Research Article

Effects of aqueous avocado pear (*Persea americana*) seed extract on alloxan induced diabetes rats

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ABSTRACT

Background: world is facing explosive increase in diabetes mellitus**.** It poses a serious challenge to primary health care in developing countries, with negative consequences on the economy. This research is aim at evaluating the effect of *Persea americana* aqueous seed extract on alloxan induced diabetes rats.
Methodology: Effects of the aqueous extract on groups of alloxan (150mg/Kg) induced diabetic rats was investigated. The blood sugar and body weight of the rats was recorded at two weeks and four weeks interval, and one week after the withdrawal of the extract. The test groups (III, IV, and V) were treated with 400mg, 800mg and 1200mg/kg body weight of the extract for 4 weeks.
Results: A significant decrease ($P<0.001$) in blood glucose were observed in all groups compared to Group II. A significant increase in blood glucose ($p<0.05$) was observed one week after withdrawal of the extract. Significant increase in body weight was recorded in groups III, IV and V compared to group II at $P < 0.01$, 0.001 and 0.05 respectively.
Conclusion: The findings may indicate anti-diabetic effects of the extract which may be due to certain mineral elements and phytochemicals, and increase in weight in could be due to proper nutrient utilization probably induced by the avocado seeds' extract. Avocado seeds may be of beneficial effects to diabetic patients.

Key Words: Avocado pear (*Persea americana*) seed, Alloxan, Diabetes, Serum glucose.

INTRODUCTION

Medicinal plant have continued to attract attention in the global search for effective methods of using plants' parts (e.g. seeds, stems, leaves, roots and bark etc) for the treatment of many diseases affecting humans (Sofowora, 1993). Many important drugs used in medicine to day are directly or indirectly derived from plants due to its bioactive constituents such as; alkaloids, steroids, tannins e.t.c (Cordeiro and Oniyangi, 1998). In recent years, secondary plant metabolites previously with unknown pharmacological activities have been extensively investigated as sources of medicinal agents (Krishnaraju *et al.,* 2005).Thus it is anticipated that phytochemicals with adequate antimicrobial efficacy will be use for treatment of bacterial infections (Balandrin *et al.,* 1985).

Persea americana (luraceae) is one of the 150 varieties of avocado pear. The tree is widely cultivated in tropical and subtropical areas (Lu *et al.,* 2005). The seed of *Persea americana* (avocado seed) has diverse application in ethno–medicine, ranging from treatment for diarrhea, dysentery, toothache, intestinal parasites, skin treatment and beautification. The avocado seed oil has several health benefit e.g. for controlling human weight (especially used for obese for weight loss) (Lopez *et al.,* 1996; Roger, 1999). *Persea americana* leaves have been reported to have or posses anti inflammatory and analgesic activities (Adeyemi *et al.,* 2002). Antioxidant activity and phenolic content of seeds of avocado pear was found to be greater than 70% (Soong and Barlow, 2004). The edible part (fruit) is very popular in vegetarian cuisine, making a substitute for meat in sandwiches and salads, because of its high fat content and high in valuable, health-promoting fats (Lu *et al.,* 2005) . The fruit is not sweet but fatty, almost distinctly, yet subtly flavored, and of smooth, almost creamy texture. Avocado fruits in many countries such as Mexico, Brazil, South Africa and India are frequently used for milkshakes and occasionally added to ice-cream (Zeldes, 2010).

Diabetes mellitus is often described as a group of complex metabolic disorders with a partial or absolute insufficiency of insulin secretion and/or its action. These disorders are generally characterized by chronic hyperglycemia and glucose intolerance (Naim *et al.,* 2001). Diabetes mellitus is considered to be a bi-hormonal disease involving insulin deficiency or defect in its utilization (e.g. receptor abnormality or presence of antibody to

