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RESEARCH ARTICLE

STREPTOZOTOCIN INDUCED DIABETES: BIOCHEMICAL PROPERTIES IN HERBS TREATED WISTAR ALBINO RATS

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ABSTRACT

The effect of aqueous extract of two herbs *Tapinanthus bengwensis* and *Oscimum gratissimum* treatment on Wistar Rats induced with diabetic streptozotocin (Zanosar) have been investigated. Their hypoglycemic properties were evaluated after treatment of separate groups of adult male and female rats weighing between 240-300g with 5% and 10% aqueous extract of the herbs for 16 days resulted in significant ($p<0.05$) reduction of fasting blood glucose (FBG) and glycated heamoglobin (HbA1c). Other paramaters investigated were insulin, glutathione (GSH) creatinine and urea. Further analysis of data with a scatter plot of individual groups according to their FBG and HbA1c shows a positive linear correlation and the linear regression had a coefficient of $r=0.69$ significant at 0.01 level ($r=0.69$, $p<0.01$). Histological examination of the kidney and pancrease pre and post induction were studied. We deduced an improvement from the weight status. Treatment with *T.bengwensis* improved GSH, insulin and creatinine levels appreciably suggesting possible credence for its efficacy in management of diabetes mellitus specifically as it has to do with protection of red blood cells and kidney functions.

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INTRODUCTION

The abundance of several herbs in our environment and the realization that they possess active ingredients with therapeutic values has made the need for their study imperative. A scientific scrutiny of the enthnobotanical information, chemical constituent and the therapeutic application of the plant potend great opportunities for humanity. Diabetes mellitus is a complex metabolic disorder characterized by chronic hyperglycemia due to dynamic interactions between varying defects of insulin secretion and action. As identified by Odia, *et al.* (1992) the burden of this non communicable disease on global morbidity and mortality has gained serious concerns. It is now known that diabetes is a leading causes of stroke, kidney failure, blindness, loss of libido and foot ulcer. Arising from this, the need for the management of diabetic patient through assessement of medication monitoring and education has become imperative. It is now known that glycation (non-enzymatic glycosylation) occurs frequenly in diabetes. Works carried out by Brown lee *et al*. (1983) and (2001), Standing *et al.* (1992) have educated the need to treat these complications as an integral part of the clinical stratification of diabetic patients. Several prospective studies have been carried out which showed that intensive blood glucose control can effectively reduce the various complications associated with diabetes. Wincor (2003) has elucidated a growing body of evidence to conclude that tight blood glucose control is possible. As shown by Buciarelli *et al.* (2005), there is evidence to support the fact that inhibition of advanced glycated end product (AGEs) formation of their downstream signaling pathway may be a promising strategy for treatment of diabetic patients. There are ongoing trials on several herbs to identify substances that may be able to prevent or reverse the diabetic process and the prospects are high. Some authors have earlier reported the antidiabetic and hypoglycemic properties of African mistletoe. Obatomi *et al.* (1994) and Didem *et al.* (2005) have both reported on the efficacy of mistletoe in the management of diabetes. Swanston Flatt *et al.* (1989) had earlier reported a reduction in some clinical parameters associated with diabetes. The current research effort determines the properties of the named herbs and compares their efficacy in induced diabetes.

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MATERIALS AND METHOD

Animals and Chemicals

Wistar strains of albino rats weighing between 240-300g derived from a colony maintained at the animal house at the Department of Biochemistry, University of Port Harcourt, Nigeria were used for the whole experiment. Twenty rats were used to determine the number of days in which streptozotocin induced diabetic rat was stable. Ten rats were used to determine the hypoglycemic properties of the reference drug daonil (Glibenclamide). Seventy two rats were used to determine the biochemical properties of *Tapinanthus bengwensis* and *Ocimum gratissimum* aqueous extract on streptozotocin-induced diabetic rats. Streptozotocin (zanosar) was obtained from Sigma Chemical Company, St. Louis M.O. USA.

