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EFFECT OF AQUEOUS EXTRACT OF *DIOSCOREABULBIFERA* ON SOME BIOCHEMICAL PARAMETERS IN ALLOXAN- INDUCED DIABETIC RATS.

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ABSTRACT
The study was carried out to investigate the effect of the aqueous extract of *Dioscoreabulbifera*tuber on blood glucose, protein, albumin, bilirubin, total cholesterol;triglyceride, high density lipoprotein and enzymes activities on normal and alloxan induced diabetic rats. The aqueous extract was administered orally at a dose of 400mg/kg body weight to both normal and alloxan induced diabetic rats. Twenty adult male rats were divided into four groups of five rats each, two groups were made diabetic and the other two groups were non diabetic. One of the diabetic groups was treated with the extract and the second serves as diabetic control. The alloxan was administeredintraperitoneal at a dose of 150mg/kg per body weight. The administration of the extract lasted for 14 days. Effect of the extract on blood glucose, protein, albumin, bilirubin, total cholesterol; triglyceride and high density lipoprotein concentrations were analysed.The toxic effect of the extract was determined using biochemical enzyme markers. The photochemical screening of the aqueous, ethanol and chloroform extracts showed the presences of alkaloid, flavonoid, tannins, saponins, cardiac glycosides, resins, terpenes and steroids. Treatment with the extract showed significant ($P<0.05$) reduction on the blood glucose level and other biochemical parameters. Theextract possesses no toxic effect as indicated by the lowering of ALP and ALT levels and may be used for the management of diabetes mellitus.
Keywords: *Dioscoreabulbifera,Alloxan, Photochemical, Diabetes Mellitus, Hypoglycaemic.*

INTRODUCTION
Plants are found everywhere and so they constitute the earth's vegetation. They have been of tremendous importance to man from time immemorial green plants are original producers of food with all nutrients to meet mans need. Plants consumed as food supplies materials needed for reproduction, body repair and medical use. Among the plants consumed as food and used for medicinal purposesare yam tubers. *Dioscoreabulbifera* is a perennialvine with broad leaves and two types of storage organs. The plant forms bulbils in the leaf axils of the twining stems, and tubers beneath the ground. These tubers are like small, oblong potatoes, and they are edible and cultivated as a food crop especially in West Africa. The tubers often have a bitter taste, which can be removed by boiling. They can then be prepared in the same way as other yams, potatoes, and sweet potatoes. The air potato is one of the most widely-consumed yam species. It can grow up to 150 feet tall. Air potato can grow extremely quickly, roughly 8 inches per day, and eventually reach over 60 feet long. It typically climbs to the tops of trees and has a tendency to take over native plants. New plants develop from bulbils that form on the plant, and these bulbils serve as a means of dispersal. The aerial stems of air potato die back in winter, but resprouting occurs from bulbils and underground tubers. The primary means of spread and reproduction are via bulbils.The smallest bulbils make control of air

potato difficult due to their ability to sprout at a very small stage. The vine produces small white flowers, however these are rarely seen when it grows in Florida, [1].In some places, such as Florida where it is considered a noxious weed, it is an invasive species because of its quick-growing, large-leafed vine that spreads tenaciously and shades out any plants growing beneath it. The bulbils on the vines sprout and become new vines, twisting around each other to form a thick mat. If the plant is cut to the ground, the tubers can survive for extended periods and send up new shoots later.*Dioscoreabulbifera* has also been widely used in China.

Diabetes mellitus, often simply referred to as diabetes, is a group of metabolic diseases in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger).Diabetes is one of the first diseases described with an Egyptian manuscript from 1500 BCE mentioning "too great emptying of the urine [2]. The first described cases are believed to be of type 1 diabetes, Indian physicians around the same time identified the disease and classified it as *madhumeha* or *honey urine* noting that the urine would attract ants. The term "diabetes" or "to pass through" was first used in 230 BCE by the Greek Apollonius of Memphis. The disease was rare during the time of the Roman Empire with Galen commenting that he had only seen two cases during his career [3]. Type 1 and type 2 diabetes where identified as separate conditions for the first time by the Indian physicians Sushruta and Charaka in 400-500 AD with type 1 associated with youth and type 2 with being overweight. The term "mellitus" or "from honey" was added by the Britain John Rolle in the late 1700s to separate the condition from diabetes insipidus which is also associated with frequent urination While many measure where tried effective treatment was not developed until the early part of the 20th century when the Canadians Frederick Bantings and Charles Best developed insulin in 1921 and 1922. This was followed by the development of the long acting insulin NPH in the 1940s.