receptors) together with glucagon excess (Naim *et al.,* 2001). Diabetes is one of the most common non–communicable diseases in the world, affecting 5 to 10% of the adult population in the western world and hence, representing a major cause of morbidity and mortality (Naim *et al.,* 2001). There are two severe types of diabetes mellitus, i.e. Type 1 or juvenile onset or insulin dependent diabetes mellitus (IDDM) and Type 2 or adult onset also known as non–insulin dependent diabetes mellitus (NIDDM). In Type I diabetes; there is absence of insulin and massive B-cells lesion and necrosis (Eisenberth, 2008). The Type 2 diabetes is characterized by a significant insulin production ranging from less than normal to above normal but always in quantities insufficient to maintain glucose homeostasis and organ resistance to insulin (Ripson, 2009). The characteristics symptoms of diabetes mellitus includes excess thirst, polyphagia, polyurea, loss of body weight, frequent occurrence of boils, itching in the limbs, and crowding around urine and impotence (WHO, 2003). This study is to evaluate effect of *Persea Americana* aqueous seed extract on alloxan induced diabetes rats.

METHODOLOGY

Experimental Design

Twenty five (25) albino wistar rats weighing 180 – 220g were kept at Animal House of the Department of Biological sciences, Bayero University, Kano Nigeria at 25 ± 2^{o}C and 45-55 relative humidity. The animals were fed with palletized commercial rat feed (Pfizer livestock co. Ltd, Aba, Nigeria) and tap water *ad libitum*. The rats were assigned into five (5) groups of five (5) rats each as given below:

GROUP I → Normal untreated albino rats (NC)
GROUP II → Diabetic untreated albino rats (DC)
GROUP III → Diabetic treated albino rats (DGI), 400mg/Kg dose
GROUP IV → Diabetic treated albino rats (DGII), 800mg/Kg dose
GROUP V → Diabetic treated albino rats (DGIII), 1200mg/Kg dose

The baseline blood glucose level was determined, before groups II to V were induced with diabetes by intraperitoneal (IP) injection of 150mg/kg body weight of alloxan monohydrate solution (Yanarday and Colac, 1998). The levels of blood glucose of the animals were determined 48 hours after the injection using Glucometer (ACCU CHEK{Aviva}) model GMbH, 68298, those found to be diabetic (serum glucose ≥ 200mg/l) were selected for the study. The weight of the experimental animals was noted before the inducement, after the inducement and at interval of two weeks for four weeks during extract administration.

Sample Collection and Preparation

Samples of ripe Avocado pears (*Persea Americana*) were purchased from Sabon Gari Market, Fagge local government Kano State, Nigeria. The plant material was authenticated at Department of Biological Sciences, Bayero University, Kano, Nigeria. The succulent part of the fruit was removed to obtain the seeds. The seeds were minced by means of a grater and dried to constant weight in an oven at 55^{o}C before being ground to powder and stored in plastic container. Aqueous extract was prepared and adjusted to concentration of 0.4 g/cm^{3}. Diabetes rats of groups; DGI, DGII and DGIII) were orally treated daily with 400mg; 800mg and 1200mg/kg body weigh with the extract respectively for four weeks, while DC and NC groups were garaged with normal tap water. Their blood glucose was determined at interval of two weeks. The extract was then withdrawn and the blood glucose level was determined one week after.

Statistical Analysis

The data was statistically analysed using GraphPad Instat3 Software (2000) version 3.05. by GraphPad Inc..

RESULTS AND DISCUSSION:

Table 1 show blood glucose mmol/l for groups of rats induced with diabetes and treated with aqueous seed extract of avocado seed for zero, two four weeks and one week after withdrawal of the extract. The One-way Analysis of Variance (ANOVA) for normal gave a P value of 0.9950 (P>0.05.) hence considered not significant; therefore variation among column means is not significantly greater than expected by chance.

Table 1: Blood glucose in mmol/l of alloxan induced diabetes rats orally treated with aqueous seed extract of Avocado Americana for one 14 days, 28 days and 7days after withdrawal of the treatment.

