Effects of 'ZPC' Polyherbal Formulation on Diabetic-Dyslipidemic Wistar Rats

ABSTRACT

In this study the antidiabetic effect of a polyherbal formulation- ZPC was investigated in Wistar rats. Diabetes was induced by intraperitoneal injection of streptozotocin at a dose of 50 mg/kg. Rats having Fasting Blood Sugar (FBS) level above 250mg/dl after 72 hrs were considered diabetic and used for the studies. Five rats served as non- diabetic control (Group 1) while twenty diabetic rats were randomized into 4 groups of 5 rats each. The four groups (Groups 2,3,4 and 5) received 1ml (diabetic control, 250 mg/kg chlorpropamide and ZPC at doses of 250 and 500ng/kg respectively for 28 days. During the treatment period, the FBS and bodyweight of rats were monitored weekly and on day 28, the rats were euthanized and blood samples collected for serum lipid profile analysis. Results obtained indicated that following administration of streptozotocin, there was a significant ($p<0.05$) increase in the FBS, total cholesterol, triglycerides and LDL concentration with a corresponding significant ($p<0.05$) decrease in HDL concentration compared to non- diabetic control. However, following the treatment with the polyherbal formulation, there was a significant ($p<0.05$) reduction in the FBS level and a significant ($p<0.05$) increase in the body weight of rats compared to the diabetic control. The polyherbal formulation also produced a significant ($p<0.05$) reduction in total cholesterol, triglycerides and LDL concentration with a corresponding significant ($p<0.05$) increase in HDL concentration compared to diabetic control. It was concluded that, ZPC might serve as a good alternative or as an adjunct to the oral hypoglycaemic agents in the management of diabetes.

Key words: Polyherbal, Formulation, Diabetic, Dyslipidemic, Wistar Rats

INTRODUCTION

Diabetes mellitus is chronic metabolic disorders that affect human body in terms of physical, psychological and social health. It is defined as a group of disorders characterized by hyperglycemia, altered metabolism of lipids, carbohydrates and proteins [1], [2]. It is becoming the third "killer" of the health of mankind along with cancer, cardiovascular and cerebrovascular diseases [3]. Among all the cases of diabetes, type 2 diabetes was found to be more prevalent [4]. Knowledge about diabetes mellitus existed in ancient Egypt and Greece. The word "diabetes" is derived from the Greek word "Diab" (meaning to pass through, referring to the cycle of heavy thirst and frequent urination); "mellitus" is the Latin word for "sweetened with honey" (refers to the presence of sugar in the urine) [2]. According to ancient Hindu physicians, "Madhumeha" is a

41   disease in which a patient passes sweet urine and exhibits sweetness all over the body, such as in

42   sweat, mucus, breath, and blood. It was recommended that the low carbohydrate diet and almost

43   total withdrawal of animal fats should be taken by the patients suffering from Madhumeha,

44   whereas obese adults should live on low calorie diet.

45   There are two major types of Diabetes: Type 1, previously known as "Juvenile onset diabetes

46   mellitus" (Insulin dependent diabetes mellitus), is hereditary and is managed via insulin

47   injection, and Type 2, "Adult type" previously known as non-insulin dependent diabetes

48   mellitus, occurs mostly in elderly people and is usually, managed via life style modification and

49   the use of oral hypoglycemic drugs [2].

50

51   Plants have always been a good source of drugs. The ethno-botanical information reports about

52   800 plants that may possess anti-diabetic potential [5], [6]. The beneficial uses of medicinal plants

53   in traditional system of medicine of many cultures are extensively documented. Several plants

54   have been used as dietary adjuvant and in treating the number of diseases even without any

55   knowledge on their proper functions and constituents. This practice may be attributed to the

56   uncompromised cost and side effects of synthetic hypoglycemic agents [4]. Although numerous

57   synthetic drugs were developed for the treatment of diabetes mellitus but the safe and effective

58   treatment paradigm is yet to be achieved. Medicinal foods are prescribed widely even when their

59   biologically active compounds are unknown, because of their safety, effectiveness, and

60   availability [7]. The World Health Organization (WHO) has recommended the evaluation of

61   traditional plant treatments for diabetes as they are effective, non-toxic, with less or no side

62   effects and are considered to be excellent candidates for oral therapy [8].