Experimental procedure

Induction of diabetes was achieved through the intraperitoneal injection of Streptozotocin (STZ) 70mg/kg body weight dissolved in 1.0ml/l citrate buffer p^H 4.5 for two days. The animals were considered diabetic when the blood glucose values exceeded10.0mol/l (range 10.0mmol/l -24.3mmol/l) two weeks after STZ induction.

Assay methods

The fasting blood glucose (FBG) was determined by the glucose oxidase method. Glycated hemoglobin HbA1c was determine by ion-exchange high performance liquid chromatography (HPLc-

Esi/ms) approach with UV detection. The Assay method for GSH included monitoring and evaluating the conjugation of CNDB with GSH at various concentration of drug extract at 340 nm and the optical density after every minute was measured. Radioimmunoassay was used for the measurement of insulin while alkaline picrate method was used to determine creatinine and read at 540nm. Urea was determined by the diacetyl monoxime method.

RESULT

A total of five parameters were evaluated to assess the properties of the extracts. Tables 1-5 shows assay result. Whereas values for the control rats were within reference range, marked variations were observed in the other groups. Test results confirmed the fact that higher mean values of FBG level in diabetics (positive) control were resulting effects of the diabetic properties of streptozotocin induction. While the rise in FBG for the diabetic control rats were sustained, that for the control were stable while post treatment values varied. Similar observation were made for glycated hemoglobin (Table 2). Post treatment effects for the other parameters are as shown in the other Tables. Statistical evaluation of data collected were summarized as mean ±SEM. Differences between individual groups were assessed by one way and 2-way analysis of variance with significant level set at $p<0.05$. Pearson Correlation Coefficient was used to determine the efficacious measure of the concurrent validity of the extract. From the tables the correlation of FBG and HbA1c at 0.9588 is very close to perfect. Histological results of the kidney and pancrease are shown elucidating pre and post treatment effects.

Table 1. FBG (mmol/l) Assay values for different groups

Day	Controls	Diabetic control rats	DTR on 10% T.beng	DTR on 10% O.grat	DTR on 5% T.beng	DTR on 5% O.grat	DTR on Daonil
0	4.6±0.03^{a}	10.3±0.03^{b}	18.3±0.06^{c}	16.5±0.05^{d}	14.4±0.05	14.4±0.06	18.3±0.06^{v}
2	4.7±0.03^{d}	10.7±0.03^{e}	16.3±0.03^{f}	16.4±0.03	14.0±0.00	14.3±0.03	16.1±0.11
4	4.4±0.03^{a}	11.4±0.00^{c}	14.2±0.05^{d}	15.3±0.03	13.8±0.03	13.2±0.00	14.2±0.11^{h}
6	4.5±0.03^{a}	13.3±0.08^{f}	12.1±0.04	15.0±0.03^{g}	13.4±0.03	14.2±0.00	12.0±0.15^{l}
8	4.7±0.03^{m}	15.5±0.05^{n}	8.9±0.11	15.0±0.03^{o}	13.0±0.05	14.0±0.00	10.0±0.22^{q}
10	5.1±0.08^{s}	16.4±0.11^{t}	8.0±0.08^{u}	14.8±0.08^{v}	11.1±0.05^{w}	13.6±0.05^{x}	9.3±0.24
12	5.3±0.08^{u}	18.2±0.08^{v}	8.5±0.28	14.4±0.03^{w}	10.4±0.05^{l}	13.3±0.03^{x}	7.0±0.27^{t}
14	4.6±0.12^{a}	22.3±0.08^{b}	6.3±0.00^{e}	14.0±0.00^{f}	10.0±0.06^{g}	13.1±0.13^{a}	5.2±0.11
16	4.7±0.08^{a}	24.3±0.93^{d}	5.6±0.03	13.3±0.03^{g}	7.6±0.05	12.7±0.12	4.4±0.27

Values are mean ± SEM triplicate determination. Values on the same row having the same superscript are not significantly different from each other.