Group	Normal	ZWKS	TWKS	FWKS	OWAFWD
I , n 0mg/Kg alloxan	4.80 ± 0.61	4.80 ± 0.41	4.70 ± 0.51	4.70 ± 0.55	4.70 ± 0.64
II, n 0mg/kg	4.7 ± 0.50	26.50 ± 4.04	27.90 ± 4.34	28.90 ± 4.25	28.90 ± 4.05
III, n 400mg/Kg	4.70 ± 0.50	25.80 ± 7.16	12.70 ± 5.01	10.50 ± 3.47	14.87 ± 1.62
IV, n 800mg/Kg	5.60 ± 0.32	26.30 ± 3.39	18.70 ± 2.49	16.90 ± 2.82	20.00 ± 3.19
V, n 1200mg/Kg	5.20 ± 0.70	24.40 ± 0.68	8.50 ± 1.15	6.60 ± 1.00	13.70 ± 3.01

Values are mean ± standard deviation, n=5

Key:

Normal= before induced with diabetes
ZWKS = 0 week after inducement with diabetes
TWKS = 2 weeks after inducement with diabetes
FWKS = 4 weeks after inducement with diabetes
OWAFWD = 1 weeks after withdrawal of treatment

ANOVA assumes that the data are sampled from populations (GI) with identical SDs. This assumption is tested using the method of Bartlett (2000) given P value of 0.9345 (P>0.05), thereby suggesting that the differences among the SDs are not significant. The statistical conclusion is expected since G I and normal columns were neither treated with alloxan nor with aqueous extract of avocado seeds. Hence whatever variation recorded in any of the; groups or columns could be as a result of the treatment.

ANOVA for ZWKS had a P < 0.0001, variation among column means (GI vs GII, III, IV and V) is considered extremely significant and this may confirm inducement of diabetic among alloxan treated groups. Table 2: Show Tukey-Kramer multiple comparisons test for Group II and variation among raw means (Normal vs Zwks, Twks, Fwks and OWAFWD) is considered significant (P<0.001), while variation in raw mean among alloxan induced diabetic rats with time is not significantly different (P>0.05). This may confirm inducement of diabetic condition in the alloxan treated groups and it lasted throughout the study period. This out come should be expected since the group was not treated with extract and show no evidence of natural healing (Table1and 2).

Table 2: Tukey-Kramer Multiple Comparisons Test, for different period in Group II

Comparison	Mean difference	q	p value
Norm vs Zwks	-21.800	13.040	*** P<0.001
Norm vs Twks	-23.200	13.877	*** P<0.001
Norm vs Fwks	-24.200	14.475	*** P<0.001
Norm vs OWAFWD	-24.200	14.475	*** P<0.001
Zwks vs Twks	-1.400	0.8374	ns P>0.05
Zwks vs Fwks	-2.400	1.436	ns P>0.05
Zwks vs OWAFWD	-2.400	1.436	ns P>0.05
Twks vs Fwks	-1.000	0.5982	ns P>0.05
Twks vs OWAFWD	-1.000	0.5982	ns P>0.05
Fwks vs OWAFWD	0.000	0.000	ns P>0.05

If the value of q is greater than 4.232 then the P value is less than 0.05 and is specified by the script ***

ANOVA for TWKS, is considered extremely significant (P < 0.0001) therefore, variation among column means is significantly greater than expected by chance. This could be due to treatment given to group III, IV and V. Table 3 show the outcomes on comparing variation in blood sugar in group III with duration of treatment. The non significant difference for Norm vs Twks, and Norm vs Fwks is indicating probable glucose lowering effect of avocado aqueous seed extract on alloxan induced diabetes rats. While, the significant different (p<0.01) in Norm vs OWAFWD is indicating that the avocado extract may have management effects but not curative. The significant difference (p< 0.001) for Zwks vs Twks, and Zwks vs Fwks had confirmed the glucose lowering effect of the extract when compared with that of the diabetes rats before treatment. The significant difference at p <0.01 for Zwks vs OWAFWD is indicating that the blood glucose level rising one week after the withdrawal of the treatment, therefore, confirmed management effect of the extract.