63   Polyherbal extracts, which are combinations of different herbal extracts/fractions, are also used

64   for the treatment of diseases. Many people believe that polyherbal extracts are just effective as

65   drugs. Herbalists suggest that nature provide other ingredients that may act as buffers, synergists

66   or counterbalances, working in harmony with the more powerful ingredients. Therefore, by using

67   herbal combination in their complete form, the body's healing process utilizes a balance of

68   ingredients provided by nature [9]. In this study one of such polyherbal formulations, ZPC has

69   been evaluated for anti-hyperglycaemic and hypolipidemic properties. ZPC is made from the

70 aqueous extracts of *Zingiber officinale* (Ginger) and the leaves of *Phyllantus spp,* and *Camellia*

71 *sinensis*.

72 *Zingiber officinale* commonly referred to as Ginger is widely used around the world as a spice. It

73 is also widely used in traditional alternative medicine in the treatment and management of

74 various disorders including catarrh, rheumatism, nervous diseases, gingivitis, toothache, asthma,

75 stroke, constipation and diabetes [10], [11]. *Phyllantus* spp is widely cultivated in Africa. Its parts

76 are considered to have antibiotic properties and also useful in the treatment of hemorrhage,

77 diarrhoea, dysentery, anaemia, jaundice, diabetes, fever, dyspepsia, bronchitis and cough [12].

78 *Camellia sinensis* commonly known as tea plant is probably the most widely consumed beverage

79 in the world [13]. Even though the tea plant is cultivated all over the world, it grows best in

80 tropical and subtropical areas with adequate rainfalls, good drainage, and a slightly acidic soil

81 [14].

82 2.0 MATERIALS

83 2.1 Collection and Identification of Plant Materials

84 The plant materials were collected from Ajaka, Igalamela/Odolu Local Government Area of

85 Kogi State, Nigeria. The identities of the five plants were confirmed at the Herbarium unit of the

86 Department of Biological Sciences, Ahmadu Bello University, Zaria, as *Zingiber officinale*

87 (Voucher No.2261), *Phyllantus* spp (Voucher No. 900351) and *Camellia sinensis* (Voucher

88 No.217).

89 2.2 Preparation of Aqueous Extract

90 The leaves were rinsed with distilled water and shade- dried for 5 days and thereafter pulverized,

91 using electric blender. The crude powders obtained from the plants materials were mixed in the

92 following proportion: *Zingiber officinale* (500g), *Phyllantus spp* (1000g) and *Camellia sinensis*

93 Stem bark (500g) and extracted with 5000 ml (5L) of distilled water. After 48 hours, the mixture

94 was filtered using muslin sieve followed by Whatmann filter paper (No 1). The filtrate was then

95 dried and the extract was stored in the refrigerator for subsequent analysis. The extract will

96 henceforth be referred to as ZPC.

97 2.3 Chemicals and Materials

Chlorpropamide (Diabenese) was purchased locally, Streptozotocin was purchased from the country representative of Sigma Chemical, St. Loius USA while a digital glucometer and corresponding test strips (ACCU- CHECK) were purchased from a pharmacy store. All other chemicals used were of analaR-grade and obtained commercially.

2.4 Animals

Twenty Male Wistar rats weighing between 120-200g were used for this study. They were fed daily with growers mash diet and were given free access to water, during the experimental period. The food and water were replaced each day except on days prior to testing for their fasting glucose level. The rats were housed in well ventilated plastic cages which were cleaned once in three days, with naturally illuminated condition of 12 hour light and 12 hour dark.

2.5 Experimental Design

2.5.1 Acute toxicity study

The oral median lethal dose (LD_{50}) of the extract was determined in rats according to the method described by [15]. The study was carried out in two phases. In the first phase, nine rats were randomized into three groups of three rats which were given 10, 100, and 1000mg extract/kg body weight. The rats were kept under the same conditions and observed for signs of toxicity which included but were not limited to paw- licking, stretching, respiratory distress and mortality for the first 4h and thereafter daily for two weeks. Based on the results of the initial phase, the following doses- 1600, 1900 and 5000mg extract/kg body weight were administered to another set of three groups of three rats in the second phase. These rats were also monitored closely for the first 4 h after treatment and subsequently daily for 4 days for signs of toxicity and/or mortality. The results obtained in the second phase were used to calculate the LD_{50}.

