals and reagents used were of analytical grade.

Acute toxicity study (LD_{50})

The oral acute toxicity of the MEAW was determined in mice as described by Lorke (1983). Nine mice randomly divided into three groups (n=3) were orally administered 10, 100 and 1000 mg/kg of MEAW respectively, and observed for 24 h for mortality. When no death occurred, 1600, 2900 and 5000 mg/kg of MEAW was administered to a fresh batch of animals (n=1) and the number of death in 24 h also noted. The LD_{50} was estimated as the geometric mean of the highest non-lethal dose and the lowest lethal dose.

Induction of diabetes

Diabetes was induced by intraperitoneal injection of 120 mg/kg of a solution alloxan monohydrate (Kannur et al., 2006). The alloxanized rats were kept for 7 days with free access to food and water. On the 8^{th} day, the rats were fasted for 12 h but allowed free access to water and their fasting blood sugar (FBS) level was determined using Accu-chek® active glucometer (Roche Diagnostic Corporation, Mannheim, Germany) and blood glucostrips (Roche Diagnostic Pvt Ltd, Mumbai)). The animals were carefully monitored every day. Rats with glucose levels above 200 mg/dl were used for the study.

Antidiabetic study in normal and diabetic rats (FBS)

The non-diabetic rats were randomly divided into four groups (n = 6). Group 1 received 2 ml/kg of 5% Tween 80 while group 2 received 5 mg/kg glibenclamide. Groups 3 and 4 received 200, 400 mg/kg of the MEAW respectively. Same procedures were performed using diabetic rats at similar doses of MEAW. All groups received various treatments orally. Blood samples were drawn at weekly intervals from the tail vein after overnight fast till the end of study. FBS levels were estimated on day 0, 7 and 14 of the study (Claudia et al., 2006). On day 14, under mild ether anesthesia blood was collected and processed for estimation of serum glucose and serum lipid profile as described below.

Collection of blood and estimation of biochemical parameters

On day 14, blood was collected from retro-orbital venous plexus of the rats under light ether anesthesia using capillary tubes into Eppendorf tubes containing heparin. The plasma was separated by centrifugation for 5 min at 5000 rpm and was analyzed for lipid profiles (serum cholesterol, serum triglyceride), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) and liver glycogen content. The plasma profiles were measured by standard enzymatic methods with an automatic analyzer (Phuong et al., 2004).

Estimation of glycogen content in liver

Glycogen content in liver was measured according to spectrophotometric determination of glycogen with o-toluidine reagent. It utilizes the o-toluidine glucose coupling reactions for the estimation of glycogen after trichloroacetic acid extraction, precipitation by alcohol and hydrolysis (Khan et al., 2010).

Body weight measurement

Body weight was measured totally four times during the course of study period (Nagappa et al., 2003), before alloxan induction (initial values), and on day 1, 7 and 14 of the treatment period, using a digital weighing scale (KERN (EMB), Tischwaage, Germany).

Oral glucose tolerance test (OGTT) in normal rats

Animals were divided into four groups of six rats each. Group 1 was kept as vehicle control which received 5% Tween 80 (p.o.). Group 2 received glibenclamide (5 mg/kg) as the reference standard while Group 3 and 4 received MEAW 200 and 400 mg/kg respectively. Blood sugar level was determined from overnight fasted animals at 0 min. After 30 min of the drug treatment, animals were fed with glucose (4 g/kg) and blood glucose was determined at 1/2, 1, 2, and 3 h after glucose load (Sellamuthu et al., 2009).

Qualitative/quantitative phytochemical analysis

Alkaliods determination

The determination was as described by Harborne (1973). 5 g of sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 2 h. This was filtered and the extract was concentrated on a water bath to one–quarter of the original volume. Concentrated NH_4OH was added drop wise to the extract and the precipitate was collected and washed with dilute NH_4OH and then filtered. The alkaloid (residue) was dried and weighed.

Flavonoids determination

Following the method described by Boham and Kocipai- Abyazan (1994), 10 g of the sample was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through a Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight.

