International Journal of Pharma Sciences
Vol. 3, No. 6 (2013): 391-393
Research Article
Open Access

ISSN: 2320-6810

Anti-Diabetic effect and RNA content of the pancreas of Albino Wistar rats treated with aqueous extract of *Vernonia amygdalina* roots

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Received: 15 October 2013 Accepted: 05 November 2013 Online: 07 November 2013

ABSTRACT

The research provided evidence on the anti-diabetic property of the root of *Vernonia amygdalina* rather than the hypoglycemic abilities showcased by the leaf of the same plant. A study on the RNA content of the pancreas of the diabetic animal model to elucidate the possible mechanism of action of this great anti-diabetic agent gave credence to the fact that the aqueous extract caused increase in the cell mass of the pancreas, enhanced proliferation of the β-cells of islets of langerhans of the pancreas as evidenced in the RNA content of the post-mitochondrial supernatant and whole homogenate obtained by the fractionation of the pancreas. The anti-diabetic property could be associated with the high presence of flavonoids, saponins, terpenoids, sterols and carbohydrate obtained during the preliminary phytochemical screening of the plant roots.

Keywords: *Vernonia amygdalina* root; diabetes mellitus; RNA Content; cell mass of Pancreas

INTRODUCTION

Medicinal plant extracts have been valuable anti-diabetic agents and may involve one or more active components responsible for blood glucose reduction [1]. Undoubtedly, medicinal plants are relevant in both developing and developed nations of the world as sources of drugs, hence, WHO [2] recommended and encouraged this practice especially in countries where access to conventional treatment of diabetes is inadequate. Nimenibo-Uuadia, R. (2003) [3] had reported the blood glucose lowering effect of aqueous extract of *Vernonia amygdalina* root in alloxan-induced diabetic rats after one week administration. Therefore, this study was designed to investigate the anti-diabetic property of the aqueous extract and the protective abilities of the extract on the pancreas to restore the damage done on the β-cells of islet of langerhans after two weeks administration as the probable mechanism of action in exerting the anti-diabetic action.

RNA concentration levels is a useful tool in determining how the transcriptional machinery of cells is affected in the presence of external signals like drug treatment and how cells differ between a healthy and diseased state [4]. The determination of the effect of the extract on the cell mass of the pancreas as well as the RNA content of the whole homogenate and the sub-cellular fractions will give evidence on the regeneration mechanism of the pancreas in the animal model.

MATERIALS AND METHODS

Preparation of Plant Materials

Fresh roots of *Vernonia amygdalina* were harvested from the endocrine research farm of Biochemistry Department, University of calabar, Nigeria. They were washed, sundried, crushed to smaller sizes and kept in an air tight container for extraction. To 150 g of the blended root, 600 ml of distilled water was added;, the mixture was shaken for 15 minutes in electric shaker and left in a refrigerator at 4^{o}C for 48 hours. The mixture was filtered and the filtrate diluted and given to rats.

Animals

Twenty four albino wistar rats (93 g - 275 g) obtained from the infection free stock of the department of

Biochemistry, University of Calabar, Nigeria were used. They were fed with standard rats feed, water *ad libitum*, but starved 12 hours prior to the commencement of the experiment.

Induction of Diabetes

A single intravenous injection of freshly prepared solution of alloxan monohydrate at a dose of 70 mg/Kg body weight of rats was given [5]. The rats fasting blood glucose levels were estimated at 0, 3, 6 and 9 hours post treatment using One Touch® glucometer (Lifescan, Johnson and Johnson, California). Ten days later, rats with blood glucose concentrations above 250 mg/dL [6] were considered diabetic and used for the study.

Administration of the Extract

The administration was done by oral gavaging twice daily for a period of 14 days at a dose of 200 mg/Kg body weight of rats. The rats were divided into four groups of six rats each. The diabetic treated as well as the normoglycemic groups were given the root extract whereas the normal control and diabetic control groups received distilled water within the period of administration. The blood glucose levels of the rats were monitored daily and on the 15th day, all the rats were sacrificed under chloroform anaesthesia and their blood and tissues collected for analysis.

Tissue Preparation

Each pancreas was washed in 0.9 % normal saline, blotted with clean filter paper and instantly weighed and their cell masses were recorded. Each pancreas was homogenized and subsequently fractionated according to the method by Tartakoff, A. M and Jamieson, J. D. (1974) [7] and Scheele, A. G et al. (1978) [8] to obtain the whole homogenate and post-mitochondrial supernatant.

Estimation of Glucose and RNA Content

Glucose estimation was determined based on the principle of Barhan, D. and Trinder, P. (1969) [9] using glucose oxidase kit (Randox, UK). RNA level was determined by the method of Fleck, A. and Begg, D. (1965) [10] as modified by [11-12]. Absorbance measurements were done using UV spectrophotometer.