2.5.2 Induction of diabetes

The animals were injected intramuscularly with a single dose of 50mg/kg of the body weight streptozotocin. Diabetes was confirmed by the presence of fasting plasma glucose level above 250mg/dl on the third day post administration of streptozotocin.

2.5.3 Grouping and treatment

Twenty five (25) diabetic rats were weighed and randomly divided into five (5) groups of five rats each and treated daily for 28 days as follows:

Group I (Non- diabetic control): Normal saline only

Group II: (Diabetic control)   Normal saline only

Group III: 250mg/kg b.w of chlorpropamide (an anti-diabetic drug)

Group IV: 250 mg/kg b.w of ZPC

Group V: 500 mg/kg b.w of ZPC

2.5.4 Assay of Fasting Blood Glucose Level

The ACCU-CHEK Glucometer with its corresponding test strips was used to determine the fasting blood glucose levels of the rats.

2.5.5 Estimation of Body Weight

The body weight of the rats was monitored weekly throughout the duration of the study.

2.5.6 Preparation of plasma for assays

After 28 days, the animals were fasted for 12 hours (overnight), after which they were sacrificed by cervical dislocation and blood collected by cardiac puncture using 5 ml syringes. A portion of the blood was dispensed into EDTA anticoagulant bottles for the estimation of haematological parameters (like pack cell volume, haemoglobin concentration, white blood cell count etc) using an automated haemoglobin machine. Another portion of the blood was dispensed into plain bottles, allowed to clot and centrifuged at 3600rpm for 15 minutes and the clear sera aspirated off using a Pasteur pipette and stored at $- 4^0$c in a refrigerator.

2.6.1.1Assay for serum total cholesterol:

The serum level of total cholesterol was quantified after enzymatic hydrolysis and oxidation of the sample as described by the method of [16].

2.6.1.2 Assay for serum triglyceride:

The serum triglyceride level was determined after enzymatic hydrolysis of the sample with lipases as described by the method of [17]

2.6.1.3 Assay for serum high density lipoprotein cholesterol:

The serum level of HDL-C was estimated by the method of [18].

2.6.1.4 Determination of serum low-density lipoprotein cholesterol:

The serum level of (LDL-C) was calculated according to the method of [19] using the equation below:

LDL-C = Tchol - TG/5 - HDL-C

2.7   Statistical Analysis

Statistical analysis was carried out using SPSS version 20.0. All the data were expressed as mean ± SEM and the statistical differences between the means were determined by one way analysis of variance (ANOVA) which was followed by Duncan test and difference between means at $P > 0.05$ were considered significant.

3.0 RESULTS

3.1 Acute Toxicity Study

The results of acute toxicity studies showed no mortality or physical changes in skin and fur, eyes and mucus membrane, respiratory rate, circulatory signs, autonomic and central nervous system effects up to a dose of 5000 mg/kg of ZPC. The oral LD_{50} of the extract was then taken to be > 5000 mg/kg.

3.2 Effect of ZPC on Fasting Blood Sugar (FBS) (mg/dl) of Streptozotocin Induced Diabetic Albino Rats

The effect of the polyherbal formulation and chlorpropamide on the FBS of diabetic Wistar rats is presented in Table 1. Administration of Streptozotocin significantly (P<0.05) elevated the FBS as seen on day 0 when the diabetic control and treatment groups are compared to the non-diabetic control. When compared to each other there was no significant (P>0.05) difference between the groups. Treatment with chlorpropamide and ZPC showed time and dose- dependent significant (P< 0.05) reduction in FBS on days 7, 14, 21 and 28 compared to Group 2 (diabetic control).

Table1: Effect of ZPC on Fasting Blood Sugar (FBS) (mg/dl) of Streptozotocin- Induced Diabetic Albino Rats

