IJASRM
International Journal of Advanced Scientific Research and Management, Vol. 2 Issue 1, Jan 2017.
www.ijasrm.com
ISSN 2455-6378

Effect of Formulated *Eleusine coracana* Diet in the Management of Alloxan-induced Diabetes Mellitus in Albino Rats

Nadro, M. S. [1] and Onotu, M.O [2]

[1&2] Department of Biochemistry, Modibbo Adama University of Technology, PMB 2076, Yola. Nigeria

Abstract

In the present study, diet was formulated; the glycemic index of the formulated diet determined and its effectiveness tested in the management of alloxan- induced diabetic rats. Diabetes was induced by single intraperitoneal injection of alloxan 60mg/kg body weight in albino rats. Normal and diabetic control rats received balanced normal nutritive diet while the treatment groups were fed with 30, 70 and 100 % of the formulated diet for a period of 28 days. There was significant decrease ($p<0.05$) in blood glucose level of the treated groups when compared to diabetic control group. High density lipoprotein significantly increase at ($p<0.05$). There was significant decrease ($p<0.05$) in triglyceride, cholesterol and low density lipoprotein values of the formulated diet treated groups when compared to the diabetic control group. The group treated with 100% formulated diet showed significantly decrease ($p<0.05$) of creatinine (7.6 mg/L) and urea (95.94 mg/L) compared to 14.00 mg/L creatinine and 331.56 mg/L of urea for the diabetic control group. The serum albumin was found to increase significantly ($p<0.05$) compared to the diabetic control group. Thus, the present animal study evidenced the hypoglycaemic, hypolipidaemic properties of the formulated diet suggesting that it could be used in management of diabetes.

Keywords: *formulated diet, finger millet, alloxan, diabetes*

1. Introduction

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia resulting from a defect in insulin action or deficiency in insulin secretion or both leading to alterations in carbohydrate, protein and lipid metabolism. The uncontrolled diabetes will leads to micro vascular such as neuropathy, nephropathy, retinopathy and macro vascular (atheroma) complications (Wild *et al.,* 2004). Development of diabetes are involved several causes, from autoimmune destruction of the pancreatic β-cells with consequent insulin deficiency to resistance of insulin action abnormalities (Nishimura *et al.*, 1998).

The impaired insulin secretion and defects in insulin action often coexist in a patient, and its abnormalities often unclear but the primary cause is hyperglycemia. Polyuria, polydipsia, weight loss, sometime with polyphagia and blurred vision are some of hyperglycemic symptoms. Chronic hyperglycemia will cause growth impairment and susceptibility to certain infections. Ketoacidosis or non ketotic hypersmolar syndrome are life threatening found in acute hypergycemia (Lakshmi and Sumathi, 2002).

Eleusine coracana (finger millet) is a plant found in tropical regions like Nigeria. The leaf is linear to lancelet shape up to 70 cm long and 20 mm width. *Eleusine coracana* is often intercropped with legumes. The grains of finger millet are tiny, with a dark brown seed coat that is rich in polyphenols compared to other grains such as barley, rice, maize, and wheat (Subhash and Hathan, 2014).

Finger millet usage is limited due to its coarse nature; the outer cover of the seed is thick which makes its processing difficult and gives a poor sensory quality (Singh and Raghuvanshi, 2012). There is declined in both consumption and production of this grain that when prepared is known for its higher sustaining power, lower glycemic response higher satiety scores when compared with other cereal foods. Finger millet has antioxidative, anti-allergic, anti-inflammatory, anti-carcinogenic and gastroprotective properties due to the presence of flavonoids (Shobana *et al.,* 2010, Kumar *et al.,* 2014). Vanillic acid and quercetin

found in finger millet inhibit cataract formation in the eye lens. This is important because it is one of the three causes of blindness worldwide; diabetes is the major risk factor (Chandrasekara and Shahidi, 2010). Feeding of finger millet and kodo millet on the antioxidant and glycemic status of alloxan-induced diabetic rats has showed reduction in blood glucose level by 36 and 42 % and cholesterol level 13 and 27 % reduction, besides improved antioxidant status (Shrinivasa, 2008).