Table 2. HbA1c (%) Assay values for different groups

Day	Controls	Diabetic control rats	DTR on 10% T.beng	DTR on 10% O.grat	DTR on 5% T.beng	DTR on 5% O.grat	DTR on Daonil
0	4.3±0.03^{m}	10.4±0.08^{n}	11.6±0.03	12.5±0.84^{o}	12.0±0.02	11.6±0.02	11.8±0.71^{v}
2	4.4±0.06^{a}	10.0±0.57^{b}	11.0±0.75	12.4±0.02^{c}	11.6±0.04	11.5±0.82	11.5±0.82^{h}
4	4.5±0.05^{a}	12.2±0.10^{t}	10.1±0.05^{u}	11.6±0.04	11.3±0.05	11.5±0.03	11.0±0.77^{w}
6	4.3±0.03^{m}	14.3±0.05^{n}	9.2±0.08^{r}	11.5±0.04s	11.0±0.02	11.3±0.83	10.1±0.82^{w}
8	4.4±0.40^{x}	15.6±0.03^{y}	9.7±0.36^{z}	11.0±0.03^{o}	10.8±0.04	11.0±0.06	10.1±0.82^{q}
10	4.6±0.03^{a}	15.9±0.35^{b}	6.8±0.05^{c}	10.5±0.05^{d}	10.5±0.6	10.8±0.85	6.3±0.84^{f}
12	4.4±0.40^{a}	16.3±0.03^{t}	6.0±0.03^{g}	9.4±0.06	9.4±0.03	10.5±0.06^{h}	5.0±0.88
14	4.4±0.30^{h}	17.4±0.02^{k}	5.5±0.03	9.0±0.04	8.0±0.03	10.0±0.06^{l}	4.2±0.86
16	4.4±0.57	18.3±0.04^{e}	4.0±0.03^{a}	8.9±0.03^{b}	7.2±0.02	9.7±0.04^{c}	3.2±0.42

Values are mean ± SEM triplicate determination. Values on the same row having the same superscript are not significantly different from each other.

DISCUSSION

Several local herbs are being used by the population as alternative therapy for the treatment of diabetes. Most of the herbs have not been subjected to scientific scrutiny to determine their potency. This study has brought to the fore the need to thoroughly examine these herbs for their efficacy. Swanston-Flatt *et al.,* (1989) had earlier reported a reduction in some clinical parameters associated with diabetes after chronic administration of mistletoe (6.25% by weight of diet, lg/400ml infusion in place of drinking water) ameliorated symptoms of polydipsia, hyperphagia and body weight loss in severely hyperglycemic streptozotocin-diabetic mice. However significant decrease of plasma glucose was not demonstrated in this insulin deficient model. In our study, a marked decrease in plasma glucose has been demonstrated. It is possible to explain that species differences in the plant used may have accounted for the variation of results. This work has given credence to the earlier study of Obatomi (1994), Didem *et al.* (2005), and George *et al.* (2006) in which the antidiabetic and hypoglycemic properties were reported. Phytochemical analysis of mistletoe as earlier reported by previous workers (Obatomi 1994; Alessi *et al.* 2003) have attributed the hypoglycemic propertied to the presence of some constituents notably saponins, tannins, lectins and choline derivatives. It was suggests that tannins could account partly for the observed reduction of glucose by the same mechanism that makes them antinutrients. The observed hypoglycemic properties must have been potentiated by the behaviours of the several secondary plants metabolites. It is known that cardiac glycosides, resins, reducing sugars, steroids, coumarines, terpenoides, flavonoids and other plant metabolites including arginine and glutamic acid possess hypoglycemic effect in various experimental animals. The effect of flavanoids quercetin and ferulic acid on pancreatice B-cells leading to their proliferation and secretion of more insulin have been proposed. Kako *et al.* (1997), Okomoto (1970), Mahesh and Menon (2004) and Sri-Balasubashini (2004) have suggested B-cell recovery as the mechanism by which hyperglycemia caused by streptozotocin reduces glucose. As shown by Gray *et al.* (1997, 1998) aqueous extract of mistletoe enhanced insulin secretion and mimicked the effect of insulin on glucose metabolism. Such dual pancreatice and extrapancreatic action would prove to be an important advance on existing therapies used to treat and control diabetes, such as hypoglycemic drugs (which act by either enhancing insulin secretion or by improving the action of insulin). These combined findings even with *T.bengwensis* and *ocimum gratissimum* illustrate the enormous potential of these plants for use as possible dietary adjuncts and the discovery of natural products for diabetes therapy. This work has elucidated antiglycation as a potential hypoglycemic property of *T.bengwensis* and *ocimum gratissimum* and strongly support the work of some early authors. It has been shown by (Buciarelli *et al.* 2002; Naka *et al.* 2004; Vlassara 2005) growing evidence to support that inhibition of advance glycated end-products (AGEs) or blockade of their downstream signaling pathway may be a promising strategy for treatment of patient with diabetics complications. In the present studies, statistical analysis indicates that significant differences at ($p<0.05$) existed between tested groups of rat for various parameters see Tables 1-5. Of particular importance is the value of glycated hemoglobin (HbA1c) which shows a positive linear correlation and the linear regression had a coefficient of $r=0.69$ significant at 0.01 ($r=0.69$, $p<0.01$) see plat 1.

