Full Length Research Paper

Effect of the methanolic extract of *Brachylaena discolor* in a streptozotocin-induced diabetic rat model

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Traditionally, leaf infusions of *Brachylaena discolor* are used for the treatment of diabetes and renal conditions. Therefore, the aim of this study was to evaluate the effect of a methanolic leaf extract of *B. discolor* in a streptozotocin (STZ)-induced diabetic rat model. Diabetes was induced in Wistar rats by an intraperitoneal injection of STZ; blood glucose levels greater than 20 mmol/L measured after 7 days confirmed a stable diabetic mellitus state. Two doses of the test extract (50 and 150 mg/ml) were administered daily via oral dosing to both STZ-induced and control rats. Blood was obtained from the tail vein and used to measure the effects of the extract on the biochemical profile of the rats over 28 days. The methanolic leaf extract of *B. discolor* at both doses (50 and 150 mg/ml) caused a significant reduction in the blood glucose levels at 7, 14, 21 and 28 days in STZ-induced diabetic rats when compared with the normal rats (negative control). Significant differences were also observed in alkaline phosphatase, creatinine, total bilirubin and body weights of extract treated diabetic rats when compared with untreated diabetic rats, normal rats and metformin treated rats. Findings suggest that *B. discolor* exhibits significant anti-hyperglycaemic activity in STZ-induced diabetic rats.

Key words: Diabetes, streptozotocin, *Brachylaena discolor*, Wistar rats.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disease which occurs as a result of insulin deficiency and/or insulin resistance and is a major cause of disability and hospitalization (Kamgang et al., 2008). Diabetes mellitus has become a major health concern in the world, with an expectation that diabetes and obesity will reach epidemic proportions, affecting third world developing countries to a far greater extent than the developed world (Rheeder, 2006). This may be attributed to the high number of African countries, which are undergoing a demographic transition and coming under increased western influence.

This has resulted in an increase in the consumption of fat, salt and sugar, coupled with a rapid increase in urbanization in African countries and increase in nutritional deficiency, thereby providing an elevated risk factor for the prevalence of diabetes (Colagiuri et al., 2006). Estimates from the World Health Organization (WHO) have projected an increase from 171 million in the year 2000 to 366 million in 2030, with approximately 70% occurring in developing countries (WHO, 2002; Wild et al., 2004). Currently, type 2 diabetes is the most prevalent form of diabetes mellitus; however, oral

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hypoglycemic drugs which are the main form of treatment, have been shown to have undesirable side-effects and high secondary failure rates (Moller, 2001). In addition to the lack of efficacy by pharmacological agents for the treatment of type 2 diabetes, there is also limited access to these drugs for people living in many rural areas as well as cost implications (Holt, 2004; Tonye Mahop and Mayet, 2007).These limitations have prompted research into investigating traditional medicines as potential replacements for conventional pharmacological agents used in the treatment of diabetes (Grover et al., 2002; Odhav et al., 2010). There are already an estimated 122 drugs from 94 plant species, which have been discovered through ethnobotanical leads such as indigenous knowledge. Plants such as *Syzygium cordatum*, *Allium sativum* and *Ficus thonningi* prevail as the treatment for diabetes in poor socio-economic conditions (Musabayane et al., 2005). These plants have been found to control diabetes mellitus by exhibiting hypoglycaemic and antioxidant effects. These ethnobotanical studies are promoted by the WHO for the discovery of novel mechanisms for diabetes treatment (WHO, 1999). Currently, in South Africa, there are no regulations with regard to the prescription and use of traditional medicine, exposing people to misadministration, especially of toxicity of plant compounds. The potential genotoxic effects that follow prolonged use of some of the more popular herbal remedies, are also cause for alarm (Fennell et al., 2004). Therefore, it becomes necessary to identify the amylase inhibitors from natural sources having lesser side-effects. The traditional African herbal medicinal system practiced for thousands of years have reports of anti-diabetic plants with no known side effects. Such plants and their products have been widely pre-scribed for diabetic treatment all around the world with less known mechanistic basis of their functioning. Therefore, these natural products need to be evaluated scientifically in order to confirm claims for their anti-diabetic properties (Iwueke and Nwodo, 2008). The aim of this study was to investigate *Brachylaena discolor* var. *discolor* as a possible dietary adjunct or therapeutic for diabetes therapy. *B. discolor* is a species of tree belonging to Asteraceae family. There are three known varieties: var. *discolor* which is commonly known as the Coast Silver Oak or Coastal Silver Oak, var. *transvaalensis* commonly known as the Forest Silver Oak or Natal Silver Oak and var. *rotundata*. *B. discolor* var. *discolor* is an evergreen shrub or small tree usually 4 to 10 m in height which is often multi-stemmed. It is confined mainly to the coastal bush and associated bushveld (van Wyk and van Wyk, 2007). These trees are distributed from the Eastern Cape in South Africa and into Southern Mozambique, but are most common in the coastal vegetation of KwaZulu-Natal. The leaf infusions of *B. discolor* var. *discolor* are used for diabetes and renal conditions by different ethnic groups in Southern Africa and are reported to act as a

