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Full Length Research Paper

Comparative effect of *Rothmannia hispida* leaves extract and protamine-zinc insulin on alloxan-induced diabetic rats

A. B. Antai[1]*, O. E. Ofem[1], O. J. Nwosu[2], S. O. Ukafia[3], K. C. Iyadi[4] and R. Nia[1]

[1]Department of Physiology, University of Calabar, Cross River, Nigeria.
[2]Department of Anatomy, University of Uyo, Akwa Ibom, Nigeria.
[3]Department of Pharmacology, University of Abuja, Abuja, Nigeria.
[4]Department of Pharmacology, University of Maiduguri, Maiduguri, Nigeria.

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Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action, or both. Insulin and *Rothmannia hispida* are among the anti-diabetic agents. This study therefore sought to establish the relative potency of insulin and *R. hispida* leaves extract in alloxan-induced diabetic rats. Four groups of 5 albino Wistar rats each were made diabetic with alloxan (150 mg/kg), then treated with either extract (100 mg/kg) or insulin (1 unit) for 7 days or left untreated. The terminal blood glucose level of diabetic extract treated (DET) (4.40 ± 0.52 mmol/L) and diabetic insulin treated (DIT) (4.10 ± 0.48 mmol/L) were significantly (P < 0.001) lower compared with diabetic untreated (DUT) (22.00 ± 0.00 mmol/L). Terminal urine glucose was only present in DUT (42.60 ± 6.14 mmol/L) . Terminal urine output was also significantly (P < 0.001) lower in DET (8.60 ± 1.17 ml) and DIT (8.80 ± 0.80 ml) compared with DUT (44.00 ± 1.48 ml). The DUT and DIT were also observed to have negative growth rates (-4.86 and -0.29 g/day respectively), whereas the controls and DET had positive growth rates (5.70 and 0.14 g/day respectively). Terminal blood glucose levels and urine output were not significantly different between DET and DIT. Therefore, 100 mg/kg body weight of the *Rothmannia hispida* leaves extract is equally as potent as 1 unit of protamine-zinc insulin as an anti-diabetic agent.

Key words: Insulin, *Rothmannia hispida,* diabetes mellitus, rats.

INTRODUCTION

From time immemorial, humans have relied on herbs for the treatment of various ailments. Roots, barks, leaves and seeds of different plant extracts provide a ready source of relief for various seemingly incurable diseases (Gonzalez et al., 1992). Among the plants used in this traditional medicine is *Rothmannia hispida* (Antai et al., 2005).

R. hispida is a perennial plant that grows into a tree of about 35 ft tall. The leaves are silky and hairy in appearance with purple marking on its white corolla tube (Lewis et al., 1977). In Nigeria, its local names include: "okukim", "obong", "asun", "asogbodu", "uriohia" and "owuruokumuo". *R. hispida* has been used to treat many ailments including diabetes mellitus, (Antai et al., 2005), and skin infections (Antai et al., 1995).

Diabetes mellitus is a metabolic disorder that precipitates disturbances in glucose, lipid and protein homeostasis (Van den Berghe et al., 2006). It is secondary to a deficiency of the number of pancreatic beta cells of islet of Langerhans or resistance of tissue cells to insulin (Kelly and Fabtus, 1995; Mogensen, 1992; Lang et al., 2005). It is characterized by hyperglycaemia, glucosuria, polyuria, body weight loss, coma, death etc. To identify genetic factors that increase the risk of diabetes, Hakonarson et al. (2007), Smyth et al. (2008) and Cooper et al. (2008) evaluated the association between type 1 diabetes and 8 loci related to the risk of

*Corresponding author. E-mail: ofemo2003@yahoo.com. Tel: 08036731746.

celiac disease in 8,064 patients with type 1.

Diabetes mellitus is one of the leading causes of disability and death in the world (Cockram et al., 1993; Park et al., 2000) and in Nigeria (Erastus, 1988). So many therapeutic agents have been produced to combat this ailment, notable among these is insulin. Since, the discovery of insulin by Banting and Best in 1921 and its first clinical use in 1922, there have been astonishing improvement in the health and strength of diabetic patients (Tattersal, 1994). Today, insulin remains about the most efficacious drug for the treatment of diabetes and its associated complications (Fore, 1995; Van den Berghe et al., 2006). Protamine zinc insulin is long acting insulin preferred to the short acting insulin for the management of diabetes.