MATERIALS AND METHODS

Animals Used

Twenty adult male albino rats weighing approximately 110g – 250g were obtained from animal house unit of university of Jos.The rats acclimatized to the laboratory conditions for five weeks before any experimental work was undertaken; they were fed with standard feed.

Preparation of Plant extracts

Tubers of potato yam *Dioscoreabulbifera* were obtained from the Bukuru area of Plateau State. The tubers were first peeled, sliced into very small bits and then dried in a shady, well aerated place. The dried tubers were pounded into fine powder and then packed into an air tight container and stored until required. The *Dioscoreabulbifera* powder was poured into a beaker, mixed properly using a shaker and water as a solvent, it was allowed to stand for a day. Then the mixture was filtered and the filtrate was placed in a beaker. They filtrate was evaporated to dryness and the dried extract was stored in a clean, air tight container. Appropriate weights of the extract were prepared to obtain the concentration needed for the study.

Induction of Experimental Diabetes
Diabetes was induced in groups A and B rats by intraperitoneal injection (IP) of Alloxan at doses of 150mg/kg body weight. Diabetes was confirmed in the animal after 48 hours by estimation of blood glucose level. Animals with blood glucose level above 120mg/dl were selected.

Phytochemical Screening
The extracts of *Dioscoreabulbifera*was screened for some phytochemical constituents using standard qualitative procedure [4].

Administration of the Extract
The *Dioscoreabulbifera* extract solution was administrated through the oral route at a dose of 400mg/kg body weight daily. The extract was administrated for 14 days, after experimental diabetes was made manifest in the rats.

Experimental Design
Twenty male rats were randomly divided into four groups of five rats each and fed with standard feed as follows:
Group A- Diabetic control rats with no administration extract (negative control). Group B- Diabetic rats given extract (400mg/kg) daily for 14 days through oral intragastric tube administration.
Group C- Normal rats no administration extract (positive control).
Group D- Normal rats given extract (400mg/kg) daily for 14 days through oral intragastric tube administration.

Sample Collection and Preparation
At the end of 14 days of extract administration, blood from the animals (both treated and control groups) was collected from the jugular vein into plan bottles.The blood in the plain bottle was allowed to clot at room temperature. The clotted blood sample was ringed and centrifuged for 10 minutes at 5,000 r.p.m. Pasteur pipette was used to separate the serum (supernatant) into clean bottles. The serum was used for the biochemical assay.

Statistical Analysis
All data are express as mean ± standard deviation (SD). The result were analysed by one-way Anova and were applicable least significant (LSD) was used to determine significant result. Differences between groups were considered significant at $P<0.005$.

Results

Phytochemical Screening

Table I: Phytochemical Screening of Extracts of *Dioscoreabulbifera*.

Phytochemicals	**Water**	**Ethanol**	**Chloroform**
Alkaloid	+	+	+
Flavonoids	+	+	+
Tannins	_	_	_
Saponins	+	_	_
Cardiac-glycosides	+	+	+
Terpenes& steroids	+	+	+
Balsam	_	_	_
Phenols	_	_	_
Resins	+	+	+

Key: + = present, - = not present

Weights of Rats

Table II: Effect of *Dioscoreabulbifera* extract on weight changes in diabetic and normal groups of Rats on days 1, 7 and 14.