tonic (Hutchings, 1996). The roots are used to treat roundworms and chest pains.

MATERIALS AND METHODS

Plant

Leaves of *B. discolor* var. *discolor* was collected in Durban, province of Kwazulu Natal, South Africa, and identified by using available floral keys. The leaves were carefully examined for insect-damage or fungus-infection and these were discarded. Healthy leaves were dried at 40°C for 48 h in a convection oven. Once completely dry, plant was ground to a fine powder using a Waring blender. The dried plant was then stored in Amber Schott bottles at room temperature until required. The methanolic extract of the dried plant material was prepared according to the procedure outlined by Jeremy and Whiteman (2003) with minor modifications. 20 g of the dried plant material was stirred for 24 h in 200 ml of 80% methanol. The slurry was filtered using Whatman No. 1 filter paper and the supernatant was collected. The supernatant was concentrated using a Buchi RE Rotoevaporator including a Buchi 461 water bath set at a temperature of 45°C. The concentrate was placed in a biofreezer and freeze-dried using a Virtis Benchtop Freeze Dryer. The freeze-dried material was used as a stock and working solutions were prepared for appropriate applications.

Animals

Male Wistar rats of 250 to 300 g body weight (BW) were obtained from the Biomedical Resource Centre (BRC) at the University of KwaZulu-Natal and divided into non-diabetic and diabetic groups of n=12 to be used for acute and chronic studies. These animals were maintained under laboratory conditions of temperature (22 to 24°C), humidity (40 to 60%) and 12 h light/12 h dark regime at the Biomedical Resource Unit (University of Kwazulu Natal, Westville Campus) where animals were exposed to both food and water *ad libitum* for the entire duration of the study. All animals used for this study were maintained according to the rules and regulations outlined by the Ethics Committee of the University of KwaZulu-Natal, South Africa (Ethics approval was obtained from the University of KwaZulu Natal, Ethics reference 089/11/Animal).

Experimental design

The two groups of animals were sub-divided into a control group, metformin group (500 mg/kg BW) and treated group, each with 12 rats per group. These animals were administered two different concentrations of the crude plant extract (200 and 600 mg/kg BW) reconstituted in 1% Tween 80 and standard treatments through feeding 1 ml of the plant extract (gavage) and standard treatment to alleviate any strain on the animal during dosage. The crude extract and standard treatment were administered to the animals for 28 days after identification of streptozotocin (STZ)-induced rats. All blood samples were obtained from the tail vein every 7 days. The study also involved a Humane Endpoint for the experiment and was conducted by veterinary support staff on a daily basis. This was used to determine the severity of the animals' well-being for the duration of the study through visual and intrusive tests (blood glucose levels, weight loss, dehydration etc).

