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Full Length Research Paper

Aloe arborescens aqueous gel extract alters the activities of key hepatic enzymes and blood concentration of triglycerides, glucose and insulin in alloxan-induced diabetic rats

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The present study investigated the antidiabetic activity and the possible mechanisms of action of aqueous extract of *Aloe arborescens* leaf gel (AALGEt) on normal and alloxan-induced diabetic rats. Diabetes was induced in 12 h fasted rats by intraperitoneal injection of 140 mg/kg body weight of alloxan. Blood glucose levels, body weight and water intake were determined on day 7, 14 and 21 of AALGEt treatment. Plasma insulin and triglycerides levels, as well as activities of hepatic glucokinase and glucose 6-phosphatase (G6Pase) were determined at the end of the study. Blood glucose levels, plasma triglyceride and insulin levels, as well as the activity of hepatic G6Pase were significantly increased in diabetic rats. With the exception of hepatic glucokinase activity, daily oral administration of AALGEt to diabetic rats significantly reversed the effects induced by alloxan. The activities of glucokinase and glucose-6-phosphatase as well as plasma insulin levels in AALGEt-treated normal rats were comparable with those observed in untreated normal rats. The results suggest that AALGEt ameliorates physiological parameters altered by the diabetic state. These effects may be mediated in part, through the protection of pancreatic beta cells from further damage by alloxan.

Keywords: *Aloe arborescens*, alloxan, diabetic rats, antidiabetic activity, hepatic enzymes.

INTRODUCTION

Ethnomedical reports suggest that more than 1200 plants are used to control diabetes mellitus in traditional medicinal systems of different cultures, worldwide (Marles and Farnsworth, 1995; Grover et al., 2002). The hypoglycemic effects of a large number of these plants have been evaluated and confirmed in animal models of diabetes (Day and Bailey, 2006; Frode and Medeiros, 2006) as well as in clinical studies (Jayawardena et al., 2005; Day and Bailey, 2006). However, many of these plants still need to be validated and characterized in terms of toxicity, active principles and hypoglycemic mechanism of action.

Aloe arborescens Miller., also known as Krantz aloe, or Kidachi aloe, is a multi-stemmed succulent shrub with a height of about 3 m. It belongs to the Asphodelaceae family (van Wyk and Smith, 2004; Smith et al., 2008). It is indigenous to southern Africa but it is reported to be cultivated all over the world (Smith et al., 2008). Some black communities in the Northwest Province (Odi district)

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Abbreviations: AALGEt, Aqueous *Aloe arborescens* leaf gel extract; **FFAs,** Free fatty acids; **G6Pase,** glucose 6-phosphatase; **HDL,** high density lipoprotein; **HSL,** hormone sensitive lipase; **LPL,** lipoprotein lipase; **SANBI,** South African National Biodiversity Institute.

of South Africa use the leaf components (gel and juice) of *A. arborescens* as antidiabetic remedies (Mogale, personal communication). The hypoglycemic activity of *A. arborescens* leaf components as evaluated and confirmed in experimental animal models of diabetes (Beppu et al., 1993; 2006) are attributed to the presence of the polysaccharides, arboran A and B in the leaves of this plant (Hikino et al., 1986; Beppu et al., 2006). Katerere and Eloff (2005) also discussed the use of Aloes in diabetes. However, the mechanism whereby *A. arborescens* leaf components exert their hypoglycaemic effect remains unknown.

An antidiabetic agent may be effective in reducing blood glucose concentration through: 1) stimulating insulin secretion from pancreatic beta-cells; 2) enhancing glucose uptake by fat and muscle cells; 3) altering the activity of some enzymes (e.g. α-glucosidase, α-amylase, glucokinase and glucose-6-phosphatase) that are involved in glucose metabolism and 4) slowing down the absorption of sugars from the gut (Tanira, 1994; Klover and Mooney, 2004; Cheng and Fantus, 2005). The present study was undertaken to investigate the effects of aqueous A. arborescens leaf gel extract on fasting blood glucose levels, insulin secretion and activities of selected hepatic enzymes in normal and alloxan-induced diabetic rats.

