SENRA Academic Publishers, British Columbia
Vol. 7, No. 1, pp. 2223-2226, Feb 2013
Online ISSN: 1920-3853; Print ISSN: 1715-9997

Canadian Journal of pure&applied sciences

Short Communication

GLYCATED HAEMOGLOBIN, GLUCOSE AND INSULIN LEVELS IN DIABETIC TREATED RATS

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ABSTRACT

In assessing the relationship between plasma glucose, insulin and glycated haemoglobin, data for these parameters were obtained after induction of Wistar albino rats with 70mg/kg body weight of streptozotocin which resulted in Type 1 model diabetes. Treatment of the rats with 10% concentration of extract of *Tapinanthus bengwensis* and *Oscimum gratissimum* was carried out for two weeks and the parameters were evaluated and compared. Rats treated with *T. benwgensis* had significant effect of reducing both plasma glucose and glycated haemoglobin (HbA1C) and increasing insulin ($P<0.05$) suggesting a higher hypoglycemic propensity.

Keywords: Glucose, glycated haemoglobin, insulin, streptozotocin, *T. bengwensis*, *O. gratissimum*

INTRODUCTION

The relative abundance of medicinal herbs in our environment and the realization that they possess active ingredients with therapeutic values has made the need for their study imperative. The World Health Organization (WHO) in 1980 released this and harped on the need to carry out scientific scrutiny of most of these herbs to elucidate their chemical constituents and pharmacological potentials by ascertaining efficiency and efficacy of the flora as a potential remedy.

Diabetes is a heterogeneous metabolic disorder characterized by chronic hyperglycemia largely due to the dynamic interactions between varying defects of insulin secretion and action. It is known that hyperglycemia itself has adverse effect on tissue insulin sensitivity and insulin secretion that make it difficult to differentiate between primary and secondary abnormalities. As identified by Ordia and Wokoma (1992) the burden of this non-communicable disease on global morbidity and mortality has gained tremendous recognition. Diabetes mellitus is now known to be a major cause of stroke, kidney failure, blindness, loss of libido etc. Arising from this knowledge, the need for the survival of diabetic patients through assessment of medication, monitoring and education has become imperative. Diabetic patients with raised plasma glucose levels have proportionally more glycation occurring both intracellularly and extracellularly. The works of Brucalla *et al*. (1992), Standing and Tailor (1992), Philips and Floege (1999) have elucidated 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 is possible and could effectively control microvascular complications among diabetics. Wincor (2003), has elucidated a growing body of evidence to conclude that tight blood glucose control is possible. As shown by Buciarelli *et al*. (2002), Naka *et al*. (2004), Melpomeni *et al*. (2003) and Vlassara (2005), there is growing evidence to support that inhibition of advanced glycated end products (AGEs) formation or blockade of their downstream signaling pathway may be a promising strategy for treatment of patients with diabetic vascular complications.

Trials arongoingng on substances that may be able to prevent these processes and possibly even reverse them and the prospects are promising. Some authors have earlier reported the antidiabetic and hypoglycemic properties of African mistletoe. Obatomi *et al*. (1994) and Didem (2005) have both reported on the efficacy of mistletoe in management of diabetes. Swanson Flatt *et al*. (1989) reported a reduction in some clinical parameters associated with diabetes. As reported by Agnaniet *et al*. (2005) and Mohammed *et al*. (2007), *Oscimum gratissimum* has a dose and time dependent effect in hyperglycemic levels of streptozotocin induced diabetic rats. This study examined the response of glucose, insulin and glycated hemoglobin which are recognized as characteristic markers in diabetes mellitus following induction with streptozotocin and treatment with the herbs.

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MATERIALS AND METHODS

Induction of diabetes mellitus was achieved through the intraperitoneal injection of 70mg/kg body weight of streptozotocin dissolved in 1M citrate buffer, p^H 4.5 twice daily for 2 days. A total of 102 rats were used, selected among those that have exceeded glucose threshold (10.0mmol/L) 2 weeks after streptozotocin induction. Samples for fasting plasma glucose, glycated hemoglobin and insulin were collected at the tail vein. Glucose was determined by glucose oxidase method, glycated hemoglobin was determined by high performance liquid chromatography (HPLC-Esi/ms) with UV detection. Insulin was determined by radioimmunoassay. Data was analyzed by two way analysis of variance using SPSS version 6.5

RESULTS

The mean fasting plasma glucose concentration, insulin and HbA1C did not show appreciable change in the control subjects. There were however marked variation in the values of these parameters among the groups following treatment with the extracts. See tables 1,2 and 3 for glucose, HbA1c and insulin respectively.

Metabolic characteristics in control, diabetic rats, diabetic test rats and rats on Daonil following treatment with *T. bengwensis* and *O. gratissimum* are shown.

