

HYPOGLYCAEMIC EFFECTS OF *BOSWELLIA DALZIELII* STEM BARK IN NORMOGLYCAEMIC AND ALLOXAN DIABETES RATS.

*AMINU A. BOBBOI AND ROLAND OLESUGUN

DEPARTMENT OF BIOCHEMISTRY
COLLEGE OF MEDICAL SCIENCES
UNIVERSITY OF MAIDUGURI
P.M.B 1069, MAIDUGURI, NIGERIA

*Correspondence author

ABSTRACT

The hypoglycaemic effects of aqueous extract of *Boswellia dalzielii* stem bark were studied in normal and alloxan induced diabetes rats. Groups of rats were administered saline (control) or the plant extract (10mg/100gm) daily for three weeks. Diabetic animals administered the plant extract regained their body weight by week 3. The fasting blood glucose was normalized from week 2 in diabetic extract treated rats. The plant extract control animals exhibited pronounced lower fasting blood glucose. Similarly, the weekly postprandial blood glucose responses were significantly reduced in diabetic rats treated with the extract. In an acute study, gastric administration of the extract lowered the postprandial blood glucose after 3h. However, following intraperitoneal administration of the plant extract, the blood glucose responses were significantly lowered after 2h, 3h and 6h. The studies suggest possible hypoglycaemic effects of *Boswellia dalzielii*. The hypoglycaemic actions of the plant may possibly involve changes in gastrointestinal, pancreatic and extrapancreatic events.

Key Words: Blood Glucose, Glucose Tolerance Test, Diabetes mellitus.

Boswellia dalzielii.

INTRODUCTION

Boswellia dalzielii Hutch (family: Burseraceae) is a tree widely distributed in tropical and subtropical countries, mainly dry regions of tropical Africa, Arabia and India (Dalziel, 1973). When strips of its bark are peeled away, *B.dalzielii* exudes a gummy oleo- resin. The gum resin and different parts of the plant are widely employed in traditional medicine. Historically, the gum resins were recommended for a variety of conditions including osteo and rheumatoid arthritis, abdominal pain, diarrhoea dysentery, asthma, bronchitis and other pulmonary diseases, skin diseases, tumours, cancers, stimulation of menstrual flow, diabetes mellitus, syphilis, and as a diuretic (Burkill, 1985, Nwinyi *et al*, 2004).

The exudates of the *Boswellia* tree contain oils, terpenoids, and gum (Pizzorno and Murray, 1999). The oleo-resin is 16% essential oil, primarily thujene and P-cymene. The terpenoids are comprised of pentacyclic triterpene acids, boswellic acids. Some experimental animal studies, *in vitro* studies and clinical trails have supported some of the medicinal uses of *B. dalzielii*. The sedative and analgesic effects were demonstrated in an *in vivo* animal study (Menon *et al.*, 1971) and its mechanism demonstrated *in vitro* (Ammon *et al.*, 1993). Boswellic acids were implicated and were found to inhibit leukotriene synthesis via 5-lipoxygenase (Safayhi *et al.*, 2000). Clinical studies with formulas containing *Boswellia* have yielded good results in both osteo arthritis and rheumatoid arthritis (Kulkani *et al.*, 1991). Boswellic acids were shown to reduce glycoaminoglycans degradation while inhibiting 5-lipoxygenase (Reddy *et al.*, 1989). *Boswellia* gum resin was shown to be a safe and effective therapy in colitis, crohn's disease and ileitis through inhibition of inflammatory leukotrienes (Gupta *et al.* 2001). Excessive humoral inflammatory response in asthma was shown to be improved by boswellia gum resin preparation in a double-blind, placebo-controlled study (Gupta *et al.*, 1998). Methanol and aqueous extracts of *B.dalzielii* stem bark were found to exhibit broad spectrum inhibiting activity against bacteria (Adelkun *et al.*, 2001). Several studies suggest that boswellic acids have anti-carcinogenic effects (Jing *et al.* 1999, Huan *et al.* 2001). *In vitro*, boswellic acids inhibited synthesis of DNA, RNA and proteins in human leukaemia HL-60 cells. (Shao *et al.* 1998). However, there appears to be no investigative report on the hypoglycaemic activity of *B.dalzielii*. This study was designed to investigate the activity of *B. dalzielii* stem bark in normal and alloxan induced diabetes animal model to justify its use in traditional-alternative medicine.