MATERIALS AND METHODS

Plant material and preparation of AALGEt

Leaves of *A. arborescens* were collected from a private garden in the North West province of the Republic of South Africa and confirmed as those of *A. arborescens* (Miller) by the South African National Biodiversity Institute (SANBI) (Genspec no. 2206- 12). Leaf gels were dried and milled into a fine powder after which AALGEt was prepared by homogenizing 25 g of the powder in 250 ml of water, followed by centrifugation at 3000 xg for 20 min. The resulting supernatants were pooled and stored at 4°C until use.

Animals and induction of diabetes

Male albino Wistar rats weighing 220 to 280 g were obtained from the animal facility of the University of Cape Town, South Africa. All animals were kept in individual cages in an environmentally controlled room with a 12 h light/12 h dark cycle. The animals had free access to water and standard rat diet. The study was approved by the Ethics Committee of the University of Limpopo, South Africa (Animal Ethics Approval number AEC09/06). Diabetes mellitus was induced in 12 h fasted animals by intraperitoneal injection of alloxan monohydrate (Sigma, St. Louis, MO., USA) dissolved in sterile normal saline at a dose of 140 mg/kg body weight. Since alloxan is capable of producing fatal hypoglycaemia as a result of massive pancreatic insulin release (Szudelski, 2001; Dhandapani et al., 2002), rats were treated with 20% glucose solution intraperitoneally, 8 h after alloxan treatment. The rats were then kept for the next 24 h in their cages with 5% glucose in dispenser bottles to prevent hypoglycaemia (Dhandapani et al., 2002). Diabetes was confirmed in rats by measuring fasting blood glucose, 72 h post alloxan treatment. Rats with marked hyperglycaemia (blood glucose level above 11.0 mM) were selected for use in the study. The highest blood glucose concentration measured in a diabetic rat was 25 mM.

Experimental procedure

The experiment was carried out using four groups of eight rats each: Group I (untreated normal rats), group II (treated normal rats), group III (untreated diabetic rats) and group IV (treated diabetic rats). Experimental animals (groups II and IV) received a daily dose of 300 mg/kg body weight of AALGEt incorporated in their drinking water for 3 weeks. Control animals received water without AALGEt during the same period. Fasting blood glucose levels were determined by means of MediSense®'s Optimum™ Xceed Diabetes Monitoring system and blood glucose test strips before treatment (day 0) and on day 1 (4 h), day 7, day 14 and day 21 after initiation of treatment. Body weights and water intake of all groups of rats were assessed on the same days that blood glucose levels were measured.

On day 22, blood was withdrawn from the heart of each rat under general anaesthesia. The collected blood was heparinised and centrifuged for 20 min at 3000 xg and used for the mea-surement of plasma glucose, plasma triglycerides and plasma insulin. Following blood collection, rats were euthanized using sodium thiopentone (200 mg/kg). Liver tissue was removed from each animal, washed with saline and stored at -70°C until used for the assay of glucokinase and glucose-6-phosphatase activities.

Determination of plasma glucose, triglycerides and insulin levels

Plasma glucose and plasma triglyceride levels were measured using commercially available kits based, respectively on the glu-cose oxidase and the glycerol blanked methods. Both methods were adapted to the Beckman Coulter®'s UniCell DXC 800 Synchron® Clinical System. Plasma insulin levels were determined by the enzyme linked immunosorbant assay (ELISA) adapted to the Beckman Coulter® Ireland Inc's UniCell DXI 800 Access® Immunoassay System.