Induction of diabetes

Diabetes was induced in male Wistar rats of 250 to 300 g BW by

intraperitoneal injection of STZ (60 mg/kg BW) in citrate buffer, at a pH of 4.5 (Musabayane et al., 2005). The maximum quantity for the STZ injection used was 1.0 ml/250 g body mass. Blood was collected via a tail prick after 48 h and tested using a Glucocheck blood glucose monitoring system. Animals that exhibited blood glucose levels greater than 20 mmol/L after 48 h were considered to be diabetic. Plasma glucose levels of 20 mmol/L measured after 7 days confirmed a stable diabetic mellitus state.

Serum biochemical analysis

For serum biochemical tests rats were warmed in a warming cabinet for 10 to 15 min in order to dilate blood vessels prior to taking the sample. During the warming process, rats were monitored for signs of hyperthermia and dehydration. After warming, the tail of the rat was washed with diluted Hibitane in order to see the blood vessel. Rats were restrained for the minimum time while the blood was being drawn from the lateral tail vein at the base of the tail. Approximately 1 ml of blood was collected from 3 rats in each of the respective group every 7 days. After tail bleeding, blood flow was stopped by applying finger pressure to the blood sampling site for approximately 30 s before the rat was returned to its cage. Blood was centrifuged and the serum was collected and analyzed immediately using a Chemvet analyzer for the evaluation of serum biochemical parameters. The parameters measured were albumin (ALB), alkaline phosphatase (ALKP), alanine amino transferase (ALT), amylase, blood urea nitrogen (Urea), calcium (Ca), cholesterol (CHOL), creatinine (CREA), globulin (GLOB), phosphorous (P), total bilirubin (TBIL), total protein (TP) and blood glucose (GLU).

Measurement of BW

All experimental animals were weighed every 7 days for the entire 28 day treatment period, to monitor any weight losses or gains as a result of extract dose.

Statistical analysis

Analysis was carried out using one-way analysis of variance (ANOVA; GraphPad Prism), followed by Tukey test for multiple comparisons. Values are expressed as a mean ± standard deviation (n = 3). A P-value less than 0.05 was considered to be statistically significant.

RESULTS

The result of the effect of *B. discolor* methanolic extract on the blood glucose levels of STZ-induced diabetic rats are as shown in Figure 2. These results show that non-diabetic rats (Groups 5 to 8) had blood glucose levels that fell within the normal range. The methanolic leaf extract of *B. discolor* in both doses (200 and 600 mg/kg BW), the reference drug used (metformin) caused a time dependant (P < 0.001) reduction in the blood glucose levels of the STZ-induced diabetic rats (Groups 1 to 3) when compared with the negative control (Group 4). There was also a variation between the different doses tested on the blood glucose levels with the higher dose (600 mg/kg BW) giving the highest activity followed by the reference drug metformin.

The activity of ALKP in diabetic treated rats is as shown in Figure 1. Rats treated with *B. discolor* extracts and the reference drug, decreased the ALKP levels in comparison to the untreated diabetic rats. However, the lower dose extract (200 mg/kg BW) decreased the ALKP levels within the acceptable range when compared with the higher dose and reference drug.

Creatinine levels as shown in Figure 1 shows that the diabetic rats (Group 4) exhibited a CREA level below the acceptable limits; however, animals that received both doses of *B. discolor* (Groups 1 and 2) extract and the reference drug (Group 3) increased the CREA levels to an acceptable limit. Urea levels also varied with the STZ-induced control group (Group 4) and the group receiving the reference drug (Group 3) having elevated urea levels, which exceeded the reference range at the end of the 28 days treatment period.

There was a significant difference in TBIL levels between the different groups as reflected in Figure 2. Diabetic rats which received the *B. discolor* extracts (Groups 1 and 2), showed TBIL levels within the normal range when compared with the diabetic negative control (Group 4) and reference drug (Group 3).

The levels between the different treatment groups for ALB, ALT, Ca, CHOL, GLOB, P, TBIL and TP were not significant, with only small variations in levels.