Nevertheless, it has been observed that insulin therapy prevents some of the diabetic symptoms, while merely reducing others (Anwana and Garland, 1990; Clark and Lee, 1995). *R. hispida* on the otherhand alleviates hyperglycaemia and prevents glycosuria. This study was therefore designed to compare the relative potency of the leaves extract of *R. hispida* and protamine-zinc insulin in diabetic rats.

MATERIALS AND METHODS

Experimental animals

20 male albino Wistar rats (initial body weight of 180 – 250 g) aged 9 - 11 weeks were used for this study. They were obtained from the animal house of the Department of Physiology, University of Calabar-Nigeria and kept in polyvinyl chloride (PVC) metabolic cages with steel wired bottom to give room for daily removal and collection of waste products. The wire bottom also served as filter for collection of clean urine samples devoid of food particle and feces. Beakers were placed at the infundibulum of the funnel and covered with black polyethylene bags to avoid evaporation. The animals were subjected to standard environmental conditions.

Experimental plant

Fresh and uninfected leaves of *R. hispida* were collected from the botanical garden of the University Calabar-Nigeria during the rainy season. The Botanical identification of the plant leaf was done at the Department of Botany, University of Calabar, Nigeria, where voucher samples were kept for reference (VN = UCDB 1245).

Preparation of crude plant extract

The leaves collected were rinsed with distilled water and dried under shade until they got brittle. The dry leaves were ground into coarse powder with an electric blender, after which 1160 g of the coarse powder was percolated in 5.8 L of distilled water and allowed for 15 h at room temperature. The aliquot was then filtered with a chess material and then with Whatman No. 1 filter paper. The filtrate was then evaporated by hot air oven (Amstel-Hearson oven, England) treatment at 60°C. This residue was reconstituted in distilled water to an appropriate concentration before administration.

Experimental protocol

20 male albino Wistar rats were randomly assigned into four groups of 5 rats each. Group 1 (control) received normal rat chow and drinking water. Group 2 (diabetic untreated, DUT) received same as controls plus alloxan treatment (150 mg/kg body weight once i.p). Group 3 (diabetic extract treated, DET) was treated same as group 2 but received extract treatment (100 mg/kg body weight). Group 4 (diabetic insulin treatment, DIT) had same as group 2 plus insulin treatment (1 unit).

After induction of diabetes with alloxan on the first day, animals were kept for 7 days without treatment. Thereafter, the animals were then treated with either the extract orally (group 3) or insulin intramuscularly (group 4) for another 7 days.

Body weight records were done daily using Boerr weighing balance (Munich, Germany), while daily urine blood glucose levels were measured using ACCU- Check glucose meter (Roche Diagnostics GmbH, Germany). A drop of blood from the tail vein was introduced on the test strips and inserted into the meter for blood glucose reading in mmol/L.

Food intake was measured daily, a known quantity (100 g) of feed was placed in each of the cages, the following morning, the amount of feed left was also measured, from which the total intake was determined and the mean calculated considering the number of animals per group.

Collection of blood sample

Rats were sacrificed using chloroform anaesthesia. Blood samples were collected by cardiac puncture into fluoride oxalate capped bottles with the aid of a 5 ml syringe. The blood samples were then used for the experiments.

Determination of terminal blood and urine glucose levels

The terminal (day 14 of the experiment) blood and plasma glucose levels were estimated using GOD PEROD Enzymatic kit (Randox Laboratories Ltd, UK) using Auto analyzer Auto Lab (Kunst et al., 1984).

Calculation:

$$\text{Glucose concentration (mmol/L)} = \frac{\text{Absorbance of test x 5.55}}{\text{Absorbance of standard}}$$

RESULTS

Body weight changes

The results on body weight changes of the different experimental groups are illustrated in Figure 1. The control rats had initial body weight of 244.00 ± 16.00 g, at the end of the experiment, their body weight increased to 324 ± 27.70 g showing an increase ($P < 0.001$) in mean body weight and a growth rate of 5.7 g/day.