Tannins determination

Tannin determination was done by Van-Burden and Robinson (1981) method. 500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h on a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to mark. 5 ml of the filtrate was pipette out into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

Cardiac glycosides determination

Cardiac glycoside content in the sample was evaluated using reagent as described by El-Olemy et al., (1994). 1 g of the fine powder was soaked in 10 ml of 70% alcohol for 2 h and then filtered. The extract obtained was then purified using lead acetate and Na_2HPO_4 solution before the addition of freshly prepared Buljet's reagent (containing 95 ml aqueous picric acid + 5 ml 10% aqueous NaOH). The difference between the intensity of colours of the experimental and blank (distilled water and Buljet's reagent) samples gives the absorbance and is proportional to the concentration of the glycosides.

Saponins determination

The method employed was that of Obadoni and Ochuko (2001). 20 g of the sample was put into a conical flask and 100 ml of 20% aqueous ethanol was added. The sample was heated over a hot water bath for 4 h with continuous stirring at 55°C. The mixture was filtered and the residue re-extracted with 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at 90°C. The concentrate was transferred into a 250 ml separating funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous NaOH. The remaining solution was heated on a water bath. After evaporation the samples were dried in the oven to a constant weight. The saponin content was calculated as percentage weight.

Terpenoids determination

50 g of the powdered sample was extracted with solvent combination of methanol and water (4:1) at room temperature for 24 h. The solution was filtered using Whatman filter paper No. 1 and the filtrate was then evaporated to 1/10 volume at 40°C. The evaporated filtrate was acidified with 2 M sulphuric acid (pH 0.89) followed by chloroform extraction (three times the volume), stirred and allowed to stand in a separating funnel. Out of the two layers formed, the non-aqueous layer was taken and evaporated till dryness. The dried extract contained components like terpenoids which were further used for thin layer chromatography analysis (Harbourne, 1984).

Steroids determination

1 ml of a methanolic solution of the MEAW was transferred into 10 mL volumetric flasks. Sulphuric acid (4 N, 2 ml) and iron (III) chloride (0.5% w/v, 2 ml), were added, followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 ml). The mixture was heated in a water-bath maintained at 70 ± 2°C for 30 min with occasional shaking and diluted to the mark with distilled water. The absorbance was measured at 780 nm against the reagent blank (Tyler, 1994).

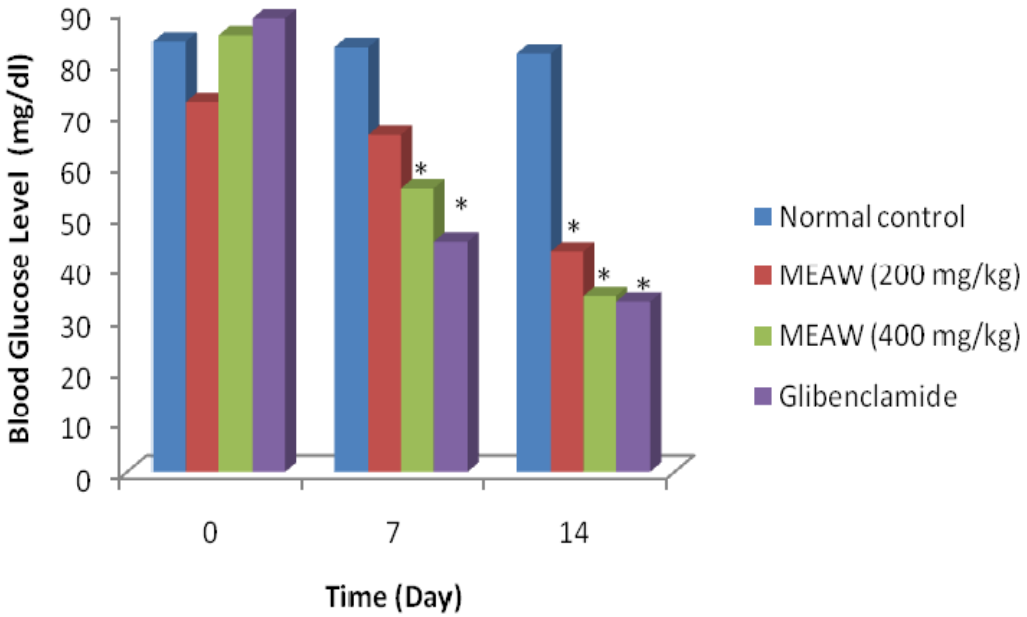

Figure 1. Effect of MEAW root and glibenclamide on FBS levels (mg/dl) of normal rats for 2 weeks.