MATERIALS AND METHODS

Animals

Twenty Wister strain male rats weighing between 100-150g, obtained from the Animal House, Department of Biochemistry, University of Maiduguri, Nigeria were used for the study. Animals were grouped into four or five animals each. They were housed in an air-conditioned controlled (18°C) with 12 hours light-dark cycle and allowed free access to feed (Sanders Feed, Jos, Nigeria) and water except during experimental periods when stated. The animals groups were as follows: group I: Normal control, group II: Plant extract control, group III: Diabetic control and group IV: Diabetic-plant extract treated. Daily food intake and body weights were recorded throughout the study period. Animals were treated humanely according to international standard protocols.

Plant extract.

The bark of *Boswellia dalzielii*, was obtained from Professor J. Akinniyi, Natural Products Unit, Department of Chemistry, University of Maiduguri, which was previously identified. The plant bark was washed with distilled water, dried and ground to fine powder. The powder (20g) was boiled in one litre distilled water for one hour, then, left for 24 hours for further extraction. The residue was filtered and concentrated by oven drying at 40°C. The plant extract was stored in refrigerator (4°C) until required.

Induction of Diabetes

Prior to induction of diabetes, all animals were subjected to glucose tolerance test to confirm their glycaemia status. Animals were fasted for 24hr without water restriction. Alloxan (70mg/kg body weight) was intramuscularly injected and feed restriction continued for further 6hr. Glucose tolerance test (2g/kg body weight) was carried out 2 days after alloxan administration, to confirm induction of diabetes. Additional confirmatory test was carried out using Benedicts test on urine collected from animals for qualitative determination of glucose.

Experimental treatments and glucose tolerance test

Rats in groups I (plant extract control) and IV (diabetic plant extract- treated) were intragastrically administered 10mg/100g body weight plant extract using feeding tube (BM1 feeding tube, size 6-8) daily, for 3 weeks. Rats in the normal control and diabetic control groups (groups I and II) were administered normal saline (0.9% NaCl) daily for the same period. Fasting blood glucose and glucose tolerance test were carried out weekly. Animals were fasted for 18hours and tail blood collected for glucose analysis (Trinder, 1969). Animals were then administered 2g/kg body glucose and blood collected at 30min intervals for 2hr. Blood glucose response was calculated as changes from the fasting levels and blood glucose total was calculated as the sum of changes at time intervals (Jenkins *et al.*, 1986).

In an acute study, the effect of route of administration was carried out in normoglycaemic rats. Animals were fasted for 18hours and fasting blood collected for glucose analysis. The plant extract in one ml saline (10mg/100g bodyweight) was gastrically or intraperitoneally administered; then after 1hr glucose (2g/kg) administered. Blood samples were then collected after 1, 2, 3, and 6 hours postprandily for glucose analysis. Saline (0.9% NaCl) was administered as the control.

Statistical Analysis

Test of statistical significance of difference between means were carried out by the students' t-test. The minimum level of statistical significance used was $p < 0.05$.

RESULTS

Body weight

The weekly body weights of rats following different treatments are presented on Table I.

Table 1. Mean body weight (g) of normal and diabetic rats following administration of extract of *Bowellia dalzielii* (10mg/100g body weight).

| Treatment | Mean body wt (g) | | | |
|------------------|------------------|-----------------------|-----------------------|-----------------------|
| | Day 0 | Day 7 | Day 14 | Day 21 |
| Normal control | 145±2.5 | 148±2.5 | 155±2.6 | 166±3.6 |
| Extract control | 130±2.5 | 132±2.1 ^c | 135±2.3 ^{ac} | 140±2.4 ^{ac} |
| Diabetic control | 145±2.4 | 126±3.2 ^{ab} | 108±3.1 ^{ab} | 103±3. ^{ab} |
| Diabetic treated | 142±1.6 | 138±1.4 ^c | 136±1.6 ^{ac} | 138±1. ^{ac} |

Significantly different (body wt. changes from day 0) from: (a) normal control (b) extract control (c) diabetes control

The normal control and plant extract control groups showed steady weight increase from wk 1 to wk 3. The diabetic control group rats showed continuous weight loss during the same period. The diabetic animals treated with the plant extract showed weight gain in the third week to the first week levels.

