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Insulin and alpha amylase levels in alloxan-induced diabetic rats and the effect of *Rothmannia hispida* (K. Schum) Fagerl leaf extract

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ABSTRACT

Objectives: *Rothmannia hispida* (*R. hispida*) is used in West African traditional medicine for the treatment of various ailments such as fever, dysentery, skin infections, abdominal pain and diabetes mellitus. To elucidate the pharmacological basis of the antidiabetic efficacy of this plant drug, the effect of *R. hispida* on insulin and alpha amylase levels were examined in alloxan-induced diabetic rats. **Method:** Diabetic rats were treated with leaf extract of *R. hipida* at dose levels of 250mg/kg and 500mg/kg respectively. The concentration of insulin in serum samples was estimated using Enzyme-Linked Immunoabsorbent Assay (ELISA) method using insulin kit (Syntron Bioresearch, USA), alpha amylase levels were estimated using routine biochemical procedures. **Results:** Treatment of alloxan diabetic rats with leaf extract of *R. hipida* significantly ($p<0.001$) reduced hyperglycaemia, significantly ($p<0.001$) attenuated alloxan-induced hypoinsulinaemia and significantly ($p<0.01$) increased alpha amylase levels compared with diabetic untreated rats. **Conclusion:** It is concluded that increased insulin secretion and/or increased alpha amylase synthesis sequel to enhanced liver glucose entry by *Rothmannia hispida* is proposed to be the mechanism by which this herbal plant exhibits its antidiabetic effect.

Keywords: Insulin, Alpha amylase, Diabetes mellitus, *Rothmannia hispida*.

INTRODUCTION

Diabetes mellitus (DM) and other ailments have been treated and managed with herbs since antiquity. Recent scientific investigations have confirmed the efficacy of some of these herbal preparations; elucidating their mechanisms of action, side effects and phytochemical components. More than 1200 plant components have been tested for their ability to lower blood sugar, and many of them have been found to contain chemical components possessing hypoglycaemic effect [1-3]. In Africa, *Rothmannia hispida* and a related plant drug, *Rothmannia longiflora* are used traditionally for the treatment of fever and as an analgesic. A decoction of the leaves, twigs, bark and roots is applied internally or externally as lotions, washes and baths. The roots are used in treating bowel complaints in Nigeria; throat abscesses, toothache and leprosy in DR of Congo [4]. The leaves of *R. hispida* and *R. longiflora* are used as enema against kidney pain and diarrhea and for the treatment of diabetes mellitus. Drinking of leaf juice is used to relieve pain during labour and child birth. It is also used for the treatment of fever, filariasis, dysentery, itching, skin diseases, ulcers, and as an emetic [4,5]. In the southern part of Nigeria, the leaves of *R. hispida* and *R. longiflora* are used traditionally for the treatment of diabetes mellitus, skin infections and for the eradication of intestinal worms. Administration of extracts of *R. hispida* to diabetic rats has been shown to reduce plasma glucose level [6,7].

Endogenously, pancreatic islets cells of Langerhans secrete insulin from β- (or B-) cells, glucagon from A-cells, somatostatin from D-cells and PP- cells secrete pancreatic polypeptide [8]. Many factors stimulate insulin secretion - amino acids, fatty acids, sulfonylureas, glucagon - but the main stimulus for insulin secretion is blood glucose [8,9]. Insulin cause reduction of blood sugar by facilitating the uptake and storage of glucose, amino acids and fats after a meal; therefore a fall in plasma insulin concentration results in an increase in blood sugar level. In the liver, insulin inhibits glycogenolysis and gluconeogenesis but stimulates glycogen synthesis [8]. In muscle, insulin increases the facilitated transport of glucose via a glucose transporter called GLUT4, stimulates glycogen synthesis and glycolysis. Insulin increases glucose uptake by GLUT4 in adipose tissue as in muscle, thus enhancing glucose metabolism.

Diabetes mellitus is marked by increased blood glucose concentration sequel to decreased insulin secretion by β-cells of the pancreas and/or decreased sensitivity of body cells to the stimulatory effect of insulin [8,12]. DM has been described as a metabolic disorder of multiple etiology and characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from

defects in insulin secretion, insulin action or both [12]. Characteristic symptoms such as thirst, increased frequency of urination, blurring of vision and weight loss may be present.