Treatment	FBS (mg/dl)				
	DAY 0	DAY 7	DAY 14	DAY 21	DAY 28
NDC (1ml dist. H_2O)	83.42 ± 3.65^a	86.1 ± 4.18^a	83.6 ± 6.33^a	81.5 ± 5.32^a	87.3 ± 4.51^a
DC (1ml dist. H_2O)	368.5 ± 77.24^c	375.4 ± 92.71^c	392.6 ± 90.76^c	373.4 ± 84.24^c	386.4 ± 77.32^c
CHLOR (250 mg/kg)	348.4 ± 67.25^c	300.4 ± 61.23^b	290.4 ± 73.45^b	289.2 ± 73.35^b	279.3 ± 62.33^b
ZPC (250 mg/kg)	352.2 ± 71.21^c	325.4 ± 58.29^{bc}	320.2 ± 43.13^{bc}	273.4 ± 31.32^b	253.0 ± 45.01^b
ZPC (500 mg/kg)	349.5 ± 45.11^c	275.2 ± 41.46^b	207.2 ± 53.45^{ab}	187.1 ± 45.23^{ab}	161.3 ± 36.44^{ab}

DC= diabetic control, NDC= non- diabetic control, CHLOR= Chlorpropamide, Data are presented as mean ± SD of FBS. Data was analysed by one- way ANOVA followed by Duncan post- hoc test for multiple comparisons, (n=5). Mean values having different lower case alphabets as superscripts are considered significant (p< 0.05) across the columns

193

194

195

3.3 Effect of ZPC on Body weight BW) (g) of Streptozotocin Induced Diabetic Albino Rats

Table 2 shows the effect of PZC and Chlorpropamide on the body weight of the Streptozotocin - induced diabetic rats. Following alloxan administration, the body weight of rats in the treatment groups was significantly ($p < 0.05$) reduced compared to the non- diabetic control. The body weight of rats in the treatment groups showed no statistically significant ($P > 0.05$) difference on days 7 and 14compared to diabetic control. However, there was no significant ($P < 0.05$) difference between ZPC- treated and chlorpropamide- treated the groups when compared to the nondiabetic control on days 21 and 28.

Table 2: Effect of ZPC on Body weight (BW) (g) of Streptozotocin Induced Diabetic Albino Rats

Treatment	BW (g)				
	DAY 0	**DAY 7**	**DAY 14**	**DAY 21**	**DAY 28**
NDC (1ml dist. H$_2$O)	162.2 ± 11.33^b	168.2 ± 13.46^b	172.7 ± 12.15^b	175.5 ± 11.19^b	179.4 ± 14.52^b
DC (1ml dist. H$_2$O)	133.2 ± 21.44^a	135.3 ± 30.21^a	130.1 ± 25.66^a	131.9 ± 11.51^a	139.2 ± 26.77^a
CHLOR (250 mg/kg)	139.3 ± 26.42^a	141.2 ± 15.26^a	144.1 ± 26.77^a	155.4 ± 22.33^{ab}	159.2 ± 99.23^{ab}
ZPC (250 mg/kg)	140.1 ± 12.78^a	139.6 ± 25.44^a	145.4 ± 19.16^a	151.3 ± 19.44^{ab}	158.2 ± 19.16^{ab}
ZPC (500 mg/kg)	138.9 ± 34.33^a	142.3 ± 9.97^a	143.3 ± 12.12^a	150.7 ± 11.14^{ab}	159.4 ± 15.44^{ab}

DC= diabetic control, NDC= non- diabetic control, CHLOR= Chlorpropamide, Data are presented as mean ± SD of body weight (g). Data was analysed by one- way ANOVA followed by Duncan post- hoc test for multiple comparisons, (n=5). Mean values having different lower case alphabets as superscripts are considered significant ($p < 0.05$) across the columns.

211

212

213

3.4 Effect of ZPC on Serum Lipid Profile of Streptozotocin Induced Diabetic Albino Rats

Table 3 shows the effect of treatment with ZPC and chlorpropamide on the serum lipid profile of the Streptozotocin - induced diabetic rats. Streptozotocin n caused a significant ($p<0.05$) elevation in total cholesterol, triglycerides and LDL concentrations and a corresponding significant ($p< 0.05$) difference in HDL concentration compared to non-diabetic control. ZPC at 250 and 500mg/kg and Chlorpropamide produced a significant ($p< 0.05$) decrease in the concentrations of cholesterol, triglyceride and LDL and a significant ($p< 0.05$) increase in the HDL concentration compared to diabetic control. The action of chlorpropamide and ZPC were comparable.