Table 1. Metabolic characteristics of glucose in control, diabetic rats, diabetic test rates and rats on Daonil following treatment with *T. bengwensis* and *O. gratissimum.*

Day	Controls	Diabetic control rats	10% *T. beng*	10% *O. grat*	DTR on Daonil	DTR on 10% *T. beng* & *O. grat*
0	4.6 ± 0.03^{e}	10.3 ± 0.03^{f}	18.3 ± 0.06^{g}	16.5 ± 0.05^{h}	18.3 ± 0.06^{i}	18.3 ± 0.41^{c}
2	4.7 ± 0.03^{j}	10.7 ± 0.03^{k}	16.3 ± 0.03^{l}	10.0 ± 0.03^{l}	16.1 ± 0.11^{l}	17.0 ± 0.32^{m}
4	4.4 ± 0.03^{n}	11.4 ± 0.00^{q}	14.2 ± 0.05^{s}	15.3 ± 0.03^{t}	14.2 ± 0.11^{u}	15.6 ± 0.33^{v}
6	4.5 ± 0.03^{w}	13.3 ± 0.08^{x}	12.1 ± 0.04^{y}	15.0 ± 0.03^{z}	12.0 ± 0.15^{g}	14.0 ± 0.51^{h}
8	4.7 ± 0.08^{i}	15.5 ± 0.05^{j}	8.8 ± 0.11^{k}	15.0 ± 0.03^{l}	10.0 ± 0.22^{m}	10.7 ± 0.41^{m}
10	5.3 ± 0.07^{k}	16.4 ± 0.11^{l}	8.0 ± 0.08^{m}	14.8 ± 0.08^{n}	9.3 ± 0.41^{v}	8.2 ± 0.25^{s}
12	4.6 ± 0.08^{t}	18.2 ± 0.08^{u}	8.5 ± 0.28^{v}	14.4 ± 0.03^{w}	7.0 ± 0.27^{x}	8.2 ± 0.25^{y}
14	4.6 ± 0.12^{z}	22.2 ± 0.08^{m}	6.3 ± 0.00^{n}	14.0 ± 0.00^{o}	5.2 ± 0.11^{p}	4.4 ± 0.14^{q}
16	4.7 ± 0.08^{r}	24.3 ± 0.93^{s}	5.6 ± 0.03^{t}	13.3 ± 0.03^{s}	4.4 ± 0.27^{v}	3.7 ± 0.15^{u}

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

Table 2. Metabolic characteristics of glycated haemoglobin in control, diabetic rats, diabetic test rats and rats on Daonil following treatment with *T. bengwensis and O. gratissimum.*

Day	Controls	Diabetic control rats	10% *T. beng*	10% *O.grat*	DTR on Daonil	DTR on 10% *T. beng* & *O. grat*
0	4.3 ± 0.03^{a}	11.6 ± 0.03^{b}	12.5 ± 0.84^{b}	12.5 ± 0.84^{b}	11.8 ± 0.71^{b}	13.3 ± 0.51^{c}
2	4.4 ± 0.06^{d}	11.0 ± 0.75^{e}	12.4 ± 0.02^{e}	12.4 ± 0.02^{e}	11.5 ± 0.82^{e}	12.0 ± 0.31^{e}
4	4.5 ± 0.05^{f}	12.2 ± 0.10^{g}	11.6 ± 0.04^{f}	11.6 ± 0.04^{f}	11.0 ± 0.77^{f}	11.0 ± 0.8^{f}
6	4.3 ± 0.03^{h}	14.3 ± 0.05^{i}	11.5 ± 0.04^{j}	11.5 ± 0.04^{j}	10.1 ± 0.82^{k}	9.3 ± 0.21^{l}
8	4.4 ± 0.04^{m}	15.6 ± 0.03^{n}	11.0 ± 0.03^{o}	11.0 ± 0.03^{o}	10.1 ± 0.82^{p}	8.0 ± 0.41^{q}
10	4.6 ± 0.03^{r}	15.9 ± 0.35^{s}	10.5 ± 0.05^{t}	10.5 ± 0.05^{t}	6.3 ± 0.84^{u}	6.2 ± 0.11^{u}
12	4.4 ± 0.04^{v}	16.3 ± 0.03^{w}	9.4 ± 0.06^{x}	9.4 ± 0.06^{x}	5.0 ± 0.88^{y}	5.1 ± 0.23^{y}
14	4.4 ± 0.03^{z}	17.4 ± 0.02^{h}	9.0 ± 0.04^{z}	9.0 ± 0.04^{m}	4.2 ± 0.86^{n}	4.6 ± 0.22^{n}
16	4.4 ± 0.57^{q}	18.3 ± 0.04^{r}	8.9 ± 0.03^{s}	8.9 ± 0.03^{s}	3.2 ± 0.42^{t}	3.0 ± 0.23^{t}

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

Table 3. Metabolic characteristics of insulin in control, diabetic rats, diabetic test rats and rats on Daonil following treatment with *T. bengwensis* and *O. gratissimum.*