Table 3. Insulin (uU/mol) Assay values for different groups

Day	Controls	Diabetic control rats	DTR on 10% T.beng	DTR on 10% O.grat	DTR on 5% T.beng	DTR on 5% O.grat	DTR on Daonil
0	5.3±0.43[a]	5.8±0.22	0.4±0.18[b]	0.63±0.13	0.5±0.16	0.6±0.12	0.5±0.17
2	5.4±0.22[b]	4.2±0.11	0.6±0.11[c]	0.60±0.15	0.5±0.17	0.6±0.12[f]	0.8±0.18
4	4.8±0.42[a]	4.0±0.12	0.6±0.11[c]	0.7±0.16	0.6±0.14	0.7±0.11	0.9±0.21
6	3.2±0.42[a]	3.0±0.13	1.3±0.13[d]	0.8±0.27[e]	0.7±0.11	0.8±0.13	1.2±0.23[r]
8	4.8±0.58[t]	2.6±0.11[g]	1.5±0.18[a]	0.93+0.12	0.9±0.17	0.8±0.13	1.6±0.22[k]
10	7.3±0.22[w]	1.4±0.12[r]	2.0±0.22	1.1±0.11	0.9±0.17[y]	0.8±0.13	2.3±0.24[t]
12	6.5±0.22[p]	1.0±0.11[q]	2.3±0.11[r]	1.0±0.12[5]	1.0±0.12[5]	0.9±0.11	3.2±0.18
14	6.8±0.27[v]	0.5±0.26[z]	3.0±0.12[5]	1.2±0.15	1.2±0.15	0.9±0.11	3.5±0.16
16	6.7±0.02[t]	0.2±0.17[u]	3.3±0.25[r]	1.3±0.16	1.3±0.16	1.1±0.12	3.8±0.13

Values are mean ± SEM triplicate determination. Values on the same row having the same superscript are not significantly different from each other.