Table 3: Tukey-Kramer Multiple Comparisons Test, for different period in Group III

Comparison	Mean Difference	q	P value
Norm vs Zwks	-21.100	1.042	*** P<0.001
Norm vs Twks	-8.000	4.187	ns P>0.05
Norm vs Fwks	-5.800	3.035	ns P>0.05
Norm vs OWAFWD	-10.170	5.322	** P<0.01
Zwks vs Twks	13.100	6.856	*** P<0.001
Zwks vs Fwks	15.300	8.007	*** P<0.001
Zwks vs OWAFWD	10.930	5.720	** P<0.01
Twks vs Fwks	2.200	1.151	ns P>0.05
Twks vs OWAFWD	-2.170	1.136	ns P>0.05
Fwks vs OWAFWD	-4.370	2.287	ns P>0.05

If the value of q is greater than 4.232 then the P values is less than 0.05 and are specify by the script (*) base on p value.

ANOVA for FWKS shows extremely significant variation (P < 0.0001) among column means greater than expected by chance, it could be due to treatment given to group III, IV and V. Table 4 gives the assumption test for GIV, ANOVA 'assumes that the data are sampled from populations with identical SDs'. This assumption is tested using the method of Bartlett (2000). Bartlett statistic (corrected) = 12.650, the P value is 0.0131, Bartlett's test suggests that the differences among the SDs is significant. From Tables 1 and 4, the significant (p<0.001) variation in Norm vs Twks, and Norm vs Fwks despite treatment with higher dose compared to Group III (Table 4, is unexpected this could be due to the time difference between feeding and glucose determination. However, the significant difference at p<0.01, 0.001 and 0.05 respectively for Zwks vs Twks, Zwks vs Fwks and Zwks vs OWAFWD also indicated the hypoglycemic effects of the extract on alloxan induced diabetes rats.

Table 4: Tukey-Kramer Multiple Comparisons Test, for different period in Group IV

Comparison	Mean Difference	q	P value
Norm vs Zwks	-20.700	17.268	*** P<0.001
Norm vs Twks	-13.100	10.928	*** P<0.001
Norm vs Fwks	-11.300	9.427	*** P<0.001
Norm vs OWAFWD	-14.400	12.013	*** P<0.001
Zwks vs Twks	7.600	6.340	** P<0.01
Zwks vs Fwks	9.400	7.842	*** P<0.001
Zwks vs OWAFWD	6.300	5.256	* P<0.05
Twks vs Fwks	1.800	1.502	ns P>0.05
Twks vs OWAFWD	-1.300	1.084	ns P>0.05
Fwks vs OWAFWD	-3.100	2.586	ns P>0.05

If the value of q is greater than 4.232 then the P values is less than 0.05 and are specify by the script (*) base on p value.

One-way Analysis of Variance (ANOVA) for OWAFWD shows that variation among column means is significantly greater than expected by chance (P < 0.0001). Table 5 shows the out comes for ANOVA that 'assumes that the data are sampled from populations with identical SDs'. This assumption is tested using the method of Bartlett. Bartlett statistic (corrected) = 12.786, the P value is 0.0124. Bartlett's test suggests that the differences among the SDs are significant. None significant (p>0.05) difference in Norm vs Fwks may suggest that the hypoglycemic effect of the avocado seed extract is based on dose and duration of exposure.

Table 5: Tukey-Kramer Multiple Comparisons Test, for different period in Group V

Comparison	Mean Difference	q	P value
Norm vs Zwks	-9.200	13.097 ***	P<0.001
Norm vs Twks	-3.300	4.698 *	P<0.05
Norm vs Fwks	-1.400	1.993 ns	P>0.05
Norm vs OWAFWD	-8.500	12.101 ***	P<0.001
Zwks vs Twks	5.900	8.399 ***	P<0.001
Zwks vs Fwks	7.800	11.104 ***	P<0.001
Zwks vs OWAFWD	0.7000	0.9965 ns	P>0.05
Twks vs Fwks	1.900	2.705 ns	P>0.05
Twks vs OWAFWD	-5.200	7.403 ***	P<0.001
Fwks vs OWAFWD	-7.100	10.108 ***	P<0.001

If the value of q is greater than 4.232 then the P value is less than 0.05 and is specify by the script (*) base on p value.