Table 3: Effect of ZPC on Serum Lipid Profile of Streptozotocin Induced Diabetic Albino Rats

Treatment	Tchol (mg/dl)	TAG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
NDC (1ml dist. H_2O)	86.43 ± 11.23^a	74.43 ± 5.37^a	47.11 ± 2.44^c	24.20 ± 3.44^a
DC (1ml dist. H_2O)	160.43 ± 88.44^c	140.56 ± 8.45^c	13.33 ± 5.66^a	118.55 ± 44.34^c
CHLOR (250 mg/kg)	110.34 ± 16.33^b	85.26 ± 7.77^{ab}	39.41 ± 6.87^b	53.88 ± 2.45^b
ZPC (250 mg/kg)	117.14 ± 13.11^b	100.45 ± 11.55^b	37.24 ± 3.23^b	59.81 ± 6.33^b
ZPC (500 mg/kg)	115.48 ± 8.66^b	91.21 ± 5.72^{ab}	30.55 ± 2.16^b	66.68 ± 8.45^b

DC= diabetic control, NDC= non- diabetic control, CHLOR= Chlorpropamide, Data are presented as mean ± SD of body weight (g). Data was analysed by one- way ANOVA followed by Duncan post- hoc test for multiple comparisons, (n=5). Mean values having different lower case alphabets as superscripts are considered significant ($p< 0.05$) across the columns.

4.0 DISCUSSION

Diabetes mellitus is possibly the world's highest metabolic disorder, and as knowledge of its heterogeneity is advancing, the need for more appropriate therapy increases [20]. This disease

232 causes many chronic complications such as vascular disease, retinopathy, neuropathy, kidney
233 disease and heart disease. There is an increase demand to use natural products (herbs) with anti-
234 diabetic activity due to the side effects associated with the use of insulin and oral hypoglycaemic
235 agent [21]. The available literature shows that there are more than 1000 plant species showing
236 hypoglycaemic activity [22]. In order to mimic the diabetic state Streptozotocin (50mg/kg) was
237 used to induce albino rats intramuscularly as experimental models. Streptozotocin is known to
238 selectively destroy the β-cells of the islet of Langerhans of the pancreas that functions in the
239 regulation of insulin secretion and thus leads to increase in the blood concentration of glucose
240 and type 1 diabetes mellitus [23]. Hence, there was evident hyperglycaemia (250-600 mg/dl)
241 consequent to establishing the diabetic state in the animals.

242 The result of this study shows that the polyherbal formulation exhibited time and dose-
243 dependent effect on the FBS of the rats. The anti-hyperglycaemic activity of the polyherbal
244 formulation might be due to the high antioxidant content of the component plants. It has been
245 reported that the co-administration of ethanolic leaf extract of Moringa oleifera and metformin
246 can be useful in ameliorating symptoms of diabetes in alloxan-induced diabetic rats [24]"ZPC
247 might have also produced anti-hyperglycaemic activity through direct release of insulin by
248 inhibiting the ATP- sensitive potassium channels in the membrane of the residual beta-cells just
249 like sulfonylureas and meglitinides. It is also possible that the extract might have potentiated the
250 action of insulin to stimulate glucose uptake and utilization by tissues, especially by the liver,
251 skeletal muscle, and adipose tissue [25]. The goal of management of diabetes is to avoid or
252 minimized chronic diabetic complications, as well as to avoid acute problems of hyperglycemia
253 [26]. Hence ZPC might serve as a good alternative or as an adjunct to the oral hypoglycaemic
254 agents.

255 In this study, the body weights of diabetic rats decreased following streptozotocin treatment.
256 This is in agreement with the symptoms of diabetes as stated by [27] to include unexplained
257 weight loss. In diabetes mellitus, the gluconeogenic pathway is activated as a result of the
258 inability of the cells to utilize glucose for energy production. Thus the weight loss in diabetes
259 mellitus is linked to the utilization of muscle protein and excessive mobilization of fats from the
260 adipose tissues for energy production in the gluconeogenic [28]. However, after treatment with
261 chlorpropamide and ZPC, probably with improvement in glucose uptake by cells and subsequent

262 reversal of gluconeogenesis, the body weights of the treated diabetic groups showed a steady
263 increase throughout the course of the experiment.