AIMS AND OBJECTIVES

General

- To study the effect of *R. hispida* leaf extract on insulin and alpha amylase levels in diabetic rats.

Specific Objectives

- To determine the effect of diabetes mellitus on body weight of alloxan-induced diabetic rats and the influence of *R. hispida* leaf extract.
- To determine the anti-hyperglycaemic effect of *R. hispida* leaf extract in diabetic rats.
- To determine the effect of *R. hispida* leaf extract on insulin levels in diabetic rats.
- To determine the effect of *R. hispida* leaf extract on alpha amylase levels in diabetic rats.

MATERIALS AND METHODS

Procurement of plant

The leaves of the sample were collected from Botanical Garden of the University of Calabar. They were identified at the Department of Biological Sciences of the same University as *R. hispida*, of the family Rubiaceae.

Preparation and extraction of the plant extract

Fresh leaves of *R. hispida* were collected and washed free of sand and debris, and then ground into a fine paste. Wet weight of the paste was measured with Mettler weighing instrument (S/N 754550, Zurich, Switzerland). The weight of 348.5g of paste was soaked in 2 litres of distilled water for 18 hours. The mixture was filtered using Whatman's No. 1 filtered paper. The filtrate was slowly evaporated to dryness in an electric oven at 40-50 °C, yielding a semisolid substance with a percentage yield of 12.7; this was stored in a refrigerator to prevent bacterial decomposition and possible loss of efficacy.

Animals / Induction of diabetes

Adult albino rats used for this study were obtained from the animal house of the Department of Pharmacology, University of Calabar, Nigeria. They were acclimatized for two weeks in well aerated cages. The rats had free access to water and were fed *ad libitum* with standard rat feeds (Vital feeds, Nigeria Limited). The protocol of the experiments in this study have been examined and approved by the Ethical Committee of the Faculty of Basic Medical Sciences, University of Calabar, Nigeria and have been performed in accordance with the ethical standards laid down in the 1964 declaration of Helsinki.

Twenty (20) rats of both sexes were divided into four groups of five rats per group. These were treated with aqueous extract of *Rothmannia hispida* leave (RHE) 250mg/100g body weight and 500mg/100g body weight for fourteen days after diabetic induction. Diabetes mellitus was induced by intramuscular injection of alloxan (Sigma, UK) at a dose of 120mg/kg body weight. Alloxan was administered within 10 minutes of preparation to avoid loss of efficacy. Diabetes mellitus developed within 24 hours of alloxan administration and was verified by the appearance of polyuria and glycosuria. The latter was confirmed by the use of Medi-Test indicator strips (Macherey-Nigel, Germany).

Determination plasma alpha-amylase level

The assay was carried out following the standard biochemical protocol [10] with slight modifications by Dineshkumar *et al* [11].

Calculations: A somogyi amylase unit is the amount of amylase which will destroy 5mg starch in 15min. Since 1ml of buffered substrate contains 0.4mg starch and 0.1ml of diluted serum is equivalent to 0.01ml of undiluted serum, then let absorbance(A) = A of blank – A of test /A of blank. The absorbance (A) is proportional to the amount of starch digested by 0.01ml of serum in 15 min., and as 1 amylase unit will destroy 5mg of starch (but only 0.04mg of starch was used), then absorbance (A) is given by:-

(A of blank – A of test/A of blank) x (0.4/5.0)(100/0.01) = amylase units per 100ml serum

OR (A of blank – A of test/A of blank) x 800 = amylase units per 100ml serum

Estimation of plasma insulin level

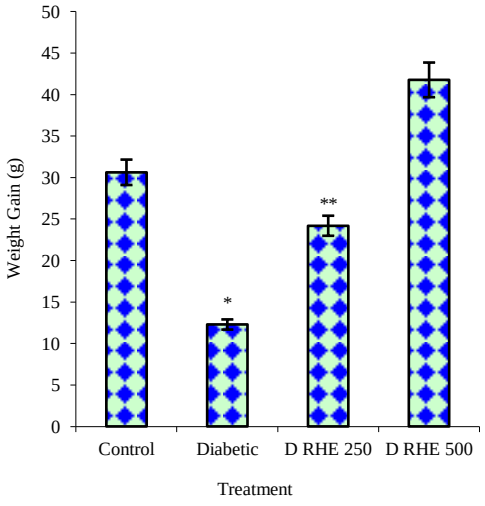
The concentration of insulin in serum samples was estimated using Enzyme-Linked Immunoabsorbent Assay (ELISA) method using insulin kit from Syntron Bioresearch (USA). The sample used was non-haemolysed serum. Following a standard procedure, a sample of the standard curve was plotted and insulin concentrations in the samples were determined by interpolation from the standard curve [12, 13].