Day	Controls	Diabetic control rats	10% *T. beng*	10% *O. grat*	DTR on Daonil	DTR on 10% *T. beng* & *O. grat*
0	5.3 ± 0.43^{a}	5.8 ± 0.22^{a}	0.4 ± 0.18^{b}	0.63 ± 0.13^{b}	0.5 ± 0.17^{b}	0.5 ± 0.12^{b}
2	5.4 ± 0.22^{c}	4.2 ± 0.11^{d}	0.6 ± 0.11^{e}	0.6 ± 0.15^{e}	0.8 ± 0.18^{e}	0.5 ± 0.13^{f}
4	4.8 ± 0.42^{g}	4.0 ± 0.12^{h}	0.6 ± 0.11^{r}	0.7 ± 0.16^{i}	0.9 ± 0.21^{i}	0.7 ± 0.12^{i}
6	4.2 ± 0.42^{k}	3.0 ± 0.13^{l}	1.3 ± 0.13^{m}	0.8 ± 0.27^{n}	1.2 ± 0.23^{o}	1.3 ± 0.13^{o}
8	4.8 ± 0.58^{p}	2.6 ± 0.11^{a}	1.5 ± 0.18^{r}	0.9 ± 0.12^{s}	1.6 ± 0.22^{t}	1.7 ± 0.12^{t}
10	4.8 ± 0.22^{u}	1.4 ± 0.12^{v}	2.0 ± 0.22^{w}	1.1 ± 0.11^{x}	2.3 ± 0.24^{y}	1.9 ± 0.34^{z}
12	4.5 ± 0.22^{n}	1.0 ± 0.11^{m}	2.3 ± 0.11^{q}	1.0 ± 0.12^{p}	3.2 ± 0.16^{r}	2.4 ± 0.11^{v}
14	4.8 ± 0.27^{g}	0.5 ± 0.26^{h}	3.0 ± 0.12^{l}	1.2 ± 0.15^{j}	3.5 ± 0.16^{k}	2.6 ± 0.14^{l}
16	4.7 ± 0.22^{d}	0.2 ± 0.17^{e}	3.3 ± 0.25^{f}	1.3 ± 0.16^{g}	3.8 ± 0.13^{h}	$3.6+0.16^{i}$

Values are mean ± SEM of triplicate determinations. Values on the same row having the same superscript are not significantly different from each other.
DTR;Diabetic Test Rat

DISCUSSION

The three parameters, glucose, glycated haemoglobin (HbA1c) and insulin play fundamental roles in the pathogenesis of diabetes depending on their levels in circulation. Glucose toxicity which could be direct or indirect may be as a result of direct interaction with proteins and membranes or indirect as a result of the production of reactive sugars acting on membranes and enhancing diabetic complications. Increased glucose concentration as evidenced in hyperglycemia is known to be a major predisposing factor in production of reactive glucose by-products in particular α-deoxyglycosone and glyoxal which are more than 10,000x more chemically reactive than glucose(Beisswenger and Thormally, 2003). While they could inhibit cell growth, they are also known to produce precursors for activated glycated end product formation as shown earlier by Brownlee (2001). It is now known that animals have mechanisms to control the damage caused by unavoidable non-enzymatic glycation. These protective mechanisms are determined by genetically encoded enzymes which determines the level of glycated agents (Fioretto and Mauer, 1996). In diabetes, these mechanism are important due to increased glycemic stress. Furthermore, these protective mechanisms are impaired by metabolic perturbation produced by the diabetic state. HbA1c provides an accurate and reliable method to assess the glycemic control in patients with diabetes. Measurement of glycated hemoglobin in patients with diabetes is accepted as a standard for assessment of recent glycemic control and is a critical element in clinical practice (Lester, 1989). Hyperglycemia is known to have an adverse effect on tissue insulin sensitivity and insulin secretion that makes it difficult to differentiate between primary and secondary abnormalities. In assessing the relationship between glucose, HbA1c and insulin, we observed a positive correlation in reduction of both glucose and HbA1c in animals treated with the extract. There was however a slight elevation of insulin after treatment. The results suggest a greater amount of insulin production in rats treated with 10% T. *bengwensis* accounting for the reduced glucose and HbA1c in comparison with the normal and diabetic test rats. It thus authenticates the fact that the insulin effect in accelerating peripheral glucose disposal was supported by additional mechanism. The insulin: glucose ratio is occasionally used as an index of insulin sensitivity. This ratio was similar in the groups suggesting an adequate insulin response to the treatment.

Phytochemical analysis of mistletoe as earlier reported by Obatomi (1994) and Allessi (2003) have attributed the hypoglycemic properties to the presence of some constituents notably cholins, lectins, tannins and saponins. A combination of the antinutrient behaviour and activity of secondary plant metabolites must have potentiated these actions. The effects of flavoniods, quercetin and ferrulic acid on pancreatic β-cells which leads to their proliferation and secretion of more insulin have earlier been reported by Kako *et al*. (1997), Okamoto (1970), Mesh and Menon (2004) and Sri-Balasubashini *et al*. (2004) suggesting β-cells 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. This dual pancreatic and extrapancreatic action would prove to be an important advance on existing therapies used to manage diabetes mellitus.

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Received: Jan 7, 2012; Revised: Aug 11, 2012; Accepted: Aug 13, 2012