264 Dyslipidemia which includes not only quantitative but also qualitative abnormalities of
265 lipoprotein plays a significant role in the proatherogenesis of vascular complications in diabetes
266 mellitus [29]. Lowering of serum lipid levels through herbal or drug therapy seems to be
267 associated with a decrease in the risk of vascular disease in diabetes [30]. In this study, following
268 streptozotocin treatment, there was an elevation in serum concentration of total cholesterol,
269 triglyceride, low-density lipoprotein cholesterol (LDL-C) and a decrease in HDL-C in rats. [31],
270 [32] also reported increased plasma cholesterol, triglycerides, LDLC and decreased HDL-C in
271 streptozocin induced hyperglycemic rats. Similar observations were reported by [24], [33], [34], [26].
272 According to [35], the observed increase in serum cholesterol level results from increased
273 intestinal absorption and synthesis of cholesterol. [36] suggested that diabetes-induced
274 hyperlipidemia is attributable to excess mobilization of fat from the adipose due to under
275 utilization of glucose. Insulin deficiency and elevations of the counter-regulatory hormones lead
276 to activation of enzymes (hormone-sensitive lipase) that stimulate lipolysis and enhanced release
277 of free fatty acids from adipose tissues which are mobilized for energy purpose [29]. The excess
278 fatty acids are afterwards accumulated in the liver and converted to triglyceride [37]. The
279 unregulated action of lipolytic hormones on the fat depots is therefore responsible for the
280 hyperlipidemia that characterizes diabetes [30]. In this study, treatment with the polyherbal
281 formulation reduced cholesterol, triglyceride and LDL concentration with a corresponding
282 increase in HDL concentration. This dyslipidemic activity of the plant might be as a result of
283 high phenolic compound of the component plants. Flavonoids are known to increase HDL
284 biosynthesis in the liver and the increase in HDL concentration possibly enhances the excretion
285 of cholesterol. Instead, a decrease in LDL concentration could possibly be due to enhanced
286 reverse cholesterol transport and bile acid excretion through inhibition of apo B production,
287 needed for LDL-C production, transport and binding [38]. Triglyceride concentration is also
288 reduced following treatment with ZPC. ZPC might have acted through a number of ways to
289 achieve this and this include an alteration in the level of interleukin-6 (IL-6) which mediates
290 energy mobilization in the muscles and fat tissues [39].

Polyherbal formulations due to the synergy between the components are potent scavenger of free radicals helpful in combating the progression of various diseases with oxidative stress components such as atherosclerosis, diabetes mellitus among others [40]. This study has lent credence to this statement by proving the effectiveness of ZPC in controlling the hyperglycaemia and dyslipidemia that are usually associated with diabetic conditions. [41] also reported that medicinal plants, individually or as a polyherbal formulation, could be useful in the management of diabetic complications. Hence ZPC might serve as a good alternative or as an adjunct to the oral hypoglycaemic agents in the management of diabetes/ diabetic complications.

5.0 CONCLUSION

In conclusion, ZPC polyherbal formulation may serve as a good candidate for alternative and/or complimentary medicine in the management of diabetes as it possesses anti- hyperglycaemic and anti-dyslipidemic activities.

Ethical Approval:

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

REFERENCES

1. Patalet, C.J. and Watt, E. (2011). Purification and identification of active components of *pobrotus edulis* L. *J. Ethnopharm.* 76: 87-91.

2. Warjet, I.J., Guinko, S., Tuquet, C. and Salle, G. (2011). Mitletoes of the agroforestry parklands of Burkina Faso. *Agroforestry Syst.* 60; 39-49.

3. Chauchan, F.W., Shieh, P., Kuo, D. and Hsieh, C. (2010). Evaluation of the antioxidant activity of *Ruellia tuberosa. Food Chemistry*. 94: 14-18.

4. Balaraman, A., Rial-Sebbag, E. and Knoppers, B.M. (2007). "Trends in ethical and legal frameworks for the use of human biobanks". *European Respiratory Journal* 30 (2): 373–382.

5. Grover, F.K., Malaisse, W.J. and Pipeleers, D.G. (2002). Selective uptake of alloxan by pancreatic B-cells. *Biochem J*; 208:513-515.

321 6. Junget, X., Liang, W., Mao, Y., Li, H., Yang, Y. and Tan H. (2009). Hepatic glucokinase
322   activity is the primary defect in alloxan-induced diabetes of mice. *Biomed Pharmacother*;
323   63:180-186.

324 7. Richard, K.B. (2008). Dietary carbohydrate restriction in type 2 diabetes mellitus and
325   metabolic syndrome: time for a critical appraisal.