The aim of this study is to investigate the effectiveness of formulated diabetic diets consisting of finger millet seed as energy source, groundnut cake, unripe plantain, salt, methionine and premix supplements (minerals, amino acids and vitamins) in right proportion in alloxan-induced diabetic rats as a means of managing diabetes mellitus in rats.

2. Materials and Methods

Plant Sample: The seed of *Eleusine coracana* (finger millet) was obtained from Sabon Gari market in Zaria, Kaduna state. The seed was authenticated in the Department of Crop Production, Modibbo Adama University of Technology, Yola.

Experimental Animals: Male Wistar strain albino rats weighing between 80-100gm needed for this study were purchased from the animal unit of the Nigeria Institute for Trypanosomiasis Research (NITR), Vom, Jos. Plateau State. Nigeria. They were fed with standard rat diet and drinking water *ad libitum.*

Chemical and Reagents: All chemicals/reagents used were of analytical grades.

2.1 Samples Preperation

The sample of finger millet was hand-picked to separate stones and unhealthy seeds from good ones, then washed, packed in sack and left for 7 days for it to germinate. The germinated finger millet was sun dried for 6 hours, milled to fine powder and stored in air tight container until when it is required.

The unripe plantain was obtained from Jimeta main Market Yola, Nigeria. Washed, peeled, sliced, dried, milled into powdered form and stored in air tight bottle. Remaining ingredients groundnut, bone meal, mixed spices was obtained from the same Market were dried and milled into powdered form and stored in air tight container

2.2 Formulation of Diet

Using the measurement based formula, finger millet, groundnut cake, unripe plantain, mixed spices powders were mixed to form homogeneous using electric mixer. The mixture was fortified with salt, methionine, premix, and bone meal powder

3. Determination of Gycemic Index of the Formulated Diet

Six albino rats per group were used for this work, blood was gotten by excision of the tail and the blood glucose level was determined using glucometer. White bread was used for control as described by international guideline. Rats were fast overnight and giving the formulated diet that contain equivalent of 50g of available carbohydrates on two different days with interval of five days. The fasting blood glucose level and the change in blood glucose level at 15, 30, 45, 60, 120, 150 minutes and after 2 hours of food intake was recorded according to Wolever *et al.*, (1987).

$$GI = \frac{incremental\ area\ under\ the\ glucose\ curve\ for\ the\ test\ meal\ (slope)}{incremental\ area\ under\ the\ glucose\ curve\ for\ the\ standard\ glucose} \times 100$$

3.1 Diabetic Induction in Rats

Diabetes was induced using single dose of freshly prepared alloxan solution at 60 mg/kg body weight intraperitoneally after 12 hours fasting. Fasting blood glucose level was tested after four days using one touch ultra glucometer. Rats with blood glucose level above 8 mmol/L or 250 mg/dl were considered diabetic and used in experiment.

Fasting blood glucose level was measured at seven days interval and recorded.

3.2 Experimental Design

Thirty six male albino rats weighing 80-100g were allowed seven days to acclimatization period before commencement of experiment. All animal procedures were in accordance with NIH guide for the care of laboratory animals. The rats were randomly divided in six groups of six rats each (group I, II, III, IV, V, VI). Groups I and II were served as normal and diabetic controls while group III served as drug (metformin) control. The remaining groups were treated with 30, 70 and 100 % of the formulated diabetic diet respectively. The rats' body weights were measured at three days interval throughout the experimental period.

After 28 days of treatment the rats were sacrificed and blood was collected through cardiac puncture. the collected blood was allowed to clot, centrifuge for 15 minutes at 3000 rpm and serum was collected into a clean bottle for biochemical analysis.

3.3 Biochemical Analysis

Diagnostic kits were employed in the analysis of the glucose (Barham and Trinde, (1972), creatinine

(Henry, 1974) and urea (Weatherburn, 1967). Total cholesterol was determined as described by Schoenheimar and Sperry, (1939), procedure of Buccolo and David (1973) was followed for triglyceride determination, HDL was determined using Rifai and Warnick, 1994, LDL using calculation method, albumin **(**Reinhold, 1953).