Statistical analysis

Results were expressed as mean values ± standard error of mean (Mean ± SEM) based on five experiments. Significant differences between control and experimental groups were assessed using student's t-test and the results were considered significant at *P*-values of less than 0.05 ($P<0.05$). Graphical representations were designed using Microsoft Excel (2007).

RESULTS

The mean body weight gains of control and RHE-treated rats are presented in Fig. 1. At the start of the experiment, the rats had comparable body weights and were placed randomly into control, diabetic, diabetic RHE 250mg/100g body weight (bwt) treated and diabetic RHE 500mg/100g bwt treated respectively, of five rats per group. The starting body weight for control, diabetic, diabetic + RHE 250mg/100g bwt and diabetic + RHE 500mg/100g bwt were 80.0 ± 3.98g, 75.84 ± 6.46g, 89.84 ± 2.07g and 87.6 ± 4.06g respectively. There were no significant ($p>0.05$) difference among the groups.

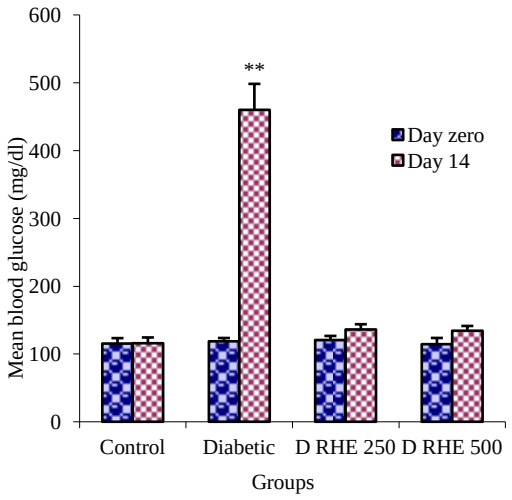


D RHE 250 = diabetic *R. hispida* (250mg/100g bwt) treated, D RHE 500 = diabetic *R. hispida* (500mg/100g bwt) treated. bwt = body weight. *P<0.01 vs control and herb treatment, ** *P*<0.01 vs high dose

Figure 1: Effect of treatment of diabetic rats with *Rothmannia hispida* extract on weight gain

At the end of the experiment, following diabetic induction and treatment, there was a significant reduction (P<0.01) in body gain in diabetic group compared with control. Herb treatment significantly (P<0.01) reversed the reduction in body weight imposed by alloxan administration. Treatment of alloxan diabetic rats with *R. hispida* resulted in significant increase (P<0.01) in weight gain and growth rate of rats compared with untreated rats (Fig. 1).

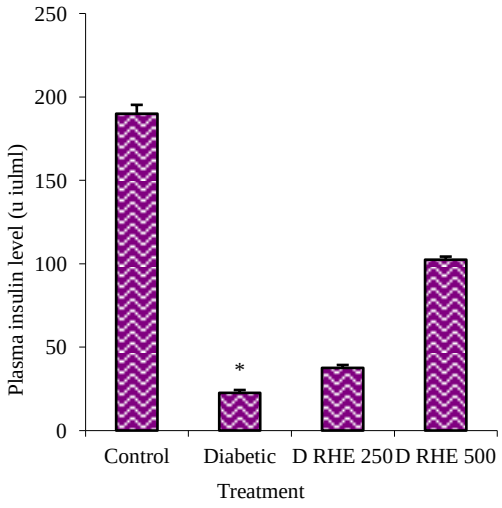
The blood glucose level of diabetic group was significantly higher (P<0.001) than that of control. The results showed a significant (P<0.001) reduction in blood glucose in herb treated groups compared with diabetic untreated group. The blood glucose of herb treated groups were comparable with that of control group at the end of the experiment (Fig. 2).