Weekly blood glucose responses

The weekly blood glucose responses are presented in Table 2.

Table 2. Weekly fasting blood glucose (mmol/l) following administration of *Bowellia dalzielii* extract (10mg/100g body weight) of normal and diabetic rats.

| Treatment | Blood glucose (mmol/l) | | | |
|------------------|--------------------------|-------------------------|-------------------------|-------------------------|
| | Day 0 | Day 7 | Day 14 | Day 21 |
| Normal control | 2.89±0.22 | 2.88±0.29 | 2.89±0.19 | 2.91±0.30 |
| Extract control | 2.54±0.16 | 1.89±0.10 ^a | 1.61±0.19 ^a | 1.60±0.31 ^a |
| Diabetes control | 9.81±0.32 ^{ab} | 9.66±0.86 ^{ab} | 9.01±0.06 ^{ab} | 9.70±0.40 ^{ab} |
| Diabetes treated | 10.26±0.41 ^{ab} | 9.40±0.49 ^{ab} | 2.35±0.16 ^{bc} | 3.23±0.76 ^{bc} |

Significantly different from (a) normal control (b) extract control (c) diabetes control (at P < 0.05)

The fasting blood glucose was maintained within the same levels in normal and control plant extract treated animals. The diabetic untreated animals maintained significantly higher blood glucose levels than normal control, extract control, and diabetic extract treated groups throughout the study period ($p < 0.001$). Diabetic plant extract treated animals showed significantly higher blood glucose values than normal in the first week but returned to normal values in the second week. Similarly the weekly postprandial blood glucose in the responses was maintained at the same levels in the normal and plant extract control groups (Table 3).

Table 3. Weekly postprandial blood glucose of normal and diabetes rats following administration of *Boswellia dalzielii* extract (10 mg/100g).

| Blood glucose concentration | | | | |
|-----------------------------|-------------------------|--------------------------|--------------------------|--------------------------|
| (mmol/l) | | | | |
| Treatment | Day 0 | Day 7 | Day 14 | Day 21 |
| Normal control | 8.34±0.44 | 7.74±0.18 | 7.76±0.24 | 8.20±0.25 |
| Extract control | 8.75±0.9 | 7.10±0.9 | 7.97±0.8 | 12.01±1.8 ^{ac} |
| Diabetes control | 13.86±0.8 ^{ab} | 13.07±0.47 ^{ab} | 20.73±1.23 ^{ab} | 27.05±1.98 ^{ab} |
| Diabetes treated | 13.82±0.7 ^{ab} | 15.5±1.3 ^{ab} | 7.12±1.4 ^c | 10.89±2.5 ^c |

Significantly different from (a) normal control (b) extract control (c) diabetes control (at P<0.05)

There were significantly persistent increased blood glucose levels in the diabetic untreated animals ($p<0.001$). The blood glucose values were significantly high in the first week for the diabetic treated animals, but returned to normal values from week 2.

The blood glucose responses following intra gastric and peritoneal administration of the plant extract are presented in table 4.

Table 4. Blood glucose concentrations following Intra gastric and intra peritoneal administration of *B. Dalzielii* (10mg / 100g) stem bark extract in normal rats.