Measurement of hepatic glucokinase activity

About 500 mg liver tissues from each rat was homogenized in 5 ml ice-cold homogenization buffer containing 100 mM KCl and 20 mM HEPES (pH 7.9). The homogenates were centrifuged at 3000 xg for 20 min at 4°C. Glucokinase activity in the supernatants was measured by the pH sensitive colorimetric assay published by Chapman and Wong (2002) in a reaction mixture containing 20 mM HEPES pH 7.9, 5 mM ATP, 5 mM $MgCl_2$, 5 mM D-glucose and 0.2 mM Cresol Red. Absorbance measurements were taken at 570 nm after incubation at 37°C for 30 min. The glucokinase activity in the supernatants was expressed as units/mg protein. One unit of glucokinase is defined as the amount of protons liberated/minute at 37°C under the specified assay conditions. Protein concentration in supernatants was assayed by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Assay of hepatic glucose-6-phosphatase activity

Liver microsomal fractions were prepared according to the ultra-centrifugation method published by Pari and Satheesh (2006). Briefly, rat liver (1.0 g) was homogenized in 5 ml ice-cold 5 mM HEPES (pH 7.4) containing 0.25 M sucrose. The homogenate was centrifuged at 10 000 ×g for 20 min, at 4°C after which the supernatant was centrifuged again at 105 000 ×g for 60 min. The microsomal pellets obtained were re-suspended in same buffer and

Table 1. Effects of 3 weeks treatment with AALGEt (300 mg/kg body weight) on blood glucose levels of normal and alloxan-induced diabetic rats.

Rat groups	Average blood glucose (mmol/L)			
	Day 0	Day 7	Day 14	Day 21
Normal control rats	4.1 ±0.5	5.9 ±0.6	4.7±0.9	4.8 ±0.7
Normal + AALGEt	4.3 ±1.3	5.2 ±0.8	4.6 ±0.5	5.1 ±0.7
Diabetic control rats	1.5 ±1.3	2.2 ±8.2	>27.8**	>27.8**
Diabetics + AALGEt	20.1 ±4.2	1.2 ±5.6¥	9.4 ±4.3¥¥	7.2 ±6.8¥¥

Each value represents ± SD, n = 8. Mean values were significantly different from those of normal control rats. *$p < 0.01$, **$p < 0.001$. Mean values were significantly different as compared to diabetic control rats, ¥$p < 0.01$, ¥¥$p < 0.001$.

their glucose-6-phosphatase enzyme activity was assayed according to the method developed by Baginski et al. (1974). In this method, glucose-6-phosphate is converted by glucose-6-phosphatase (G6Pase) in liver microsomal fractions into glucose and inorganic phosphate. The inorganic phosphate liberated is allowed to react with ammonium molybdate in the presence of ascorbic acid (Fiske and Subbarow, 1925). The amount of phosphate liberated per unit time determined as the blue phosphomolybdenum complex at 700 nm is a measure of G6Pase. G6Pase activity was expressed as units/mg of protein. One unit of glucose-6-phosphatase activity was defined as the amount of Pi liberated/min at 37°C under the specified assay conditions.

Water consumption and body weight determination

Daily water consumption was determined by calculating the difference between amount of water (200 ml) supplied and the volume of water remaining after 24 h. Body weight was determined gravimetrically.

Statistical analysis

Data expressed as mean ± SD were analyzed using the Sigma Stat statistical program (version 8.0). Comparisons were made between normal and alloxan-induced diabetic rats as well as between treated and untreated alloxan-induced diabetic rats by means of unpaired Student's t-test and their significance were established by analysis of variance analysis (ANOVA). Differences of $p < 0.05$ were considered statistically significant.

RESULTS

There was no significant difference in the fasting blood glucose levels of normal rats treated with AALGEt when compared with that of normal untreated rats (Table 1). However, a steady increase in the blood glucose levels of diabetic control rats was observed throughout the entire experiment. The increase in the blood glucose levels of untreated diabetic rats was significantly higher on day 7 ($p < 0.01$), day 14 ($p < 0.001$) and day 21 ($p < 0.001$) when compared with normal control rats. Treatment of diabetic rats with AALGEt for 3 weeks significantly reduced their blood glucose levels when compared with those of diabetic control rats on day 7 ($p < 0.01$), day 14 ($p < 0.001$) and day 21 ($p < 0.001$).

At the end of the experimental period, plasma glucose, triglyceride and insulin were measured. Plasma glucose and triglyceride levels in untreated diabetic animals were significantly higher ($p < 0.001$ and $p < 0.05$, respectively) (Figure 1A and B).