The data for BW change is represented in Figure 3. Although, there was no significant difference in the initial BW (Day 0) between the different groups, the BW gains for the diabetic negative control (Group 4) and the STZ-induced rats which received the low *B. discolor* dose (200 mg/kg BW, Group 1) were significantly lower when compared with the other groups. It is important to note that the STZ-induced rats which received the higher *B. discolor* dose (Group 3) had a better BW gain in comparison to those STZ-induced rats which received the reference drug (Group 4).

DISCUSSION

There are currently no regulations controlling the prescription and use of traditional medicine in South Africa resulting in possible misadministration, especially of toxic plants. The potential genotoxic effects that follow prolonged use of some of the more popular herbal remedies, are also cause for alarm (Fennell et al., 2004). Therefore, it becomes necessary to identify the amylase inhibitors from natural sources having lesser side-effects. The purpose of this study was to investigate the anti-diabetic activity of a methanolic extract of *B. discolor* in a STZ-induced diabetic rat model. This was achieved by monitoring the blood glucose levels over a 28 day period using a Chemvet analyzer. Results from the study show that the methanolic extract of *B. discolor* at the test doses and the reference drug (metformin) exhibited a significant time dependant decrease in blood glucose levels of the STZ-induced diabetic rats with different levels of
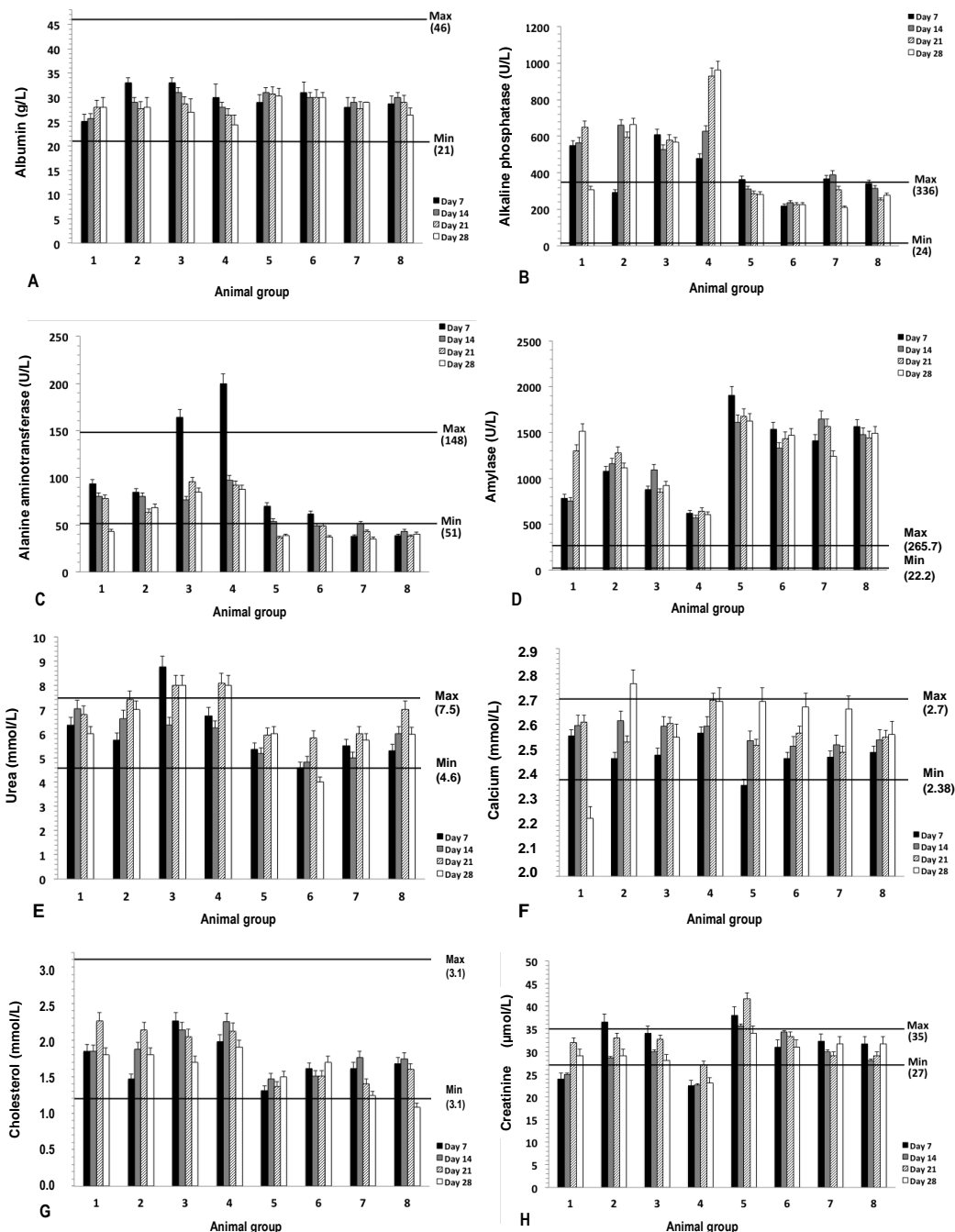
Figure 1. Effect of oral administration of *B. discolor* crude methanolic extract at doses of 200 (Ext 1) and 150 mg/kg BW (Ext 2) BW on albumin (A), alkaline phosphatase (B), alanine amino transferase (C), amylase (D), blood urea nitrogen (E), calcium (F), cholesterol (G) and creatinine (H) levels in normal and diabetic male Wistar rats. Each column represents mean ± SD (n = 3). Group 1: Diabetic + Ext 1; Group 2: diabetic + Ext 2; Group 3: diabetic + metformin; Group 4: diabetic control; Group 5: normal + Ext 1; Group 6: normal + Ext 2; Group 7: normal control; Group 8: normal + 1% Tween 80.