	Weights of rats in grams		
Groups	**Day 1**	**Day 7**	**Day 14**
Diabetic Control	150 ± 10.6	159 ± 9.8	164 ± 7’4
Diabetic + Extract	155 ± 7.9	163 ± 7.7	172 ± 7.1
Normal Control	140 ± 9.4	155 ± 12.3	161 ± 9.4
Normal + Extract	145 ±13.7	152 ± 13.9	165 ± 10.8

Data are mean ± standard deviation for the five animals in each group.

Blood Glucose Level

Table III: Effect of *Dioscoreabulbifera*extract on blood glucose level in normal and diabetic groups.

GROUPS	BLOOD GLUCOSE LEVEL IN mMol/L
Diabetic Control	22.9 ± 1.41
Diabetic + Extract	9.8 ± 0.71[a]
Normal Control	5.6 ± 0.41
Normal + Extract	4.6 ± 2.08[b]

Data are mean ± standard deviation for the five animals in each group

a. Statistically significant (P<0.05) decrease comparedto the Diabetic control.
b. Statistically significant (P<0.05) differences when compared to the Normal control.

Enzyme Activity

Table IV: Effect of *Dioscoreabulbifera* extract on enzyme activity in the blood serum of both normal and diabetic rats.

GROUPS	**ENZYME ACTIVITY (** IU/L)		
	ALP	**ALT**	**AST**
Diabetic Control	34 ± 11.1	15 ± 9.2	21 ± 10.5
Diabetic+ Extract	30 ± 11.7[a]	14 ± 11.7[a]	12 ± 2.7[a]
Normal Control	20 ± 14.3	10 ± 3.9	10 ± 8.9
Normal + Extract	21 ± 14.8[b]	11 ± 3.8[b]	9 ± 5.8[b]

Data are mean ± standard deviation for the five animals in each group

a. Statisticallysignificant (P< 0.05) decrease comparedto the Diabetic control.
b. Statistically significant (P<0.05) differences when compared to the Normal control.

Protein Profile

Table V: Effect of *Dioscoreabulbifera* extract on total protein and albumin levels in blood serum of normal and diabetic rats in g/L.

GROUPS	**Total Proteins**	**Albumin**
Diabetic Control	56 ± 4.69	27 ± 3.6
Diabetic + Extract	58 ± 4.89[a]	28 ± 3.4[a]
Normal Control	65 ± 5.21	33 ± 3.9
Normal + Extract	62 ± 5.0[b]	31 ± 3.5[b]

Data are mean ± standard derivative for the five animals in each group

a. Statisticallysignificant (P<0.05) decrease comparedto the Diabetic control.
b. Statisticallysignificant (P<0.05) differences when compared to the Normal control.

Bilirubin Profile

Table VI: Effectof Dioscorea*bulbifera* extract on bilirubin profile in serumof normal and diabetic rats in mMol/L

GROUPS	Total Bilirubin	Conjugated Bilirubin
Diabetic Control	10.2 ± 2.7	5.0 ± 2.5
Diabetic + Control	10.2 ± 2.7	5.1 ± 2.4
Normal Control	10.2 ± 2.7	5.5 ± 2.4
Normal + Extract	10.0 ± 2.6	5.1 ± 2.4

Data are mean ± standard derivative for the five animals in each group

Lipid Profile

Table VII: Effect of Dioscorea*bulbifera* extract onlipidprofile in serum levels of normal and diabetic rats in mMol/L.

GROUPS	Total Cholesterol	HDL Cholesterol	Triglyceride
Diabetic Control	3.4 ± 1.59	0.37 ± 0.23	2.6 ± 1.74
Diabetic+ Extract	2.6 ± 1.19 [a]	1.39 ± 0.92[a]	1.29± 0.69[a]
Normal Control	2.7 ± 1.07	1.21 ± 0.61	1.61 ± 1.03
Normal+ Extract	2.4 ± 1.2[b]	1.26 ± 0.72[b]	1.35± 1.08[b]

Data are mean ± standard deviation for the five animals in each group.
HDL – High density lipoprotein
a. Statistically significant (P<0.05) decrease comparedto the Diabetic control.
b. Statistically significant (P<0.05) differences when compared to the Normal control.