Phytochemical Screening

Standard protocols by Trease, G. E. and Evans, W. C. (1986) [13] were used in determining the presence of the different phytochemical constituents in the aqueous extract of the plant roots.

Statistical Analysis

The student t-test was used to determine the statistical differences at $P < 0.05$ between the normal control and normoglycemic groups as well as diabetic control and diabetic treated groups for their differences of means. This was tested for serum glucose levels (mg/dL), RNA content (mg/dL) of the whole homogenate (Wh) and post-mitochondrial supernatant (PMS).

RESULTS AND DISCUSSION

Diabetes mellitus is an endocrine disease associated with an elevated blood glucose level [14], this was evident in the diabetic control group of the study. The significant reduction ($P < 0.05$) in the blood glucose levels of rats in the diabetic treated group holds credence to the antidiabetic agents present in the aqueous root extract of the plant and this was in consonance with the earlier reports [15] as well as showing similar effects to other medicinal plants [6, 16-17]. Arising from the phytochemical screening of the plant roots, the heavy presence of flavonoids and terpenoids may be associated with the antidiabetic activity noted in this study, hence, flavonoids of different plant origin shows promising antidiabetic activity as demonstrated in diabetic animal models [10]. Also, the significant increase ($P < 0.05$) in blood glucose levels observed in the normoglycemic group when compared with the normal control group implies the aqueous root extract of the plant may not have hypoglycemic effect in normal rats, this differed with the reports [16] about the leaves of the same plant.

Table 1. Effect of Aqueous Root Extract of *Vernonia amygdalina* on Blood Glucose and RNA Content of Wistar Rats

Groups	RNA content of Wh(mg/dl)	RNA content of PMS(mg/dl)	Serum glucose (mg/dl)
Normal control	2.96±4.19	1.37±1.11	55.22±4.30
Diabetic control	1.66±0.33	1.37±1.07	276.56±41.6
Normoglycemic	6.34±5.92^{ns}	2.31±0.62^{ns}	63.12±3.29**
Diabetic treated	5.79±3.75^{ns}	2.63±1.14^{ns}	92.69±10.60*

Values are presented as mean± SEM at $P < 0.05$, n = 7, ns => non-significant increase, ** => significant increase, * => significant decrease

Table 2. Effect of aqueous root extract of *vernonia amygdalina* body weight changes and cell mass of pancreas of rats

Groups	Body weight changes (g)		Pancreas weight (g)
	Initial	Final	
Normal control	208.0±14.2	274.1±3.8	5.06±0.08
Diabetic control	194.1±18.6	107.0±13.8	2.64±0.36
Normoglycemic	212.3±24.1	291.7±31.4**	6.97±0.87^{ns}
Diabetic treated	192.5±311.7	222.5±16.0**	8.22±0.03**

Values are presented as mean± SEM at $P < 0.05$, n = 7, ns => non-significant increase, ** => significant increase, * => significant decrease

Nevertheless, the catabolic effect often resulting from tissue wasting [18] and a resultant weight loss was noted in the diabetic control group of rats. More over, the extract abilities to significantly increase ($P < 0.05$) the body weight of rats in the diabetic treated and normoglycemic groups when compared with their respective control groups showed the added advantage of this extract in weight gain in rats.

The resultant increase ($P < 0.05$) in the pancreas weight of the diabetic treated group of rats when compared with the control was in consonant with the report by [19], that diabetic drugs administration resulted in increase in pancreas weight and that the pancreas weight in diabetic rats was less than that in normal rats.

Further more, Greenbaun, D *et al.* (2003) [4] reported that the measure of RNA level of a cell could give an idea on the transcriptional machinery of that cell, hence, the insignificant increases ($P > 0.05$) in RNA level of both the whole homogenate and post mitochondrial supernatant of the pancreas in both diabetic treated and normoglycemic groups when compared with diabetic control and normal control groups respectively is an indication that the extract actually caused regeneration of the β-cells of islet of langerhans of the pancreas but may be the period of administration was inadequate. Hence, the report that the integrity of RNA and DNA as the most extensively used biomarkers for cell proliferation and death [20] has been seen in this study in the various groups of rats models considered.

CONCLUSION

Considering the high presence of flavonoids in the extract of the plant, its increase effect in cell mass of the pancreas and increase in the RNA content of the sub-cellular fractions of the pancreas of the diabetic rat models studied, the regeneration of the β-cells of islets of langerhans may be the probable mechanism of action of this *Vernonia amygdalina* root extract.

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