reduction on day 28 when compared with the negative control rats. In this experiment, the extract dose of 600 mg/kg BW produced the highest antidiabetic effect and suggests that this dose may be an effective antidiabetic dose for the crude *B. discolor* extract. The variation

between the 200 and 600 mg/kg BW doses may be due to the concentrations of phytochemicals and active compounds within the extract. The exact mechanism by which *B. discolor* reduces blood glucose levels are unclear, but some attribute the anti-hyperglycaemic
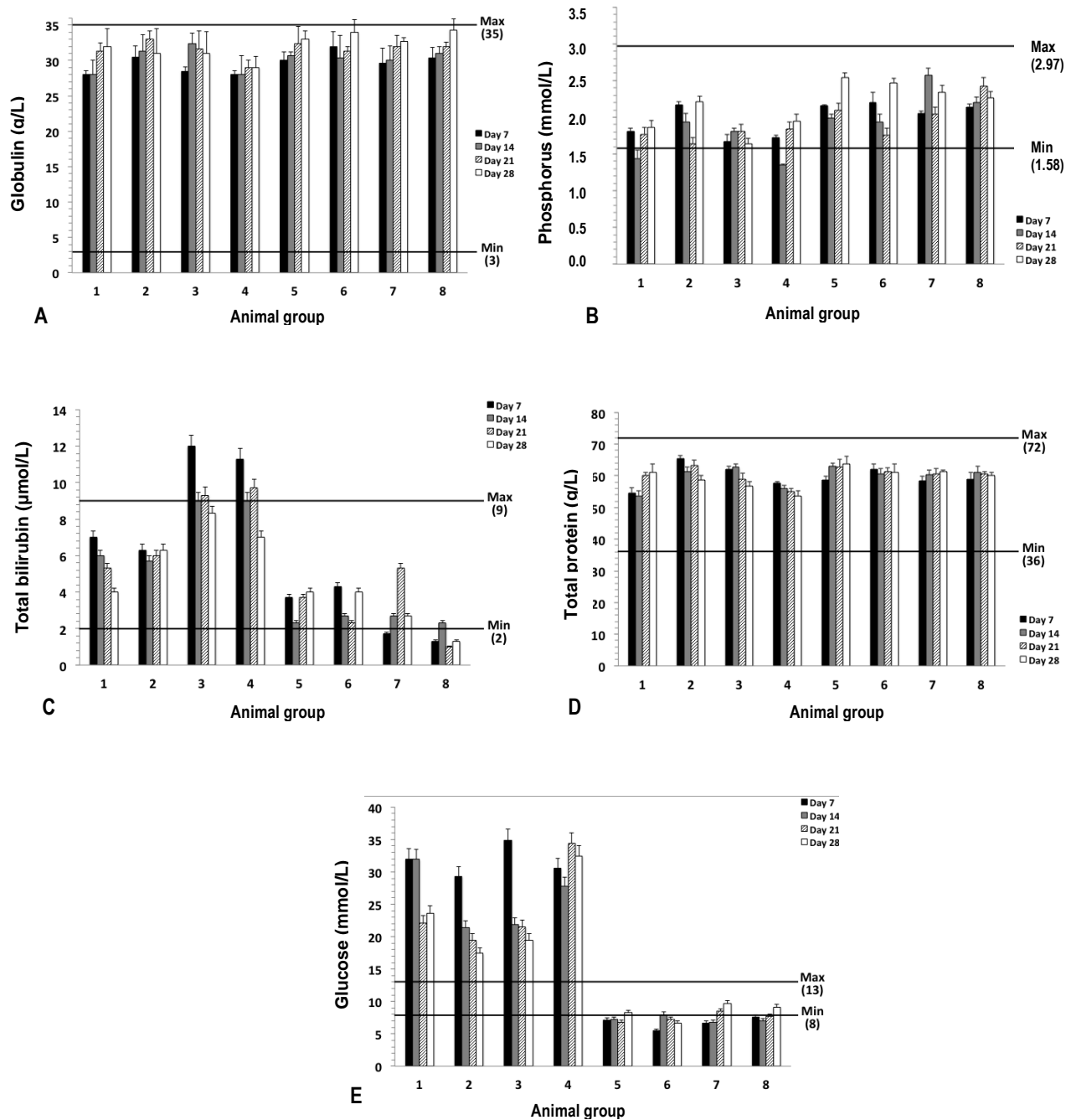
Figure 2. Effect of oral administration of *B. discolor* crude methanolic extract at doses of 50 (Ext 1) and 150 mg/kg (Ext 2) BW on globulin (A), blood glucose (B), phosphorous (C), total bilirubin (D) and total protein levels in normal and diabetic male Wistar rats. Each column represents mean ± SD (n = 3). Group 1: diabetic + Ext 1; Group 2: diabetic + Ext 2; Group 3: diabetic + metformin; Group 4: diabetic control; Group 5: normal + Ext 1; Group 6 normal + Ext 2; Group 7: normal control; Group 8: normal + 1% Tween 80.

activity of other studied medicinal plants to their ability to restore the function of pancreatic tissue, by causing an increase in the insulin output or by a decrease in the intestinal absorption of glucose (Ibeh and Ezeaja, 2011; Neelesh et al., 2010). Biomarker enzymes such as alkaline phosphatase and alanine aminotransferase may