Statistical analysis

The values are expressed as mean ± SEM (standard error of the mean). The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test. $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

In acute toxicity study, the extract was found to be safe at the tested dose level of 5000 mg/kg b. wt. The safety of the extract is the basis for the choice of the doses of the extract in this study. Previous study has shown that oral administration of the plant leaf extract at high dose of 10,000 mg/kg daily for 14 days does not produce any mortality in rats. All animals display normal behavioral, neurological and autonomic profiles (Iniaghe et al., 2013).

To ascertain a scientific base for the usefulness of this plant in the treatment of diabetes, it was decided to evaluate experimental design of antidiabetic activity by following normal, alloxan-induced and glucose tolerance test models. In the normoglycemic animals, the extract administration for 14 days produced a marked reduction in the FBS of the animals in a dose-related fashion and the effect was comparable to that of glibenclamide (Figure 1). The extract and glibenclamide caused hypoglycemia (<40 mg/dl) in the animals after 2 weeks of administration. It has been reported that provided the β-cells are fully functional, sulphonylureas, such as glibenclamide, can cause hypoglycemia since insulin release is initiated even when glucose concentrations are below the normal threshold for glucose-stimulated insulin release (approximately 5 mmol/L or 90 mg/dL) (Krentz and Bailey, 2005). This suggests that the plant may have similar mode of action to glibenclamide, an insulin secretagogue, with respect to blood glucose lowering effect.

Compared with the diabetic animals, the extract-treated groups exhibited remarkable lowering in the elevated blood glucose levels (Figure 2) in a dose-related fashion. The 400 mg/kg dose produced higher reduction (74.06%) than the 200 mg/kg dose (67.81%). The effect of the 400 mg/kg of the extract was similar to that of glibenclamide which brought back the blood glucose to normal values after 14 days. It has been suggested that the *A. wilkesiana* leaf extract might possess insulin like effect on peripheral tissues either by promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis (Al-Attar, 2010).

Effective blood glucose control is the key for preventing or reversing diabetic complications and improving quality of life in patients with diabetes. Thus sustained reduction in hyperglycemia will decrease the risk of developing microvascular complications and most likely reduce the risk of macrovascular complications (Muniappan et al., 2004). On the basis of this statement we have selected the glucose-induced hyperglycemic model to screen the anti-hyperglycemic activity of the plant extract. Any drug that is effective in diabetes will have the ability to control the rise in glucose level by different mechanisms and the ability of the extract to prevent hyperglycemia could be determined by glucose- loaded hyperglycemic model (Senthilkumar et al., 2011). In the glucose-loaded hyperglycemic (OGTT) model, the plant exhibited significant ($p<0.05$) antihyperglycemic activity which was more at a dose level of 400 mg/kg (Figure 3).