Table 4. Glutathione (GSH) (uU/mol Hb) Assay value for different groups

Day	Controls	Diabetic control rats	DTR on 10% T.beng	DTR on 10% O.grat	DTR on 5% T.beng	DTR on 5% O.grat	DTR on Daonil
0	0.4±0.03[a]	0.32±0.00	0.33±0.00	0.53±0.01[c]	0.35±0.02	0.36±0.11	0.34±0.11
2	0.44±0.00[a]	0.30±0.12	0.44±0.12[a]	0.3±0.02[b]	0.30±0.02[b]	0.35±0.12	0.34±0.12
4	0.42±0.01	0.33±0.01	0.48±0.02	0.37±0.03	0.30±0.00	0.33±0.00	0.4±0.01
6	0.36±0.00[a]	0.30±0.01	0.52±0.02[b]	0.39±0.02	0.28±0.00[c]	0.31±0.01	0.5±0.11[d]
8	0.37±0.00[e]	0.25±0.12[l]	0.55±0.22[g]	0.32±0.02	0.34±0.03	0.33±0.02	0.68±0.17[l]
10	0.41±0.01[w]	0.20±0.13[l]	0.55±0.02[q]	0.39±0.02[r]	0.54±0.02[t]	0.31±0.11[u]	0.93±0.21
12	0.42±0.02[r]	0.15±0.13[s]	0.77±0.02[l]	0.38±0.02[g]	0.52±0.02[l]	0.33±0.01[l]	0.93±0.12
14	0.43±0.00[l]	0.09±0.12[m]	0.82±0.12[n]	0.39±0.02[o]	0.52±0.02[p]	0.32±0.12[q]	1.3±0.11[s]
16	0.44±0.00[k]	0.10±0.11[l]	0.91±0.02[m]	0.39±0.02[n]	0.50±0.02[p]	0.30±0.14[q]	1.5±0.31

Values are mean ± SEM triplicate determination. Values on the same row having the same superscript are not significantly different from each other.

Table 5. Insulin (uMol/l) Assay values for different groups

Day	Controls	Diabetic control rats	DTR on 10% T.beng	DTR on 10% O.grat	DTR on 5% T.beng	DTR on 5% O.grat	DTR on Daonil
0	85±4.4[a]	135±0.85[b]	140±2.3	135+4.2	140±3.3	140±4.2	140±5.3[c]
2	90±5.8[m]	140±4.0[n]	76±0,12[b]	130+4.2[p]	145±4.6	145±4.4	135±2.5
4	95±3.4[r]	139±2.5[s]	145±2.5[t]	135+3.6	145±4.2	145±4.2	130±2.3[v]
6	85±0.87[s]	140±2.0[n]	135±2.2[w]	135+3.8	150±4.5[x]	150±4.5[x]	130±2.5[y]
8	100±0.85[a]	150±2.0[b]	130±2.3[d]	140+3.8[f]	153±3.2	15±33.2	120±2.5
10	95±0.59[x]	145±2.4[y]	130±2.3[z]	130+3.3	150±3.3	150±3.3	100±0.5[o]
12	95±0.85[l]	155±2.2[l]	120±2.0[k]	135+2.5[l]	140±4.2	140±4.2	80±2.4
14	85±0.80[q]	150±3.3[r]	100±2.5[s]	130+3.4	145±5.2[u]	145±5.2[u]	75±2.3
16	85±2.5[a]	165±2.5[t]	110±3.5[u]	125+3.3[v]	135±5.3	153±5.3	60±3.4[x]

Values are mean ± SEM triplicate determination. Values on the same row having the same superscript are not significantly different from each other.