Treatment of diabetic rats with AALGEt for 3 weeks significantly reduced plasma glucose ($p < 0.01$) by 66.7% and plasma triglycerides by 25% when compared with untreated diabetic rats. Oral administration of AALGEt to diabetic rats for 3 weeks significantly increased plasma insulin levels when compared to diabetic controls (Figure 1C).

Changes in body weight and water intake of normal control, treated normal rats, diabetic controls and AALGEt treated diabetic rats on days 1, 14 and 21 of treatment are shown in Figure 2A and B. The changes in the activities of hepatic glucokinase (GK) and G6Pase in normal and diabetic control are shown in Table 2. Alloxan treatment caused a decrease in the hepatic GK activity and a significant increase ($p < 0.05$) in the activity of hepatic G6Pase of diabetic rats when compared to normal control rats (Table 2). Furthermore, oral administration of AALGEt to normal and diabetic rats did not cause any significant difference in the activity of hepatic GK. On the other hand, oral administration of AALGEt for 3 weeks resulted in a significant decrease ($p < 0.05$) in hepatic G6Pase activity.

A significant decrease in body weight was observed in untreated diabetic rats when compared with normal control rats on day 14 ($p < 0.01$) and day 21 ($p < 0.001$) (Figure 2A). There was a significant increase in the water intake of diabetic rats than in normal control rats on days 7, 14 and 21 ($p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively) (Figure 2B). Treatment of diabetic rats with AALGEt did not significantly attenuate the observed weight loss. However, treatment of diabetic rats with AALGEt significantly reduced the water intake to lower levels than untreated diabetic rats ($p < 0.01$ on day 14 and $p < 0.001$ on day 21).