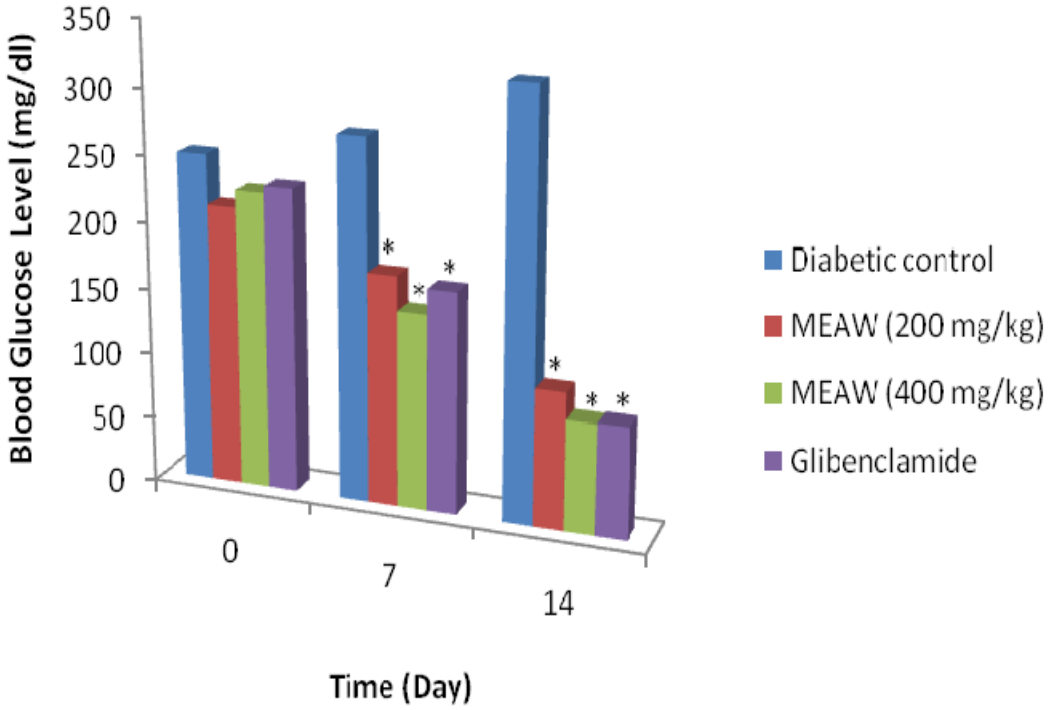

Figure 2. Effect of MEAW root and glibenclamide on FBS levels (mg/dl) of diabetic rats for 2 weeks.

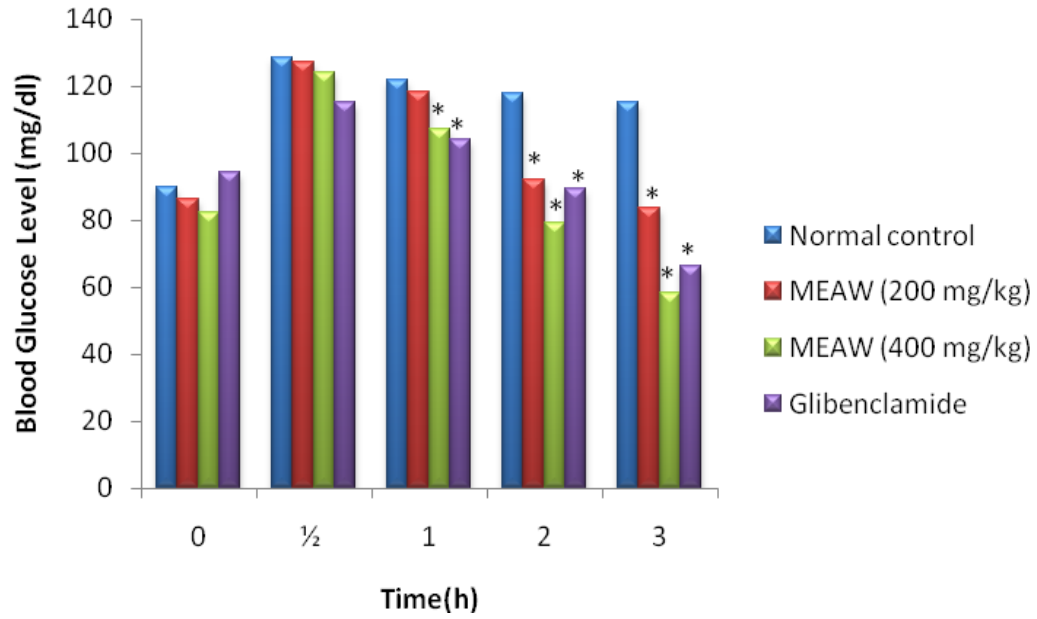

Figure 3. Effect of MEAW root on glucose loaded hyperglycemic rats (OGTT).

Remarkable blood glucose lowering effect was observed from 2 to 3 h post extract administration. At ½ h after glucose administration, the peak of blood glucose level increased rapidly from the fasting value and then subsequently decreased. Excessive amount of glucose in the blood induces insulin secretion. This secreted insulin will stimulate peripheral glucose consumption and control the production of glucose through different mechanisms (Andrew, 2000). Oral glucose tolerance test (OGTT) measures the body's ability to use glucose, the body's

Table 1. Effect of MEAW on the biochemical parameters of alloxan-induced diabetic rats after 14 days of treatment.