D RHE 250 = diabetic *R. hispida* (250mg/100g bwt) treated, D RHE 500 = diabetic *R. hispida* (500mg/100g bwt) treated. bwt = body weight. *P<0.001 vs control and herb treatment

Figure 2: Hypoglycaemic efect of *Rothmannia hispida* extract on diabetic rats

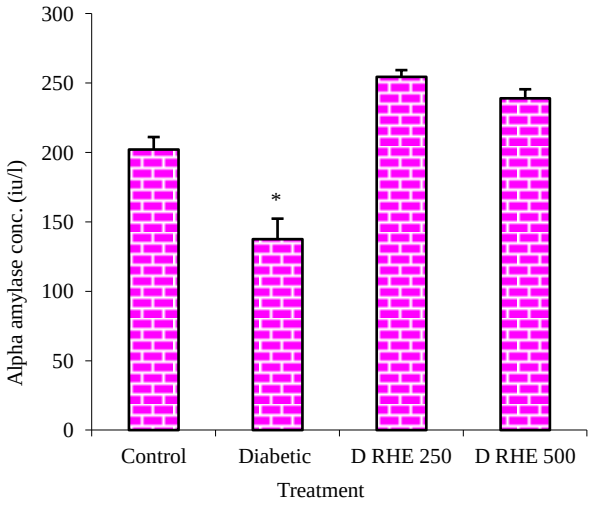
The results from this study indicated that the plasma insulin level of diabetic group was significantly (P<0.001) reduced when compared with control group. The results show a dose-dependent increase in plasma insulin levels of diabetic rats treated with leaf extract of *R. hispida* (RHE). RHE 500mg/100g bwt significantly (P<0.001) attenuated alloxan-induced hypoinsulinaemia (Fig. 3).



D RHE 250 = diabetic *R. hispida* (250mg/100g bwt) treated, D RHE 500 = diabetic *R. hispida* (500mg/100g bwt) treated. bwt = body weight. *P<0.001 vs control and high dose herb treatment

Figure 3: Effects of *Rothmannia hispida* extract on insulin levels in alloxan diabetic rats

Plasma alpha amylase levels were estimated at the end of treatment period. The results showed that plasma amylase level was significantly (P<0.01) depressed in diabetic rats compared with control. Treatment with *R. hispida* extract resulted in significant (P<0.01) increase in plasma alpha amylase level when compared with diabetic rats (Fig. 4).



D RHE 250 = diabetic *R. hispida* (250mg/100g bwt) treated, D RHE 500 = diabetic *R. hispida* (500mg/100g bwt) treated. bwt = body weight. *P<0.01 vs control and herb treatment

Figure 4: Plasma alpha amylase levels in diabetic and *Rothmannia hispida* treated rats

DISCUSSION

The principal symptoms of diabetes mellitus (DM) in both humans and in experimental conditions are glycosuria, hyperglycaemia and weight loss [14]. In this study, the use of extracts of the leaves of *Rothmannia hispida* (RHE) on alloxan-induced diabetic rats was to investigate the relationship between this herb and some of the diabetic symptoms. The potentiality of establishing the use of medicinal herbs in the treatment of this universal disease especially in poor resource regions is a welcome development. Although insulin and oral hypoglycaemic agents are the mainstay in the treatment of diabetes, this study was carried out to seek an alternative to the treatment of DM. The aim of drug treatment in DM is the lowering of high blood glucose concentration to normal or near normal with the consequent hopeful reversal of diabetic complications [8, 14-16].

The manifestations of DM – hyperglycaemia and decreased body weight gain – were observed in the diabetic untreated group. This agrees with the reports of several workers that alloxan exerts a cytotoxic effect on pancreatic β-cells, resulting in Type 1 diabetes mellitus [17-19]. Szkudelski [20] indicated that the mechanism of cytotoxic action of alloxan on β-cells involve oxidation of essential sulphydryl (-SH) group, inhibition of glucokinase, generation of toxic free radicals and disturbances in intracellular calcium homeostasis. The resulting damage to β-cells, responsible for reduced secretion of insulin, results in a decrease in insulin release and the attendant hyperglycaemia with metabolic and other associated diabetic complications.