3.4 Statistical Analysis

All values are expressed in mean ± SEM. The results are analysed for statistical significance using one way ANOVA followed by Dunnett's't' test, $P<0.05$ was considered significant.

4. Results and Discussion

Dietary modification is probably the simplest and cheapest form of diabetes treatment. For this reason patients with diabetes need dietary recommendations that can be easily understood and translated into everyday life. To achieve the goals and objectives of dietary therapy, it is important that diabetic patients are provided with dietary guidelines appropriate to their culture situations (Lorenzo *et al.,* 2007). In this study, oral administration of a formulated diabetic diet which comprises of finger millet as the bulk energy source, unripe plantain, groundnut cake, bone meal, some amino acids and vitamin substituent. Finger millet is consumed in some communities in Nigeria. **Table 1** shows formulated diet composition used with a percentage crude proteins to be 16.60 %. The diet was formulated using a diet formulation method based on Pearson's square method. The glycemic index of the formulated diet determined was 57 %**,** this shows that the diet falls within the range of medium glycemic index (56 – 69 %) based on the internationally acceptable protocol (Jenkin *et al.,* 1987). Assumption regarding GI and glycaemic load food is that these foods produce a lesser increase in the plasma glucose concentration as a result of rates of gastric emptying and digestion of carbohydrates in the intestinal lumen and subsequently, a lower rate of absorption of glucose into the portal and systemic circulation (Khan *et al*., 2008).

Several authors reported flavonoids, saponin, alkaloids and phenolics as bioactive antidiabetic principles (Punitha & Manoharan, 2006, Mohammed *et al.,* 2006). Fortunately the formulated diet contains most of these bioactive antidiabetic principles in reasonable quantities (**table 2**). Probably that is why the formulated diet lowered significantly most of the raised biochemical parameters in the diabetic rats.

Uncontrolled diabetes mellitus is a common cause of weight loss with increased appetite particularly with new –onset type1 diabetes mellitus (Ozougwu *et al.,* 2013). In this study there were significant increases ($P<0.05$) in the bodyweights **(table 3)** of the diet treated groups compared to the diabetic control, drug control and normal control. The change in body weight showed that the rats fed the formulated diet had a significantly higher weight gained compared to the diabetic rats. The weight gained may be as a result of the ability of the diet to reduce hyperglycaemia within the period of this study *(*Jaiswal *et al.,* 2009).

The blood glucose of the diet treated groups significant decrease ($P<0.05$) compared to the normal control and diabetic control group. The diet treated group blood glucose decreased significantly ($P<0.05$) compared to the normal control group. The blood glucose increases significantly for the diabetic control compared to the normal control group **(table 4)** was observed with the formulated diet treatment in contrast to the diabetic control, although this reduction in serum glucose as shown in **table 4**, was observed not enough to reach normal glucose levels in rats, but it was not significant higher when compared with normal group. This is in line with some findings which show that intakes of high fibre diets like millets were associated with lower incidence of diabetes mellitus, lower body mass index (BMI), blood pressure and serum total cholesterol compared with lower intakes (Frank, 1993).

The effect of formulated diet on kidney function was assessed by the determination of the levels of serum urea and creatinine, as level of urea and creatinine **(table 4)** are often regarded as reliable markers of renal function. Thus, elevations in the serum concentrations of these markers in diabetic rats which are significantly higher ($P<0.05$) compared to the normal control group are indication of renal injury, that is diabetes could lead to renal dysfunction. The treatment of alloxan-induced diabetic rats with formulated diet significantly reduced ($P<0.05$) urea in serum compared to the mean value of diabetic group **(table 4).** Similarly, the elevation of creatinine level caused by diabetes was declined after administration of the diet compared with the diabetic control. From the data obtained it can be concluded that treatment with 100 % of the formulated diet produced a significant improvement of the impaired kidney functions in alloxan-induced diabetic rats. Administration of the diet maintained the protein level near normal. This is in line with the observation that high blood urea levels obtained are associated with excessive tissue catabolism.