Group	Parameter			
	Total cholesterol (mmol/L)	Triglycerides (mmol/L)	SGOT (AST) (iu/L)	SGPT (ALT) (iu/L)
Normal control	33.00 ± 2.05	36.20 ± 0.22	30.00 ± 0.30	19.00 ± 2.18
Diabetic control	82. 40± 3.01	113.60 ± 0.14	47.30± 3.60	35.70 ± 1.09
Glibenclamide	35.36 ± 1.20*	40.60 ± 1.06*	35.60± 1.15*	28.18±1.33*
MEAW(200 mg/kg)	49.90 ± 0.42*	79.20 ± 0.92*	40.60± 1.15*	30.05±1.33*
MEAW(400 mg/kg)	36.50 ± 2.14*	45.70 ± 3.10*	32.20±2.16*	21.20±0.01*

Each value represents the mean ± SEM of five observations. *P < 0.05, Vs diabetic control (ANOVA followed by Dunnett's test); MEAW, Methanol extract of *Acalypha wilkesiana* , SGOT – serum glutamate oxaloacetate transaminase; SGPT, serum glutamate pyruvate transaminae.

main source of energy. It can be used to diagnose pre-diabetes and diabetes. In this study, it is found that the MEAW has hypoglycemic effect in glucose induced hyperglycemic rats, thus further giving credence to the antidiabetic effect of the plant.

The effects of the plant extract on the lipid profile and serum enzymes are presented in Table 1. The diabetic rats showed hypercholesterolemia and hypertriglyceridemia but the treatment with MEAW significantly ($p<0.05$) decreased both cholesterol and triglyceride levels with percentage reduction of 50.43 and 58.05% respectively (by the 400 mg/kg dose of the extract). The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease. Similar elevations in the serum triglycerides and cholesterol have been obtained in different experimental models (Al-Attar, 2010; Salahuddin et al., 2010). This abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots mainly due to the action of insulin (Pushparaj et al., 2007). Under normal circumstances, insulin activates the enzyme lipoprotein lipase, which hydrolyses triglycerides. However, in diabetic state lipoprotein lipase is not activated due to insulin deficiency resulting in hypertriglyceridemia (Pushparaj et al., 2007). Also insulin deficiency is associated with hypercholesterolemia. Insulin deficiency may be responsible for dyslipidemia, because insulin has an inhibitory action on HMG-CoA reductase, a key rate-limiting enzyme responsible for the metabolism of cholesterol-rich LDL particles. The mechanisms responsible for the development of hypertriglyceridemia and hypercholesterolemia in uncontrolled diabetes in humans are due to a number of metabolic abnormalities that occur sequentially (Murali et al., 2002). This implies that MEAW can prevent or be helpful in ameliorating the complications of lipid profile seen in some diabetics in whom hyperglycemia and hypercholesterolemia coexist quite often (Sharma et al., 2003).

In the diabetic rats there was a significant rise in SGOT and SGPT levels in comparison to normal rats, which could relate to excessive accumulation of amino acids (glutamate and alanine) in the serum of diabetic animals as a result of amino acids mobilization from protein stores (Colev et al., 1994) (Table 1). The higher levels of SGOT and SGPT, may give rise to a high concentration of glucose. In other words, the gluconeogenic action of SGOT and SGPT plays the role of providing new supplies of glucose from other sources such as amino acids. Following administration of the plant extract, SGOT and SGPT levels were significantly ($p<0.05$) reduced in a dose-related fashion with 400 mg/kg dose of the extract exhibiting a more significant effect.

In the present study, it was found that the level of glycogen in liver was reduced in diabetic rats when compared to the normal control group (Figure 4). Induction of diabetes with alloxan was associated with decrease in hepatic glycogen, which could be attributed to the decrease in the availability of the active form of the enzyme, glycogen synthetase, possibly because of low levels of insulin (Goel et al., 2004). Treatment of diabetic rats with glibenclamide and the extract improved the level of glycogen content remarkably in relation to the diabetic control group. *A. wilkesiana* restored the depressed hepatic glycogen levels possibly by increasing the level of insulin. Decreased activities of the enzymes involved in glucose homeostasis in liver and kidney such as hexokinase has been reported in diabetic animals resulting in depletion of liver and muscle glycogen content (Grover et al., 2000). It is possible that treatment with the plant extracts might increase the level of enzyme to the control level indicating an over-all increase in glucose influx.