326 8. Rosenzweig, S., Reibel, D.K., Greeson, J.M., Edman, J.S., Jasser, S.A., McMearty, K.D. and
327   Goldstein, B.J. (2007). Mindfulness-based stress reduction is associated with improved
328   glycemic control in type 2 diabetes mellitus; 13:36-38.

329 9. Tsao AS, Kim ES, Hong WK. Chemoprevention of cancer. *Cancer Journal*. 2004;54:150-
330 180.

331 10. Wang WH, Wang ZM, Studies of commonly used traditional medicine ginger.(2005)
332   Zhongguo Zhong Yao Za Zhi. 2005;30:1569–1573.

333 11. Tapsell LC, Hemphill I, Cobiac L. et al. Health benefits of herbs and spices: The past, the
334   present, the future. The Medical Journal of Australia. 2006;185 (Suppl. 4): S4–S24.

335 12. Unander DW, Vankates PC, Waran I. et al. *Phyllantus* species: Sources of new antiviral
336   compounds. In: J Simons (eds). Advances in new crops. Timber press, Portland; 1990.

337 13. Muktar H, Ahmad N. Tea polyphenols: Prevention of cancer and optimizing health.(2000)
338   *Am. J. Clin. Nutr.*;71:1698S 1702S.

339 14. Graham HN. Tea. In: Wiley encyclopedia of food science and technology. 2nd ed. Frederick
340   JF, Ed. John Wiley & Sons: New York. 1999;1-4:2292-2305.

341 15. Lorke D. A new Approach to Practical Acute Toxicity Testing. *Archives of Toxicology* .
342   1983; 54(4): 275-287.

343   16. Crook, M. A (2006). Clinical Chemistry and Metabolic Medicine. 7th Edn. Hodder Arnold,
344   London: p 426.

345

346 17. Jerry, R. M, Christina N. H. and Paul, F. S. (2010). *Techniques in organic chemistry*. Third
347   Editn. W. H. Freeman and Company, pp 176

348 18. Tyrberg, B., Andersson, A. and Borg, L.A. (2001). "Species Differences in Susceptibility of
349   Transplanted and Cultured Pancreatic Islets to the β-Cell Toxin Alloxan". *General and
350   Comparative Endocrinology* **122** (3): 238–251.

351   19. Wassan, K. M., Najafi, S., Wong, J. and Kwong, M. (2001). Assessing plasma lipid levels,
352   body weight, and hepatic and renal toxicity following chronic oral administration of a water
353   soluble phytostanol compound FM-VP4, to gerbils. *Journal of Pharmaceutical Science,* **4**(3):
354   228-234.

355 20. Murthly, K.B., Nammi, S., Kola, M.K., Roa, K.R.V., Rao, K.N. and Annapuma, A. (2004).
356   Evaluation of Hypoglyceamic and Anti-hypoglyceamic effect of Dasura metel (linn)

seeds in normal and alloxan–induced Diabetic rats. Journal of Ethnopharmacology 91: 95-98

21. Kameswara, M.A., Mathew, L. and Radha, A. (1997). A report on the antioxidant activities of leaves and rhizomes of *Costus pictus* D. Don. *International Journal of Integretive Biology*. 5(1): 20-26.

22. Rai, S.A., Larijani, B., Akhoondzadeh, S., Fakhrzadeh, H., Dastpak, A., Rezai, A., Bandarian, F., Badi, H.N. and Emami T. (1994). *J. Ethnopharmacol*., 14, 202.

23. Eleazu, H., Peschke, D., Bromme, H.J., Morke, W., Blume, R. and Peschke, E. (2000). Influence of melatonin on free radical-induced changes in rat pancreatic beta-cells in vitro. J Pineal Res; 28: 65-72.