The diabetic untreated group had initial body weight of 212.00 ± 4.89 g. They steadily lost weight through out the duration of the experiment. Their mean final body weight

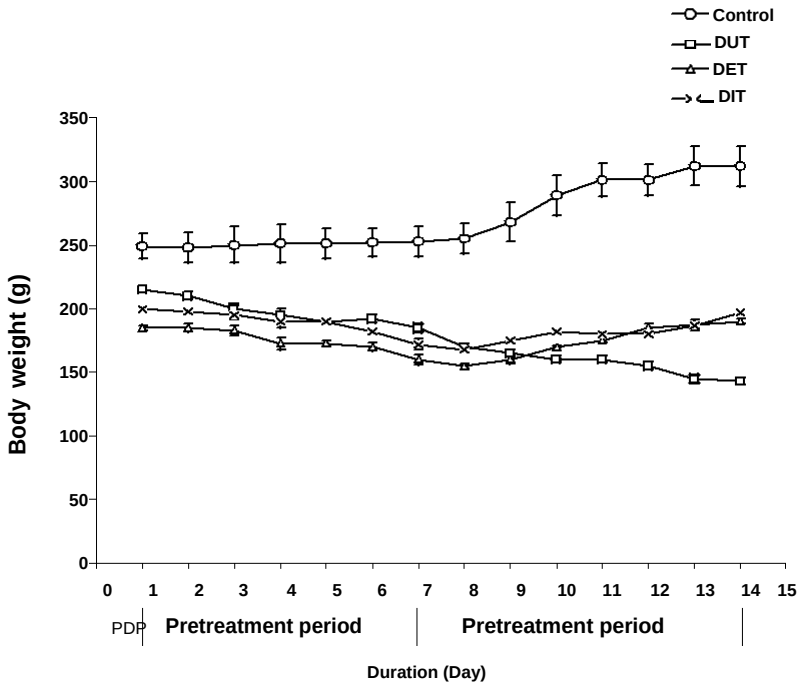

Figure 1. Body weight changes of the various experimental groups of rats. Values are mean + SEM, n = 5. DUT = diabetic untreated; DET = diabetic extract treated; DIT = diabetic insulin treated.

was 144.00 ± 4.00 g which was significantly (P < 0.001) lower compared to the initial body weight. They had a negative growth rate of -4.86 g/day.

The initial mean body weight of the diabetic extract treated group was 184.00 ± 4.00 g. They had a significant (P < 0.05) weight lost at day 7 (160.00 ± 5.47 g) prior to commencement of treatment with the extract. At the end of the experiment, their mean body weight was observed to have increased significantly (P < 0.05) to 186.00 ± 6.00 g, near the control value. They had a positive growth rate, 0.14 g/day (from day 1 to 14).

The group four rats that had insulin treatment also had a fall in mean body weight after treatment with alloxan but their body weight gradually increased thereafter following insulin treatment. Their body weight on days 1, 7 and 14 were 200.00 ± 0.10, 172.00 ± 5.83, and 196.00 ± 2.45 g, respectively. Their growth rate was -0.29 g/day (from day 1 to 14).

Plasma glucose

Figure 2 shows results of mean plasma glucose of the different groups. The initial and final plasma glucose levels of the control group were 4.48 ± 0.14 and 4.20 ± 0.52 mmol/L, respectively showing no significant differences.

24 h after alloxan treatment, the mean blood glucose levels of the DUT, DET and DIT were 12.70 ± 2.49, 10.40 ± 0.45 and 15.80 ± 2.68mmol/L respectively. Seven days after, the blood glucose level of DET and DIT increased to 17.50 ± 1.31 and 18.50 ± 2.13mmol/L respectively.

The mean final blood glucose levels of the DUT, DET and DIT were 22.20 ± 0.10, 4.40 ± 0.10 and 4.10 ± 0.48 mmol/L, respectively, showing significant reduction (P < 0.001) in DET and DIT compared with DUT.

Food intake

Results of daily food intake are shown in Figure 3. At the beginning of the experiment, the mean food intake of the control, DUT, DET and DIT groups were 19.40 ± 0.25, 17.00 ± 1.22, 17.40 ± 0.81 and 17.00 ± 0.98 g respectively, showing no significant differences among the groups. The mean food intake of the DUT rats increased significantly (P < 0.001) to 39.00 ± 0.29 g at the end of the experiment higher than the control value (21.80 ± 0.49 g).