be used for the evaluation of hepatic disorders (Ghandi et al., 2012). This may be substantiated by the ALKP results in Figure 1 depicting elevated ALKP levels found in the STZ-induced diabetic groups (Groups 1 to 4). A signifi-cant reduction in the activity of ALKP in *B. discolor* treated diabetic rats indicates a potential hepatoprotective
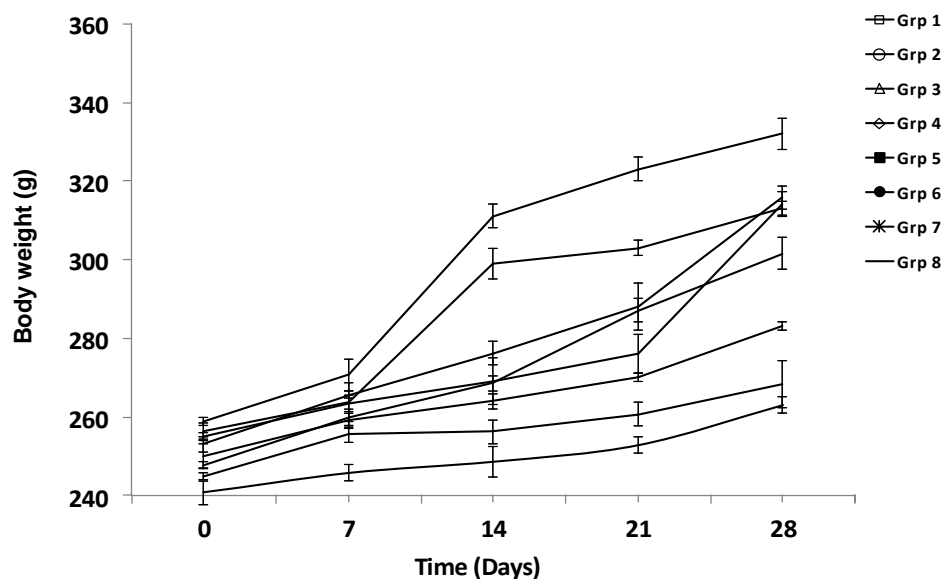
Figure 3. Body weight gains in the different animal groups during the 28 day experimental period. Data is expressed as a mean of n = 4. Group 1: Diabetic + Ext 1; Group 2: diabetic + Ext 2; Group 3: diabetic + metformin; Group 4: diabetic control; Group 5: normal + Ext 1; Group 6: normal + Ext 2; Group 7: normal control; Group 8: normal + 1% Tween 80.

role in preventing possible diabetic complications. The elevation of urea, creatinine and total proteins may be considered as a significant marker for renal dysfunction (Carlo et al., 1996). Results in Figure 1 show a decrease in the urea levels of animals treated with *B. discolor* extract. The increase in urea levels for the STZ-induced control rats (Group 4) and the STZ-induced rats receiving the reference drug (Group 3) may be due to the high protein present in *B. discolor* (Haschick and Kerley, 1997). Blood urea nitrogen is a metabolite produced from dietary protein (Al Faris et al., 2011). Hyperglycaemia results in the generation of free radicals which may exhaust antioxidant defenses, thereby leading to the disruption of cellular functions and oxidative damage to membranes (Dangi and Mishra, 2011). Decreased protein levels may be attributed to the antioxidant activity of organic compounds within the *B. discolor* extract which may play a role in reducing the protein levels as can be seen in Figure 2 (Al Faris et al., 2011). Creatinine levels for STZ-induced rats receiving the *B. discolor* extract and those receiving the reference drug (metformin) all had creatinine levels which were within the normal range when compared with the STZ-induced control group whose levels remained below the acceptable minimum range. By the end of the 28 days treatment period, the serum levels for ALB, Amyl, ALT, Ca, CHOL, GLOB, P, TBIL and TP were not significantly different in comparison to the control group. The induction of diabetes by STZ has been associated with a loss in BW due to increased muscle wasting and loss of tissue protein (Chatterjee and Shinde, 2002; Gupta et al., 2012). The use of *B. discolor* at the 600 mg/kg BW dose improved the

BW in diabetic rats over the 28 days study period. This increase in the BW of diabetic rats may be attributed to an improvement in insulin secretion and glycemic control (Eliza et al., 2009; Genet et al., 1999). It has also been reported by Rajkumar et al. (1997) that increased anabolic reactions may be responsible for weight gain in diabetic rats.

Conclusively, the results of this study have demonstrated that *B. discolor* has significant antidiabetic potential and may be acting through the restoration of pancreatic tissue, stimulation of β-cells or by decreasing intestinal glucose absorption. Further studies are required to isolate and characterize antidiabetic bioactive compounds, and establish the mechanism(s) of action.

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