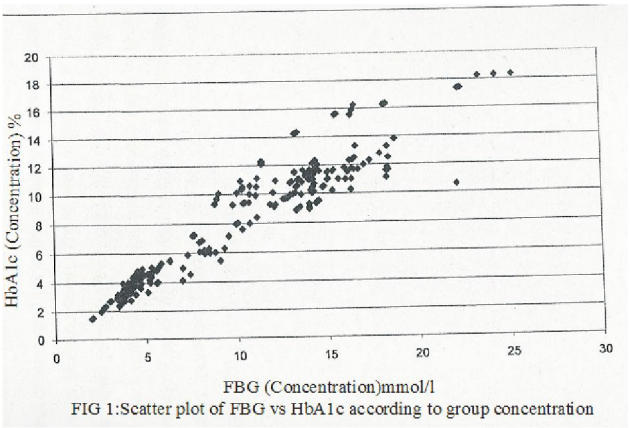
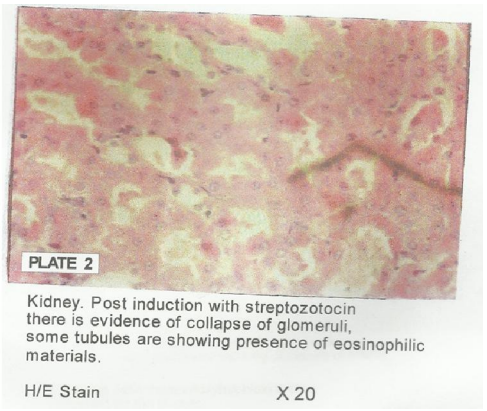
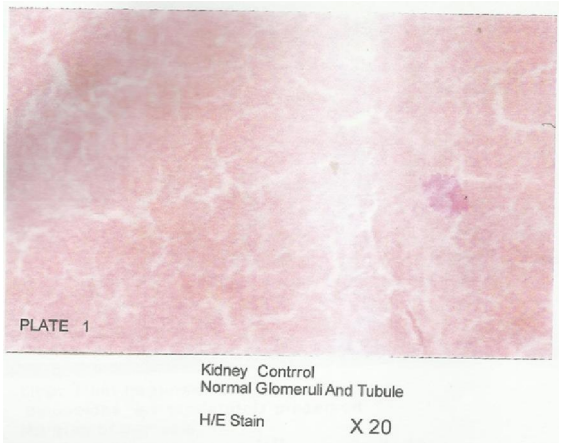
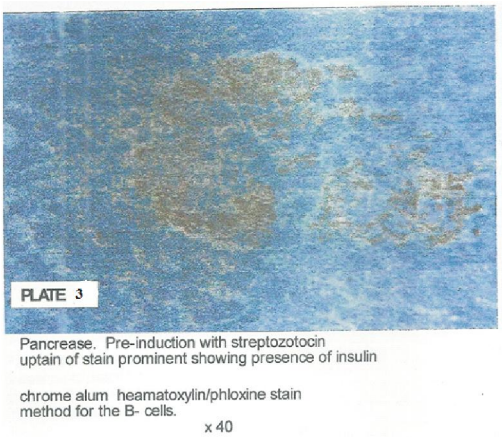


FIG 1:Scatter plot of FBG vs HbA1c according to group concentration







The fact that a significant decrease in both the glucose and HbA1c was noticed after treatment with the extract especially with *T.bengwensis* exhibited a potent hypoglycemic property of the herb. It is possible that the synthesis of HbA1c may represent a model reaction to explain the biochemical basis for many of the long term sequlae of diabetes. The tissues that suffer the most noticeable dysfunction in diabetes (e.g. retina, lens, peripheral nerves, kidney) appear to be insulin independent for glucose uptake. Perhaps in diabetes, intra-cellular proteins of these tissues undergo excess non-enzymatic glycosylation analogous to that seen within red cells. Such glycosylation might alter the enzymatic activity, solubility, antigenicity and other function of protein and thereby result in the observed clinical dysfunction. Again HbA1c has decreased reactivity with 2,3-diphosphoglycerate so red cells of diabetics will have increased affinity for oxygen. This study included the analysis of insulin and GSH parameters to investigate the hypoglycemic properties of the herbs. As shown in tables 3 and 4 respectively for insulin and GSH, a significant ($p<0.05$) was observed for both parameters. While there was an improvement in the insulin concentration, an increase in the concentration of GSH was also noticed.The increased concentration of GSH shows that the redox potentials of the red blood cell and other cells could be enhanced. Reduced concentration of GSH would mean an exposure of the cells to free radicals/antioxidants and thus to oxidant damage with accompanying complications.

An improvement in the creatinine concentration was noticed particularly with the *T.bengwensis* treatment laying credence to the fact that there is a beneficial effect on the kidney. The histology of the tissue revealed moderate recovery especially for the kidney and pancrease. The correlation coefficient done for all tested groups shows that the hypoglycemic properties exhibited by the herbs were both concentration and time dependent with *T.bengwensis* showing stronger potentials.

Conclusion

This study has elucidated the hypoglycemic properties of our local herbs and the need for their further evaluations, modification and use as a means to effective monitoring, control and management of diabetes meallitus.

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