At the day 7 of the experiment, the mean food intakes of the DET and DIT groups were 28.90 ± 1.55 and 25.10 ± 1.09 g respectively but at day 14 it reduced to 14.10 ± 0.46 and 15.60 ± 0.49 g respectively. These final mean food intakes were significantly lower compared with the

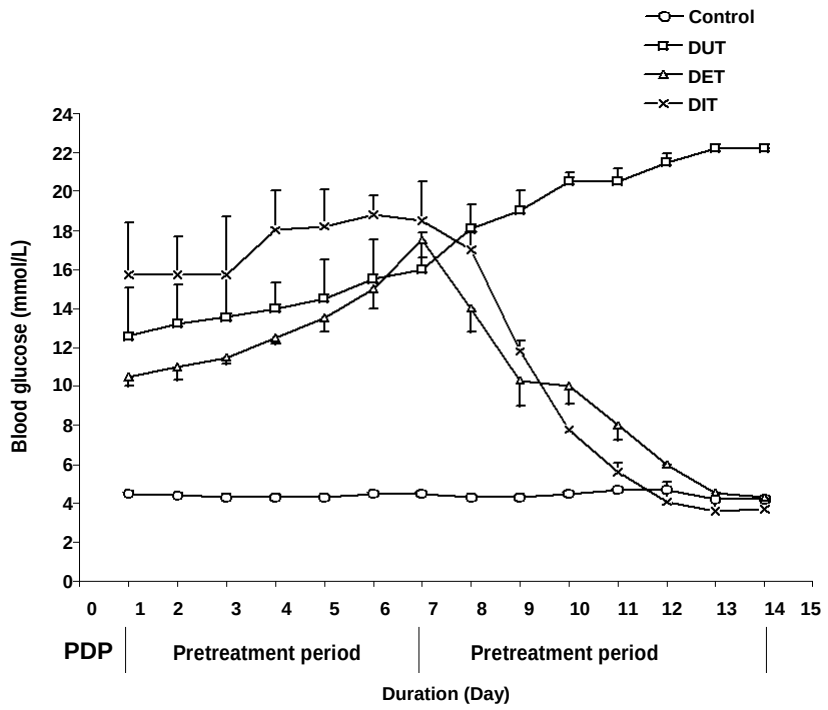

Figure 2. Blood glucose levels of the various experimental groups of rats. Values are mean + SEM, n = 5. PDP = prediabetic period. DUT = diabetic untreated; DET = diabetic extract treated; DIT = diabetic insulin treated.

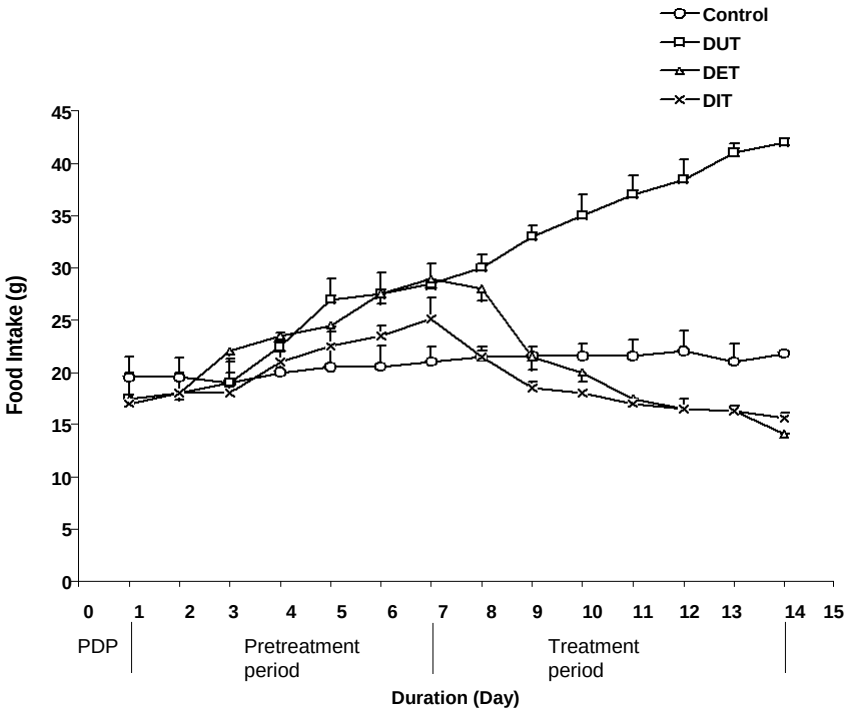

Figure 3. Daily food intake of the various experimental groups of rats. Values are mean + SEM, n = 5. PDP = prediabetic period. DUT = diabetic untreated; DET = diabetic extract treated; DIT = diabetic insulin treated.