| Blood glucose concentration | | | | | |
|-------------------------------|---------------|----------------------------|----------------------------|----------------------------|----------------------------|
| (mmol/l) | | | | | |
| Treatment | 0 hr | 1hr | 2hr | 3hr | 6hr |
| Intragastric | | | | | |
| Glucose + saline (Control) | 2.34 ±0.17 | 3.50 ±0.08 | 1.83 ±0.10 | 1.38 ±0.13 | 1.04 ±0.19 |
| Glucose +Extract | 2.13 ±0.05 | 4.99 ^a ±0.02 | 2.18 ±0.04 | 2.13 ^a ±0.10 | 2.10 ^a ±0.06 |
| Intraperitoneal | | | | | |
| Glucose + saline (Control) | 3.63 ±0.77 | 6.45 ±0.12 | 6.30 ±0.13 | 5.96 ±0.08 | 5.40 ±0.12 |
| Glucose +Extract | 2.26 ±0.13 | 5.27 ±0.02 | 2.39 ^a ±0.05 | 2.23 ^a ±0.06 | 2.03 ^a ±0.12 |

Significantly different from Control ($P < 0.05$)

*Changes in blood glucose concentrations from the basal values were calculated and used for the statistical comparison.

During gastric administration, blood glucose concentrations were higher in the first hour but returned to normal values within the second hour in the control (saline treated) animals. In animals treated with plant extract, the blood glucose peaked after 1hr and returned to pre treatment levels after the third hour. The blood glucose levels were steadily maintained at higher values up to the third hour in the control animals following intra peritoneal administration. The blood glucose initially increased in the first hour and significantly lowered in the third and sixth hour following intra peritoneal administration of the extract ($P < 0.001$).

DISCUSSION

Diabetic rats treated with extract of *Boswellia dalzielii* regained their body weight to pre induction levels by week 3. In diabetes every tissue continue to play a catabolic role (Ward *et al.*, 1984). Hyperglycaemia results in hepatic gluconeogenesis from amino acids derived from muscle protein, increased lipolysis in the adipose tissue and accelerated fatty acid oxidation in the liver. The fasting blood glucose of diabetic rats administered the plant extract was reduced to levels comparable to the control group by week 2, suggesting improved hyperglycaemic response. The weekly blood glucose responses following oral glucose tolerance further demonstrated this improved hyperglycaemic control.

Some plants have been reported to induce pancreatic beta cells regeneration and repair. Chakravarti *et al* (1980) reported the pancreatic beta cell regenerative action of *Pterocarpus marsupium* in diabetes rats. The leaf extract of *Gymnema sylvestres* was also shown to have hypoglycaemic effects in non-insulin dependent diabetes mellitus (Baskaran *et al.*, 1999). The action of the plant was ascribed to regeneration / revitalization and repair of the pancreatic islets. Similarly Shammugasundaram *et al.*, (1990) suggested possible regeneration of the islets of Langerhans in streptozotocin diabetic rats by *Gymnema sylvestre* leaf extract. The anti diabetic effect of *Gymnema sylvestre* was also ascribed to suppression of glucose absorption (Shimizu *et al.*, 1997). The plant extract was also shown to stimulate insulin release by increasing cell permeability than stimulating exocytosis (Persuard *et al.*, 1999). *Gymnema* contain tri-terpenoid gymnemic acid as the active component (Shimizu *et al.*, 1997). In this study, diabetes was induced with alloxan, by selectively damaging the insulin-secreting beta cells of pancreatic islets and inducing impairment of islets, glucose oxidation and glucose induced insulin secretion. There is increasing evidence to suggest that free radicals play role in the beta cell damage (Wacker *et al.*, 1995). It has been demonstrated that alloxan stimulates super oxide generation in beta cells (Oberley, 1988). The super oxide can be further converted to more active hydroxyl radical, which attacks cellular membrane and causes DNA breaks (Colman *et al.*, 1989). Interleukin I is the major factor in the damage of beta cells, by inducing free radicals especially super oxide and nitric oxide (Gerbitz, 1992).