Table 6: weight (g) of alloxan induced diabetes rat orally treated with aqueous extract of avocado seeds for 2 and 4 weeks, and 1 week after withdrawal of treatment

Group	Normal	ZWKS	TWKS	FWKS	OWAFWD
I	180.0	180.0	183.3	206.7	220.0
0mg/Kg alloxan	±	±	±	±	±
	1.13	1.20	4.08	8.17	14.14
II	180.0	176.7	170.0	163.3	156.7
0mg/kg	±	±	±	±	±
	1.300	4.08	7.07	3.7.3	3.73
III	193.3	186.7	216.7	220.0	213.3
400mg/Kg	±	±	±	±	±
	7.45	17	10.80.	14.14	16.33
IV	200.0	186.7	206.7	220.0	220.0
800mg/Kg	±	±	±	±	±
	0.00	17	8.17	14.14	14.14
V	200.0	193.3	200.0	201.3	206.7
1200mg/kg	±	±	±	±	±
	0.00	8. 17	0.00	8.17	17

Values are mean standard deviation, n=5

Table 7: Tukey-Kramer Multiple Comparisons Test for weight (g) of normal, diabetes; treated and untreated, and one week after withdrawal of the treatment diabetes rats

Comparison	Mean Difference	q	P value
GI vs GII	24.660	4.137 ns	P>0.05
GI vs GIII	-11.980	2.010 ns	P>0.05
GI vs GIV	-16.680	2.799 ns	P>0.05
GI vs GV	-6.266	1.051 ns	P>0.05
GII vs GIII	-36.640	6.147 **	P<0.01
GII vs GIV	-41.340	6.936 ***	P<0.001
GII vs GV	-30.926	5.189 *	P<0.05
GIII vs GIV	-4.700	0.7886 ns	P>0.05
GIII vs GV	5.714	0.9587 ns	P>0.05
GIV vs GV	10.414	1.747 ns	P>0.05

If the value of q is greater than 4.232 then the P value is less than 0.05 and is specify by the script ( ) base on p value*

Hypoglycemic effect of the avocado seed extract may be due probable contents of elements such as calcium, magnesium, potassium, sodium, zinc, chromium e.t.c that play key role in blood glucose homeostasis by regulating the key enzymes involved in gluconeogenesis in the liver e.g. glucose-6- phosphatase, fruitcose-1, 6-bisphosphatatase and phosphoenolpyruvate carboxykinase, thereby blocking gluconeogenesis and enhancing glucose utilization in the body (Broadhust, 1997). The seed may in addition to these elements contains certain hypoglycemic agents such as phytochemicals (e.g. flavonoids, saponins, steroids, terpenoids, tannins and alkaloids etc) which contain insulin stimulatory substances such as insulin receptors substrate (IRS), prohornone convertase, glycogen synthase, the b3 adrenergic receptor, glucose dependent insulinotrophic polypeptide (GIP) receptor and peroxisome proliferators – activated receptor gamma e.t.c. (Broadhust, 1997). However, the mechanism by which the extract lowered the blood glucose level in alloxan induced diabetic rats is still unclear. It could be by stimulating peripheral utilization of glucose by inhibiting absorption in the gastrointestinal tract (GIT), increasing glucose metabolism, or regenerating the pancreatic tissue or potentiating the insulin secretion by the surviving B- cells. A prolonged administration of the extract shows higher hypoglycemic effects on alloxan induced diabetic rats than are shorter period. And after withdrawal of the treatment for one week the blood glucose gradually rised, however below that of the untreated group, this signifies the management effect of the avocado seed extract.

The increase in weight of diabetic rats treated with avocado seed extract (Table 6) was found to be significant between diabetes groups treated with avocado seed and diabetic non treated (Group II). This could be due to certain compounds and or mineral elements that may stimulate effective utilization of nutrients. In addition, the seed may contain nutrients such as protein and fat this coupled with their effective utilization, may be responsible for the weight gain.

CONCLUSION

The findings of this study indicate that consumption of the aqueous extract of avocado pear seed (*Persea americana*) exerts significant hypoglycemic effects on alloxan induced diabetic rats. The findings may support acclaimed traditional use of avocado pear seed for controlling hyperglycemia in diabetes. The findings also indicate that prolonged oral administration of aqueous extracts of avocado pear seed has a greater effect in management of diabetes mellitus than short term administration.

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