IJASRM International Journal of Advanced Scientific Research and Management, Vol. 2 Issue 1, Jan 2017.
www.ijasrm.com
ISSN 2455-6378

Injection of alloxan caused an increase of serum total cholesterol (TC); the marked hyperlipidaemic that characterized the diabetic state may therefore be as a result of the uninhibited actions of lipolytic hormones on the fat depots due to the absence of insulin (Kissebah *et al.,* 2002). **Table 5** shows a significant increase in HDL ($P<0.05$) for the diet treated groups compared to the diabetic control, drug control and normal control. Also a significant decrease in serum HDL in diabetic control ($P<0.05$) compared to the normal control group and drug control group. While LDL, triglycerides (TG) and total cholesterol level decrease ($P<0.05$) for the diet treated groups was also observed compared to the diabetic control group, as the diet dosage increases, the cholesterol decrease compared to the diabetic control and drug control and thus bring it below normal. The triglycerides decrease significantly ($P<0.05$) compared to the diabetic control but could not bring it to normal the treatment of the induced-diabetic rats with formulated diet in different proportions caused reduction in the levels of serum cholesterol **(table 5).** As the body needs more cholesterol for bile acid production used for digestion, the liver removes cholesterol from the blood stream through increase hepatic LDL-receptor levels, increase hepatic uptake of LDL cholesterol and aid its catabolism to bile acids, thus lowers serum cholesterol (Stephen, 2009). The fibre content of the diet could have a contributory role in the reduction of cholesterol levels as the role of dietary fibre in the reduction of cholesterol has been reported by Brown *et al.*, (1999). Therefore, the presence of saponins and fibre in the diet is probably responsible for the cholesterol lowering ability of the diet.

In conclusion, the reduction in the levels of glucose, total cholesterol, triglycerides and LDL on administration of the formulated diets suggest that these diets have hypoglycaemic and hypolipidemic properties and could be used in the management of diabetes mellitus.

Table 1: Composition of the Diet Formulation (%)

Ingredients	Content
inger Millet	58.93
Groundnut Cake	25.15
Unripe Plantain	8.42
Bone Meal	2.5
Mixed Spices	4.0
Common Salt	0.3
Methionine	0.2
Premix	0.3

(Diabetes care, 2008)

The percentage of protein in the mixture is 16.60
GI=57%

Table 2: Quantitative determination of Phytochemical Constituents (%) on *Eleusine oracana* and Formulated Diabetic Diet

	Flavonoids	Tannins	Saponins	Alkaloids	Phytate	Oxalates
finger millet	2.02 ±0.01	0.41 ±0.02	2.99 ±0.02	3.04 ±0.15	0.42 ±0.02	1.32 ±0.01
formulated diet	6.17 ±0.01*	0.75 ±0.03*	8.26 ±0.03*	3.39 ±0.01	0.59 ±0.02	4.84 ±0.01*

Values are mean ± SEM; n=3

*significantly increased at ($p<0.05$) compared to unprocessed finger millet

Table 3: Effect of Formulated Diet on the Body Weights of Alloxan-induced Diabetic Rats

Treatment	Initial Body Weight (g)	Final Body Weight (g)	Weight Difference (%)
Normal control	95.39 ± 9.76	107.47 ± 5.67	12.08
Diabetic control	99.23 ± 18.19	75.40 ± 7.20	- 23.83
Drug(Metformin) control	85.68 ± 8.30	101.71 ± 3.57	16.03*
30 % Formulated Diet	91.82 ± 5.80	121.04 ± 7.12	29.22*
70 % Formulated Diet	92.12 ± 9.86	146.30 ± 9.76	54.18*
100 % Formulated Diet	98.89 ± 6.52	169.00 ± 6.92	71.07*

Values are means ± SEM; n = 6
*Significant increase compared to diabetic control at $P<0.05$

Table 4: Effect of Different Percentage of Formulated Diet on some Biochemical Parameters in Alloxan-induced Diabetic Rats