Table 1. Urine volume and urine glucose levels of the different experimental groups of rats.

Variable	Urine output (ml)			Urine glucose level (mmol/L)		
	Day 1	Day 7	Day 14	Day 1	Day 7	Day 14
Control	9.00 ± 0.32	8.01 ± 0.98	8.20 ± 1.43	Nil	Nil	Nil
Diabetic untreated	10.80 ± 2.04	28.76 ±1.98***	44.00 ±1.48***	24.30 ± 11.20	35.10 ± 3.45	42.60 ± 6.14
Diabetic extract treated	13.60 ± 2.91	31.80 ± 3.07***	8.60 ± 1.17	23.10 ± 9.98	26.80 ± 1.20	Nil
Diabetic insulin treated	10.20 ± 0.20	35.00 ± 3.36***	8.80 ± 0.80	18.70 ± 7.44	25.00 ± 7.98	Nil

***P<0.001 vs control, Values are mean ± SEM, n = 5.

untreated group compared with controls too.

Urine output

Results of the 24 h urine output for all groups are shown in Table 1. Urine output was not significantly different among the groups on day one.

Control rats had initial urine output of 9.00 ± 0.32 ml which dropped slightly to 8.20 ± 1.43 ml at the end of the experiment. The diabetic untreated rats had an initial mean urine output of 10.80 ± 2.04 ml. This increased to 28.60 ± 1.81 ml at day 7, further still to 44.00 ± 1.48 ml at the end of the experiment. Their mean urine output at the end of the experiment was significantly ($P < 0.001$) higher compared with control values.

Diabetic extract treated group had an initial mean urine output of 13.6 ± 2.90 ml. This increased significantly to 31.80 ± 3.08 ml at day 7. At day 14 of the experiment, it reduced significantly ($P < 0.01$) to 8.60 ± 1.17 ml. This final mean urine output was not significantly different from that of controls.

The initial mean urine output of diabetic insulin treated rats was 10.20 ± 0.20 ml. At day 7 of the experiment it increased significantly ($P < 0.001$) to 35.00 ± 3.36 ml. When treatment with insulin commenced, the urine volume fell and gradually reduced to control levels at day 14 (8.80 ± 0.80 ml). The final urine output of the insulin treated diabetic rats was not significantly different from values obtained for extract treated diabetic group or control. The changes in urine output of all groups throughout the duration of the study are shown in Figure 4

Urine glucose

Table 1 also shows result obtained of urine glucose levels of the different experimental groups. Glucose was not detected in the urine of control rats. Diabetic untreated rats were highly glycosuric. Their mean urine glucose following induction of diabetic was 24.30 ± 11.20 mmol/L. It increased to 34.60 ± 8.55 mmol/L at day 7, it increased further to 42.60 ± 6.14mmol/L at day 14 of the experiment.

Diabetic extract treated group had an initial mean urine glucose level of 23.10 ± 9.98 mmol/L 24 h after alloxan treatment. It increased to 26.80 ± 1.20 mmol/L at day 7. No glucose was recorded in the urine of these animals after 7 days of treatment with the crude extract.

The diabetic insulin treated rats had urine glucose levels of 18.70 ± 7.44 and 25.00 ± 7.98 mmol/L at days 1 and 7 after treatment with alloxan. At the end of the experiment, no glucose was observed in the urine of these rats.

DISCUSSION

Diabetes mellitus unleashes a lot of stress resulting in death of most of its victims. Studies have been directed not only on checking its mortality rate but also on an alternative to the orthodox use of insulin in its treatment. A survey of herbalist in south eastern Nigeria revealed the use of plant *Rothmannia hispida* in the treatment of diabetes mellitus; it has been shown to be a potent hypoglycaemic agent (Antai et al., 2005). Its efficacy has been further elucidated by intravenous infusion of acute doses of the extract (Antai et al., 1995).