In this study, treatment of alloxan-induced diabetic rats with leave extracts of *Rothmannia hispida* (RHE) effectively reversed alloxan-induced hyperglycaemia and body weight loss consequent upon diabetic induction. Previous reports by other workers have demonstrated the blood glucose lowering effect of plant extracts in experimental models [7, 21-27]. Rothmannia and other alkaloid and flavonoid containing plants should manifest a high level of biological activity. While some of the activities are desirable, others may be unwanted or side effects of these plant drugs, which are attributable to

their bioactive components. Song *et al* [28] indicated that flavonoids, as antioxidants, may prevent the progressive impairment of pancreatic β-cell function due to oxidative stress and may reduce the incidence of diabetes. The results of this study confirm the reports of Antai *et al* [6] on blood glucose lowering effect of *Rothmannia hispida* leaf extract on alloxan-induced diabetic rats. Treatment of alloxan-diabetic rats with RHE resulted in reversal of hyperglycaemia and reversal of weight loss consequent upon diabetic induction. The mechanism of antidiabetic action of this extract is proposed to be increased insulin synthesis and release resulting from antioxidant effect of the bioactive principles present in this plant drug [7, 29].

The basic marker of DM is high blood levels of glucose sequel to decrease insulin secretion by the pancreas or defective insulin action – insulin resistance by target cells [8,30,31]. Type 1 DM results from idiopathic or cellular – mediated autoimmune destruction of pancreatic β- cells, patients thus depend totally on exogenous insulin [14,32,33]. In this study, Type 1 model of DM was induced by administration of alloxan to rats [20]. The result from this study showed a significant ($P<0.001$) lowered plasma insulin level in alloxan treated rats compared to control rats. Treatment of alloxan – induced diabetic rats with leaf extracts of *Rothmannia hispida* resulted in a dose – dependent significant ($P<0.001$) increase in plasma insulin level compared to diabetic non-treated rats. Lack of insulin in DM is associated with diabetic complications, conditions that are reversed or arrested when DM is properly managed with insulin injections or with oral hypoglycaemic agents [8,34].

Investigations conducted to test herbals for their ability to lower blood sugar have indicated the presence of chemical components with hypoglycaemic potentials [25,35]. These components include epicatechin, a flavonoid with the potential to regenerate pancreatic β-cells [1,35,36]; a sulphur compound (thioproanal-S-oxide) with the ability of blocking the breakdown of insulin in the liver [23,37,38]; charantin (a mixture of steroidal saponins), an insulin –like peptide (polypeptide P), flavonoids and alkaloids [25,35,39,40]. Since the phytochemical screening of the leaves of *Rothmannia hispida* revealed the presence the above components [29], it is proposed that the increased insulin levels noted in the present study results from the ability of the phytochemicals in *R. hispida* to stimulate insulin release, inhibit insulin breakdown and/or regenerate pancreatic β- cells that were damaged by alloxan in the diabetic state.

Alpha amylase is a digestive enzyme found in the saliva and pancreatic juice; it functions in the hydrolysis of starch [41]. Diabetes mellitus is known to result in significant decrease in plasma-amylase level [42,43]. Panchbhai *et al* [44] reported a lower mean salivary amylase levels in diabetics than in non-diabetic group. In their report, Yavuzilmaz *et al* [45] linked the low alpha-amylase level in diabetics to hormonal and metabolic alterations sequel to diabetes mellitus. In addition, Ewadh *et al* [46] indicated that the low alpha-amylase activity in diabetics correlated negatively with hyperglycaemia and duration of diabetes. However, increased levels of alpha-amylase have also been reported in diabetics [47,48].

The results from this research work showed a significant ($P<0.01$) decrease in α-amylase level in diabetic rats compared with control rats, a condition that was reversed by oral administration of extracts from leaves of *Rothmannia hispida*. It has been reported that altered serum amylase activity in the course of DM results from impaired pancreatic exocrine secretion sequel to a decrease in insulin stimulatory action and hyperglycaemia [49,50]. Thus increased insulin level and the associated normoglycaemia resulting from oral administration of *Rothmannia hispida* leaf extracts resulted in normalization of plasma α-amylase level and function in the diabetic treated rats.

CONCLUSION

It is concluded that the increase in body weight gain, reversal of hyperglycaemia and reversal of lowered alpha-amylase level noted in the present study results from the ability of the phytochemicals in *R. hispida* to stimulate insulin release, inhibit insulin breakdown and/or regenerate pancreatic β- cells that were damaged by alloxan in the diabetic state.

Conflicts of interest

There are no conflicts of interest.

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