Treatment	Glucose (mg/dl)	Albumin (g/l)	Creatinine (mg/l)	UREA (mg/l)
Normal control	94.14 ± 3.06	38.00 ± 0.77	8.80 ± 0.15	108.9±11.55
Diabetic control	489.78 ± 8.64	23.00 ± 0.79	14.00 ± 0.03	331.56±20.41
Drug (Metformin) control	108.36 ±1.62[b]	23.00 ± 0.79	11.98 ± 0.32	127.26±6.93
30 % Formulated Diet	102.60 ±2.70[b]	28.00 ± 0.70[a]	8.90 ± 0.26[b]	112.01±15.47[b]
70 % Formulated Diet	76.14 ± 4.86[b]	33.00 ± 1.17[a]	7.67 ± 0.23[b]	105.32±10.75[b]
100 % Formulated Diet	60.66 ± 3.60[b]	35.00 ± 0.65[a]	7.60 ± 0.20[b]	95.94±3.58[b]

Values are means ± SEM; n = 6
[a] Significant increase compared to diabetic control at P<0.05
[b] Significant decrease compared to diabetic control at P<0.05

Table 5: Effect of Different Percentage of Formulated Diet on Lipid Profile Parameters in Alloxan-induced Diabetic Rats

Treatment	T C (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Normal control	39.60 ± 2.70	16.20 ± 0.90	27.00 ± 1.80	9.36 ± 0.72
Diabetic control	45.00 ± 1.98	48.60 ± 0.54	12.42 ± 0.54	23.22 ± 0.36
Drug (Metformin) control	44.10 ± 1.44	31.14 ± 1.62[b]	18.54 ± 0.54	18.36 ± 0.72
30 % Formulated Diet	41.40 ± 0.18[b]	40.86 ± 0.54[b]	26.10 ± 0.72[a]	2.88 ± 0.18[b]
70 % Formulated Diet	36.54 ± 0.36[b]	37.80 ± 2.16[b]	25.20 ± 0.36[a]	4.50 ± 0.36[b]
100 % Formulated Diet	36.18 ± 0.72[b]	36.54 ± 0.36[b]	24.66 ± 0.54[a]	8.10 ± 0.18[b]

Values are means ± SEM; n = 6
[a] Significant increase compared to diabetic control at P<0.05
[b] Significant decrease compared to diabetic control at P<0.05

References

[1] Barham, D. and Trinder, P. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst. 97(151):142–145, (1972).
[2] Buccolo, G. and David, H. Quantitative determination of serum triglycerides by the use of enzymes. *Clin. Chem.*. 19:476, (1973).
[3] Brown L., Bernard, R., Walter, W.W. and Frank, M.S. Cholesterol-lowering effects of dietary fibre; a meta-analysis. Am. J. Clin. Nutr. 69: 30-42, (1999).
[4] Chandrasekara, A. and Shahidi, F. Content of insoluble bound phenolics in millets and their contribution to antioxidant capacity. *J. Agric. Food Chem.* 58: 6706-14, (2010).
[5] Frank, Q. N. Dietary fibre in management of diabetes. Diabetes. 42:503-508, (1993).
[6] Henry, R. J. Clinical Chemistry. Principle ant Techniques, 2nd Edition. Harper and Row. Pp 525, (1974).
[7] Jaiswal, D., Kumar, P. R. and Watal, G. Antidiabetic effect of *Withania coagulans* in experimental rats. Indian J. clin. Biochem 24(1)**:** 88-93, (2009).
[8] Jenkin, DJ, Wolever, T. M., Collier G. R., Ocana, A., Rao, A. V., Buckley, G., Lam, Y., Mayer, A., Thompson, L. U. Metabolic effects of low glycemic index diet. Am. J. Clin. Nutr.46(6): 968-75, (1987).
[9] Khan, I., Farukh, T., and Khan, A. Glycemic indices and glycemic loads of varios types of pulses. Pak. J. Nutr. 3(1): 104-108, (2008).
[10] Kissebah, A.H., Vydelingum, N., Murray, R., Evans, D.F., Hartz, A.J., Kalkhoff, R.K *et al.*, Relationship of body fat distribution to metabolic complications of obesity. *J. Clin. Endocrinol. Metab.* 54:254-60, (2002).
[11] Kumari, N., Gupta, A. and Sheikh, S. Development of low glycemic foods with the use of pearl millet and finger millet. Intl. J. Sci. Res. 3(8): 193-195, (2014).
[12] Lakshmi, K.P. and Sumathi, S. Effect of consumption of finger millet on hyperglycemia in non-insulin dependent diabetes mellitus (NIDDM) subjects. *Plant Food Human Nutr.* 57:205-213, (2002).
[13] Lorenzo, C., William, K., Hunt, K.J., Haffner, S.M. The National cholesterol education program- adult treatment panel III, international diabetes federation and world health organization definations of the metabolic syndrome as predictors of incident cardiovascular disease and diabetes. *Diabetes care*. 30: 1-5, (2007).
[14] Mohammed, B., Abderrahim, Z., Hassane, M., Abdelhafid, T. and Abdelkhaleq, L. Medicinal plants with potential antidiabetic activity - A review of ten years of herbal medicine research (1990-2000). Int. J. Diabetes 14:1-25, (2006).
[15] Nishimura, F., Takahashi, K., Kurihara, M. Periodontal disease as a complication of