The main constituents of *Boswellia* are essential oils, terpenoids and gums. Pentacyclic triterpenes from the 11-keto –boswellic acid series have been identified as the active principal ingredient responsible for inhibiting the key enzyme of leukotriene synthesis, 5-lipoxygenase, which is involved in the pro-inflammatory processes (Safayhi *et al.*, 2000). Leukotrienes cause chemotaxis, chemokinesis, and release of lysosomal enzymes by phagocytes and synthesis of super oxide radicals. These factors are implicated in beta-cell destruction. Boswellic acids have been shown to be specific, non-redox, non-competitive inhibitors of 5-lipoxygenase (Schweitzer *et al.*, 2000). This may be of significance in the maintenance of functional beta cells. Similarly, lipoxygenation of endogenously released arachidonic acid is a critical step in stimulus secretion coupling in the pancreatic beta cells (Metz *et al.*, 1984). Inhibitors of lipoxygenase pathway prevented the stimulatory effect of arachidonic acid. However, the unstable intermediate in leukotriene biosynthesis, 12-hydroperoxyeicosatetraenoic acid potentiated glucose insulin releases.

Animals treated with *B.dalzielii* extract exhibited lower fasting blood glucose in both normal and diabetic animals. The effect appeared to be more pronounced in non-diabetic animals. This suggests at least some involvement in the non-beta site of action of the plant extract. In the acute study, gastric administration of *B. dalzielii* did not produce cumulative lower blood glucose values compared with the control, but rather flatter responses, suggesting slower and complete absorption of glucose. When the route of administration was intra peritoneal, a hypoglycaemic state was reached after 3 hours with *B.dalzielii* extract and the blood glucose was steadily maintained up to 6 hour in the control group, suggesting possible changes in the extra gastrointestinal events. The influence of route of administration on blood glucose homeostasis is of significance. Orally administered glucose is a more potent stimulus of insulin secretion than that administered parentally, because gastric inhibitory polypeptide is released from the intestinal mucosa (Munro, 1984).

We are also suggesting the direct effects of boswellic phytochemicals in structure-affinity relationship as another possible mechanism, at least in the short-term effects. Boswellic acids combined with glucuronic acids and galactose as its component may have suppressive activity on glucose absorption, by binding to the glucose binding sites. Similarly tannins are reported to impair the absorption of nutrients and minerals in whole animal and in semi-isolated intestinal preparations (Silverstein *et al.*, 1996). In the everted sacs, procyanidin tannin inhibited Na uptake of glucose by a non-competitive mechanism. The gum component of the plant may also have some significance in blood glucose regulation. Viscous gums are known to modify blood glucose response by slowing gastric emptying (Holt *et al.*, 1979) inhibits intestinal absorption of glucose (Blackburn *et al.*, 1979) and effects secretory activity of gastrointestinal tract (Tadesse, 1986). Studies have shown that *B.dalzielii* stem bark extract slowed gastrointestinal motility in mice (Nwinyi *et al.* 2004). This may be of significance in glucose intestinal delivery, since gastric emptying and secretions are likely to be effected. This is consistent with the results obtained following gastric administration of the extract. Glucose absorption appeared to be delayed possibly as a result of slowed gastrointestinal motility (Meyer and Doty, 1988). Boswellic acids, lipoxygenase inhibitor and leukotriens synthesis are associated and implicated in non-vascular muscle contractile activity of the intestine (Brash, 1999) and also in intracellular calcium mobilization

(Mayetepek and Hoffman, 1995), some important factors in insulin secretion and glucose utilization.

Most of the oral antidiabetic drugs, the sulphonylurease and biguanids normally act in the presence of functioning beta cells (Naghmi, 1993). The extent and severity of damage to the beta cells by alloxan cannot easily be predicted in the present study. Furthermore, the exact mode by which alloxan exerts its cytotoxic effects is not completely revealed. It is possible to have some residual beta cells to support some glycaemia control and how far the hypoglycaemic action of the plant extract be sustained will only be apparent in a prolonged chronic study. In this regard, it may be hypothesized that *B. dalzielii* extract possibly act in a manner similar to insulin or assuming that the beta cells are not destroyed completely thus potentiating the effects of insulin.

The action of boswellic acids on the parameters of blood glucose homeostasis has not been reported. The present preliminary investigation suggests possible hypoglycaemic action of *Boswellia dalzielii*. The mechanisms by which it induces antidiabetic-hypoglycaemic effects possibly involve changes in intestinal glucose absorption, hormonal alterations or at levels of peripheral glucose metabolism, and these parameters are under examination.

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