The effect of the plant extract on the body weight of the diabetic animals is shown in Figure 5. Treatment with the plant extract improved the average body weights of rats indicating control over polyphagia and muscle wasting due to hyperglycemic condition. The diabetic rats had lower body weights as compared to the normal rats. In spite of the increased food consumption, loss of body weight due to defect in glucose metabolism and excessive breakdown of tissue protein is a characteristic condition in diabetics.

Phytochemical screening of the MEAW showed the

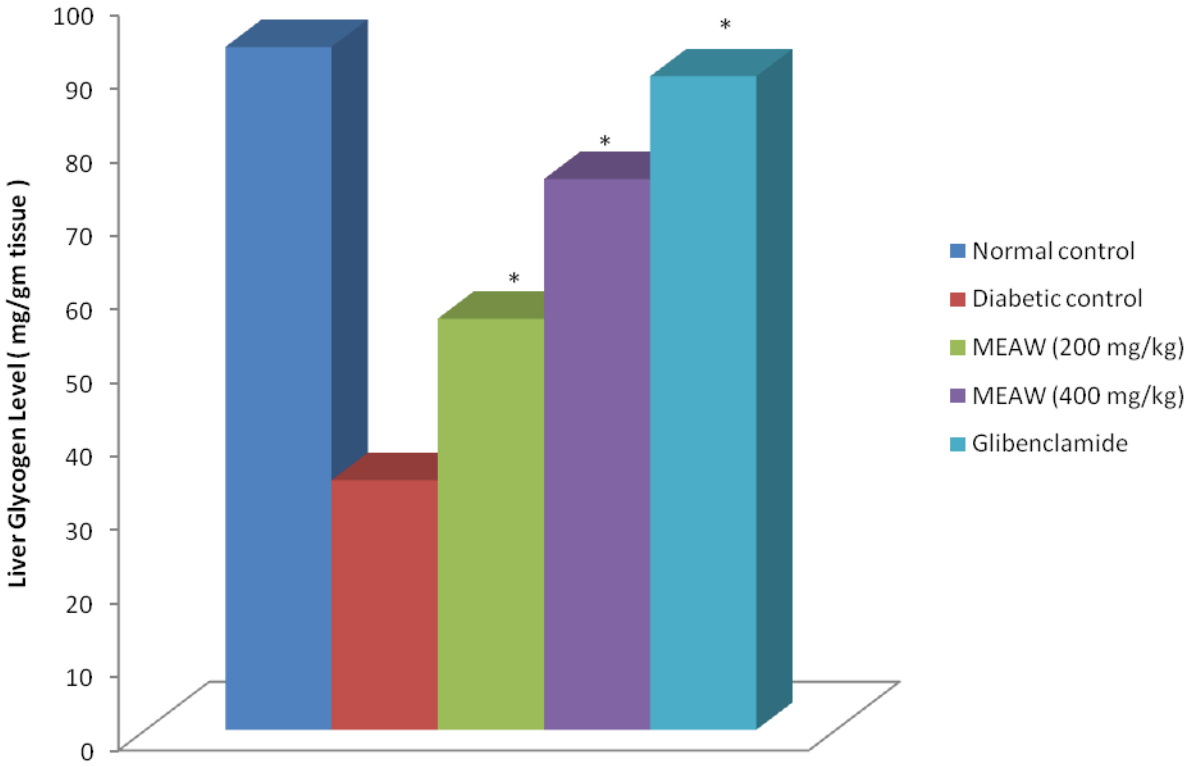

Figure 4. Effect of MEAW on the glycogen level in liver of diabetic rats.