This study was therefore designed to compare of the effects of *Rothmannia hispida* crude leaves extract and insulin on diabetic rats. Protamine- zinc insulin is long acting insulin that has been proven to be more potent than the short acting insulin.

The variation in the initial body weight of the groups was due to inability of obtaining rat with uniform weight, hence the need for their body weight change or growth rate to nullify the disparity in body weight. Results on body weight revealed that control rats gained weight throughout the duration of the study. Untreated diabetic rats on the other hand steadily lost weight throughout the study duration. This is consistent with the report of Anwana and Garland (1991). Weight loss is a prominent symptom of diabetes. The liver, during impaired glucose utilization breaks down muscle glycogen, protein and fat stores of the body for the generation of glucose to meet the body's

need. A further combination of polyuria, retarded growth and repair process leads to emaciation and weight loss (Guyton and Hall, 2004). Insulin and herb treatment restored weight gain. However, only the diabetic extract treated group regained pre-diabetic weight. This result is consistent with those of Anwana and Garland (1991) in streptozotocin diabetic rats. Other workers have reported failure of insulin treated rats to regain weight (Hedben et al., 1986).

The urine output of the controls was normal throughout the study period as expected. On the other hand, the diabetic untreated rats were markedly polyuric. Their final mean urine output was significantly higher compared with controls and diabetic treated rats. Polyuria is a result of osmotic and water diuresis due to excess glucose in the plasma (Van den Berghe et al., 2006). It results in loss of water and electrolytes from the body. Treatment with insulin was observed to significantly reduce urine output of diabetic rats to near control values. Also, treatment with *Rothmannia hispida* extract reduced urine output to control values in diabetic rats.

Urine output of both the extract and insulin treated diabetic rats were not significantly different. It therefore implies that 1 unit of long acting insulin is therefore equally as potent as 100 mg/kg of the extract in abolishing polyuria.

Since diabetes mellitus precipitates glycosuria, a symptom common in some non-diabetic pregnant females, believed to be due to an increased glomerular filtration rate (Ardawi, 2000; Alto, 2005); this study took care to eliminate this coincidence by using only male albino Wistar rats. Also, normal individuals can sometimes become glycosuric while others who are hyperglycaemic show no glycosuria due to variation in renal tubular threshold for glucose. This was however eliminated by a 24 h urine test using clinistix reagent strip before induction of diabetes mellitus.

Although, glucose was not detected in the urine of controls, the diabetic untreated rats were glycosuric. Their mean urine glucose concentration at the end of the study was significantly higher than that of the treated groups. In this study, 1 unit of protamine- zinc insulin and 100 mg/kg body weight of *Rothmannia hispida* leaves extract were equally effective in abolishing glycosuria in diabetic rats, since there were no traces of glucose in the urine of the insulin and extract treated diabetic rats at the end of the study.

Food intake of control rats had a uniform pattern throughout the duration of the study. Diabetic untreated rats were markedly hyperphagic and their mean food intake at the end of the study was significantly higher compared with other groups. Hyperphagia is also a prominent symptom of diabetes mellitus resulting from non utilization of glucose by cells of the body (Craighead, 1978; Field, 1988). This is the sole reason the diabetics always experience a sense of hunger despite hyperglycaemia. Treatment with the leaves extract restored feeding to control levels. Mean food intake of the insulin and extract treated diabetic rats were not significantly different at the end of the study, hence, both treatment regimens could be said to be equally effective in this regard.

The blood glucose of the control group was steady throughout the study period. Diabetic untreated rats were however hyperglycaemic 24 h after alloxan treatment and the trend continued to the end of the study. Hyperglycaemia is one of the most important feature of diabetes and remains the best and most reliable diagnosis for the disease (Cheung et al., 2000). Hyperglycaemia results from a total failure of glucose homeostatic mechanism of the body due to insulin absence or insensitivity of the tissues to insulin. Both the extract and insulin were equally effective in restoring the normoglycaemic state of diabetic rats. Thus, 100 mg/kg body weight of *Rothmannia hispida* was as potent as 1 unit of insulin (protamine zinc insulin) in establishing normoglycaemia in diabetic Wistar rats.

We therefore conclude that 100 mg/kg body weight of *Rothmannia hispida* leaves extract is equally as potent as 1 unit of long acting insulin in restoring normal urine output, food intake, urine glucose and blood glucose levels in diabetic rats.

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