diabetes mellitus. *Ann Peiodontol.* 3:20-29, (1998).
[16] Ozougwu, J. C., Obimba, K. C., Belonwu, C. D. and Unakalamba, C. B. The pathogenesis and pathophysiology of type 1 and 2 diabetes mellitus. J. Physiol and Pathol. 4(4): 46-57, (2013).
[17] Punitha, R. and Manoharan, S. Antihyperglycemic and antilipidperoxidative effects of *Pongamia pinnata* (Linn) Pierre flowers in alloxan - induced diabetic rats. J. Ethanopharm. 105:39-46, (2006).
[18] Reinhold, J G. Determination of total protein and albumin in: Standard methods of.
clinical chemistry, edited M Reiner, academic press, New York and London p 88. (1953):
[19] Rifai, N. and Warnick, G.R. eds. Laboratory measurement of lipids, lipoproteins and apoproteins. Washington DC, AACC press. Pp 91-105, (1994).
[20] Schoenheimer, R. And Sperry, W.M. *J. Bioz.Chem.*108:745, (1939).
[21] Shobana, S., Harsha, M.R., Platel, K. Amelioration of hyperglycemia and its associated complications of finger millet (*Eleusine coracana L*) seed coat matter in alloxan-induced diabetic rats. *Br. J. Nutr.* 104:1787-95, (2010).
[22] Singh, P. and Raghuvanshi, R. S. Finger millet for food security. Afri.J. food Sci. 6(4): 77-84, (2012).
[23] Srinivasan, K. In Pasupuleti, V. And Anderson, J. Eds Fenugreek and traditional antibiotic herbs of Indian origin: In Nutraceuticals, glycemic health and type II diabetes. Ames IA Blackwell publishing co. Pp 311-78, (2008).
[24] Subhash, B. K. and Hathan, B. S. Finger millet processing; Review. Int. J. Agric. Inno. and Res.3(4): 1003-1008, (2014).
[25] Shobana, S, Harsha, MR, Platel, K. *et al.* Amelioration of hyperglycaemia and its associated complications by finger millet (*Eleusine coracana* L.) seed coat matter in alloxan-induced diabetic rats. *Br J Nutr* 104:1787–95, (2010).
[26] Stephen P. Current trends in dietary management of diabetes mellitus and its complications. J. Postgrad. Med. 11(1):108-111, (2009).
[27] Weatherburn, M. W. Phenol hypochlorite reaction for determination of ammonia. Anal. Chem. 39: 971- 974, (1967).
[28] Wild, S., Roglic, G., Green, A., Sicree, R., and King, H. Global Prevalence of Diabetes: Estimates for year 2000 and projection for 2030. *Diabetes care* 27 (5): 1047-1053, (2004).