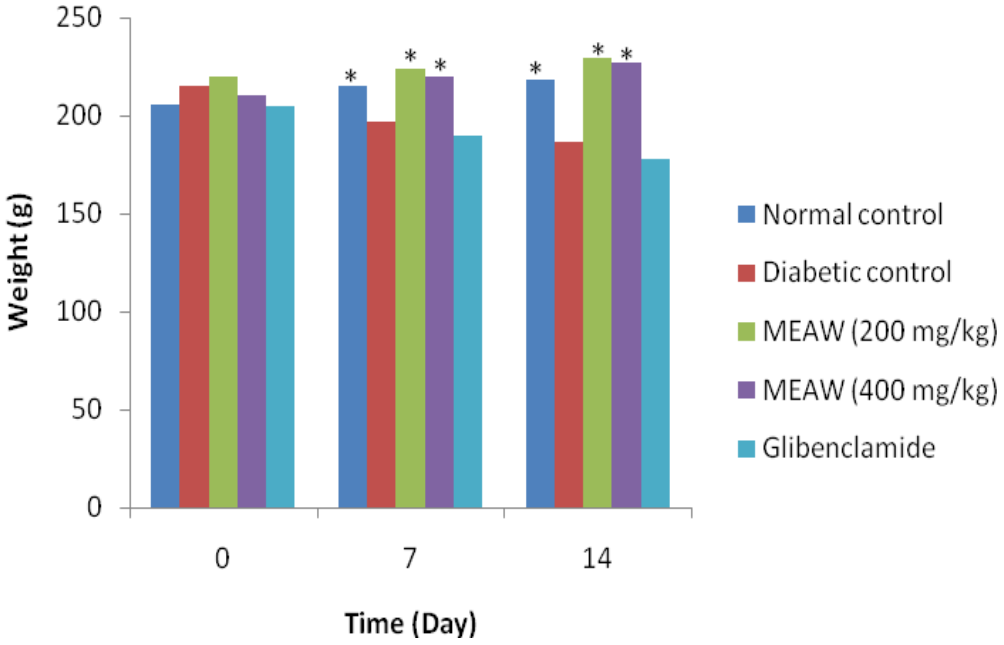

Figure 5. Effect of MEAW on the body weight of rats.

presence of various chemical constituents, including alkaloids, saponins, triterpenoids, flavonoids, tannins and steroids (Table 2) which may be responsible for its antidiabetic properties. Alkaloids (128.90±0.40 mg/100g) and terpenoids (115.5±1.20 mg/100 g) are more abundant while cardiac glycosides are lowest (0.652±1.48

Table 2. Results of quantitative phytochemical analysis of *A. wilkesiana* roots.

Constituents	Value (mg/100 g)[a]
Alkaloids	128.90 ± 0.40
Flavonoids	6.417 ± 0.22
Tannins	6.055 ± 1.08
Cardiac glycosides	0.652 ± 1.48
Saponins	9.780 ± 0.55
Steroids	16.4 ± 0.30
Terpenoids	115.5 ± 1.20

[a]Values are mean ± SEM of three replicate analyses.

mg/100 g). Similar phytoconstituents have been obtained from the leaves of this plant (Ikewuchi et al., 2010). Flavonoids are known for their diverse biological activities including hypoglycemic and hypolipidemic activities resulting from their antioxidant activity (Afanas'ev et al., 1995). Tannins have been reported to reduce blood cholesterol (Basu et al., 2007). It could be conceived that any of these biomolecules may sensitize the insulin receptor or stimulate the β-cells of islets of langerhans to release insulin which may finally lead to improvement of carbohydrate metabolizing enzymes towards the re-establishment of normal blood glucose level. The significant antidiabetic effect of methanol extract of *A. wilkesiana* in alloxan diabetic rats may also be due to enhanced glucose utilization by peripheral tissues. It is also possible that any of these phytochemicals could be associated with the antihyperlidipemic effect of the plant. The antihyperlipidaemic effect might have resulted from decreased fatty acid concentration in the circulation and reduced cholesterol synthesis possibly due to the flavonoid constituents of the plant extract (Ojewunmi et al., 2014). The actual mechanism of action and the phytoconstituent responsible for the observed effects of the plant, however, needs to be investigated further.

Conclusion

We conclude that the methanol extract of *A. wilkesiana* roots has potent antidiabetic effects in alloxan-induced diabetic rats. The extract also possesses ameliorating effect on the lipid profile and other biochemical parameters in diabetic rats. The present investigation has also opened avenues for further research especially with reference to the development of potent formulation for diabetes mellitus from the roots of *A. wilkesiana*. Activity guided fractionation and its evaluation is in progress in our laboratory.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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