24. Idakwoji, P. A., Akuba, O. B. and Elah, O. S. (2015) Co-Administration of Ethanolic Leaf Extract of *Moringa Oleifera* and Metformin Reverses Polyphagia, Polydipsia and Stabilizes Body Weight in Alloxan- Induced Diabetic Rats; *Global J Res. Med. Plants & Indigen. Med.* (9) 193–202

25. Gerich, J. E. (2000). Physiology of glucose homeostasis. *Diabetes Obesity and Metabolism* .2: 345–350

26. Idakwoji, P. A., Okafor, S. C., Akuba, O. B., Ajayi, O. I., Hassan, S. M. (2016). *In vivo* and *in vitro* comparative evaluation of the anti-diabetic potentials of the parts of *Moringa oleifera* tree. *European Journal of Biotechnology and Bioscience*; 4 (1); 14-22

27. American Diabetic Association. (2000). Standards of medical care in diabetes. *Diabetes Care*. 33 (1), S11–S61

28. Champe, P., Harvey, R and Ferrier, D. (2005). *Lipid metabolism*. Lippicott's Illustrated Review: Biochemistry. Indian edition, Jaypee Brothers: Medical Publisher (P) Ltd.: Pp 171– 217.

29. Rotimi, S. O., Omotosho, O. E. and Roimi, O.A. (2011). Persistence of acidosis in alloxan induced diabetic rats treated with the juice of *Asystasia gangetica* leaves. *Phcog. Mag.,* 7: 25-30.

30. Claudia, E. N. M., Julius, E. O., Dagobert, T. and Etienne, D., (2006) Antidiabetic and hypolipidemic effects of *Laportea ovalifolia* (*Urticaceae*) in alloxan-induced diabetic rats. *Afr. J. Trad. Complementary Alternative Med*., 3: 36-43.

31. Daisy, P., Lily, V. and Cecelia, E. P. (2009) Comparative Studies on the Different Leaf Extract of *Elephanto pusscaber. L.* on Streptozocin-Induced Diabetic Rats. *European J. Scientific Res*. 32: 304-313.

32. Eze, E. D., Mohammed, A. Musa, K. Y. Tanko, Y. and Isa A .S. (2012) Effect of ethanolic leaf extract of *Mucuna pruriens* (*fabaceae*) on lipid profile in alloxan-induced diabetic Wistar rats. *British Journal of Pharmacology and Toxicology* 3: 102- 109.

393 33. Idakwoji, P. A., O. A. Salawu, B. B. Maiha, I. Obidike and A. Y. Tijani (2015). Co-
394   administeration of Ethanolic Leaf Extract of *Moringa oleifera* and Metformin Improves
395   Glucose, Lipid and Protein Profiles of Diabetic Wistar rats. *Biokemistri;* 27 (3) 123–138

396 34. Idakwoji, P. A., Salawu, O. A., Maiha, B. B., Obidike, I., Tijani, A. Y. (2015) Assessment
397   of Biochemical and Histopathological Changes in Diabetic Wistar Rats Co-Administered
398   Ethanolic Leaf Extract of *Moringa Oleifera* And Metformin. *International Journal of*
399   *Biological & Pharmaceutical Research*; 6(12): 1008-1019

400 35. Mathe, J. D., (1995). Dyslipidemia and diabetes animal models. Mettab., 21: 106.

401 36. Nimenibo-uadia, R., (2003). Effect of aqueous extract of Canavalia ensiformis seeds on
402   hyperlipidemic and hyperkotonaemia in alloxan-induced diabetic rats. Biokemistri. 15: 7-15.

403 37. Suryawanshi, N. P., A. K. Bhutey, A. N. Nagdeote, A. A. Jadhav and G. S. Manoorkar,
404   (2006) Study of lipid peroxide and lipid profile in diabetes mellitus .Indian J. Clinic.
405   Biochem., 1: 126-130.

406 38. Libby, P. (2011). Current concepts of the pathogenesis of the acute coronary syndromes.
407   Circulation 104: 365-372.

408 39. Oyewo, E. B and Akanji, M. A. (2010) Immune modulation potentials of aqueous extract of
409   Andrographis paniculata leaves in male rat. Researcher. 5: 928–935.

410 40. Idakwoji, P. A., Akuba, O. B. and Okafor, S. C. (2016). Comparative Anti-radical Activity of
411   Five Indigenous Herbal Plants and their Polyherbal Extract *International Journal of*
412   *Biochemistry Research & Review;* 11(1): 1-10

413 41. Idakwoji, P. A. and Uzuazokaro, M. M. A. (2018). Anti- radical and Inhibitory Effect of
414   some Common Nigerian Medicinal Plants on Alpha Glucosidase, Aldose Reductase and
415   Angiotensin Converting Enzyme: Potential Protective Mechanisms against Diabetic
416   Complications. *Int. J. Adv. Res. Biol. Sci.* 5(3): 188-201.
417