Discussion

The Table 1 shows result obtained from the preliminary phytochemical screening of the plant extract studies showed the presence of cardiac glycosides, alkaloid, flavonoids, tannins, saponins, resins, terpenes and steroids. The presences of cardiac glycosides support the usage of potatoes yam as anti –hypertensive agent as it has been reported by [5].Cardiac glycosides are known to reduce the effect of diabetic complication [6]. Also reported are the saponins,steroidal glycosides with hypoglycaemic effect [7]. Flavonoids and alkaloids obtained in the extract are reported to be found in the plant with hypoglycaemic activity [8]. The changes in the body weight in the normal and diabetic groups of the rats as shown in Table 2 shows significant increase in the body weight of the rats. The results in Table 3 showed the fasting serum glucose levels in both the diabetic and normal groups of rats, with significant (P<0.05) increase in the blood glucose levels of the alloxan-induced diabetic rats. The administration of the extract showed a marked decrease in blood glucose levels when compared with the diabetic control group also there was significant (P<0.005) decrease in the normal blood glucose levels when compared to the normal controls. This finding is in line with the result obtained by Luka *et al* [9]. The enzyme activity (AST, ALT and ALP respectively) in the serum of both normal and diabetic rats as shown in Table 4 shows significant increase in the transaminase

activity (AST and ALP) of the diabetic control as compared to the normal control ($P<0.05$). On administration of the extract, there was significant decrease when compared to the diabetic + extract group. The increase in the ALT activity in the diabetic control was due to the hepatocellular damage and it is usually accompanied by AST activity [10]. The reversals in the AST and ALT activity after the treatment with the extract is evident of the prevention of cellular and tissue damage under diabetic conditions [11] which the ALP showed no significant difference when compared to the others.

The Alloxan- induced diabetic rats showed significant increase in the elevation of the serum total protein and albumin levels respectively as when compared to the normal rats as shown in Table 5 When the diabetic rats were placed on the extract the high levels of the total protein and albumin reduced significantly ($P< 0.05$).The bilirubin analysis in the total and conjugated bilirubin levels showed significant difference in the levels of the conjugated bilirubin as compared between the normal groups and the diabetic groups, as shown in Table 6 with little difference as compared to the total bilirubin levels.

High serum levels of blood total cholesterol were obtained in the diabetic control rats as compared to the normal rats, but a significant decrease ($P< 0.05$) of the serum total cholesterol levels was obtained in the diabetic + extract group and the normal + extract group of rats, after the administration of extract. High density cholesterol and triglycerides levels were obtained in the diabetic control and normal control groups of rats as shown in Table 7 with significant decrease ($P< 0.05$) in the serum high densitycholesterol and triglycerides levels as obtained in the diabetic + extract group and the normal + extract group of rats, after the administration of extract.In experimental diabetes the severity of the disease is dependent on the dose of the alloxan administration to the animals, low or high doses of the drug produces incomplete or complete destruction of the β-cells of the pancreas respectively [12]. Thus not only is it possible for the active substance in the plant to exert insulin like effect but also to induce insulin synthesis release from undamaged pancreatic β-cells. This study establishes the anti-diabetic properties of the extract used.Hypercholesterolemia associated with alloxan induced diabetic rats as obtained in this study was significantly lowered in the rats that received these plant, a similar fall in serum total cholesterol has been reported in patients during insulin therapy [13]. The hypercholesterolemia observed in the diabetic rats generally might be due to increased intestinal cholesterogenesisresulting from increased activity of the β-hydroxyl-β-methylglutaryl CoA reductase(the rate limiting enzyme in the biosynthesis of cholesterol).

CONCLUSION

In conclusion, the ability of*Dioscoreabulbifera* tuber to significantly decrease the raised in concentration of blood glucose, cholesterol, triglyceride, protein in diabetic rats proves that *Dioscoreabulbifera* tuber have anti-diabetic effect. It possesses no serious toxicity as indicated by the lowered AST, ALT and ALP.

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