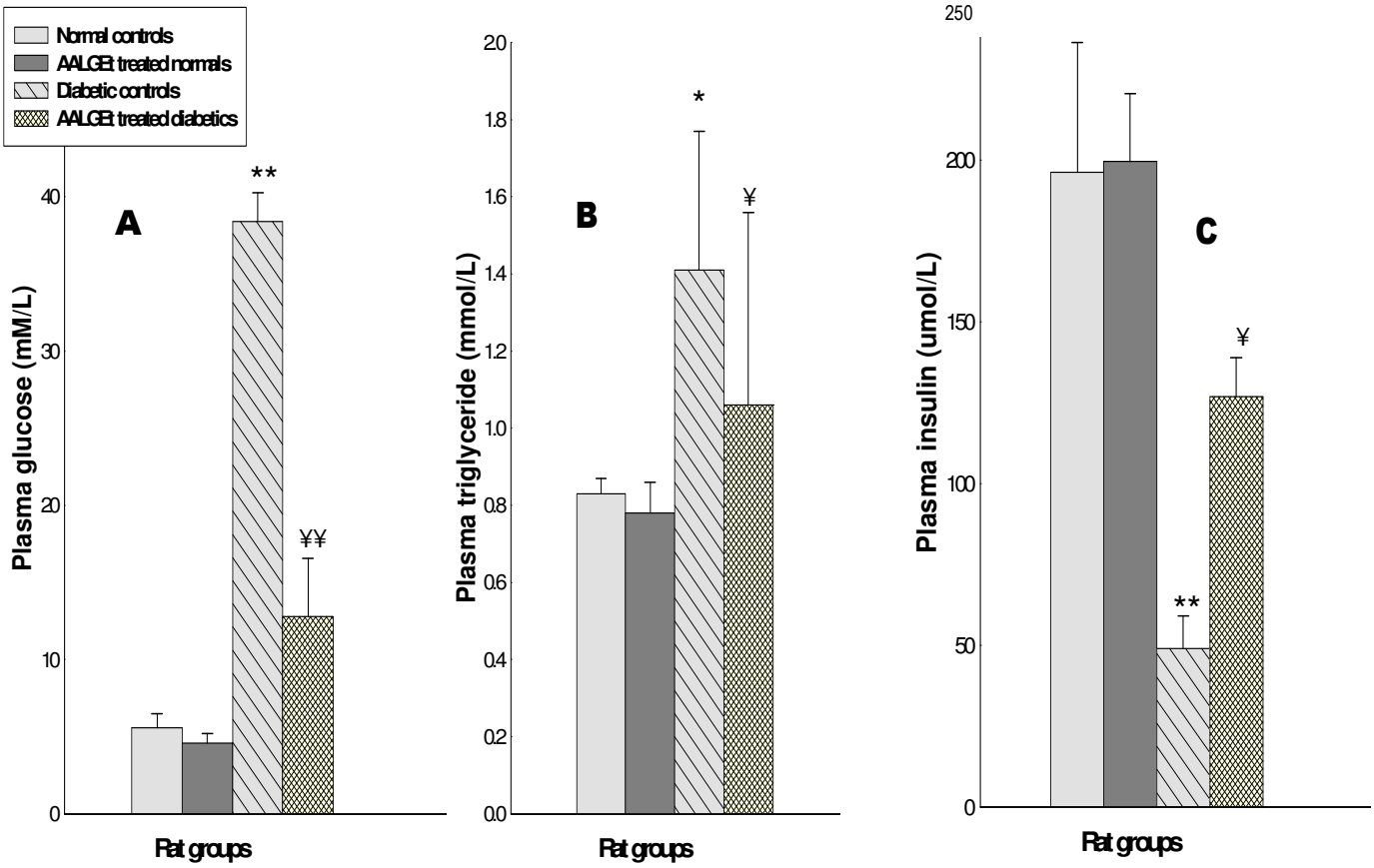

Figure 1. Effects of 3 weeks treatment with AALGEt (300 mg/kg body weight) on plasma glucose (A), plasma triglyceride (B) and plasma insulin (C) levels of normal and alloxan-induced diabetic rats. Mean values were significantly different as compared to normal control rats, *p < 0.05, **p < 0.01. Mean values were significantly different as compared to diabetic control rats, ¥P < 0.05, ¥¥p < 0.01.

DISCUSSION

Alloxan induces diabetes by damaging the insulin secreting cells of the pancreas leading to hypoinsulinemia and hyperglycaemia (Szudelski, 2001). In agreement with these known effects of alloxan, the blood glucose levels of untreated alloxan-induced diabetic rats were significantly increased throughout the current study when compared to those of normal control rats. Continuous treatment of alloxan-induced diabetic rats with AALGEt (300 mg/kg body weight) for a period of 3 weeks resulted in a significant decrease in the blood glucose levels of treated diabetic rats when compared to diabetic controls but no such effect was observed in normal treated rats. Thus, unlike the use of insulin or sulfonylurea drugs, which cause severe hypoglycaemia when taken in excessive doses (Bastaki, 2005; Cheng and Fantus, 2005), continuous use of AALGEt or its accidental overdose will not result in hypoglycaemic shock. Furthermore, results of the current study suggest that the blood glucose lowering effect of AALGEt can last for a longer period of time. These observations concur with the findings of Beppu et al. (1993, 2006) in alloxan-induced diabetic mice.

Continuous treatment of alloxan-induced diabetic rats with AALGEt (300 mg/kg body weight) for 3 weeks also significantly increased (but did not normalize) the plasma insulin levels of diabetic rats as compared to diabetic controls. However, AALGEt did not significantly alter plasma insulin levels of normal rats. Thus, like the aloe vera leaf gel extract (Reynolds and Dweck,1999), AALGEt might exert its blood glucose lowering effects through stimulation of insulin secretion from pancreatic beta cells. However, the fact that AALGEt did not significantly alter the plasma insulin levels of normal rats but significantly increased plasma insulin levels of alloxan induced-diabetic rats towards the normal value, suggest that, AALGEt may either regenerate damaged pancreatic beta cells or protect them from further damage by alloxan. Since alloxan is known to exert its diabetogenic effect through generation of oxygen free radicals, this hypothesis is in agreement with the findings of Beppu et al. (2003) and Chacko et al. (2008), who reported that *A. arborescens* components possess free radical scavenging effects that may prevent pancreatic islet beta-cell destruction by free radicals generated by alloxan. Thus,

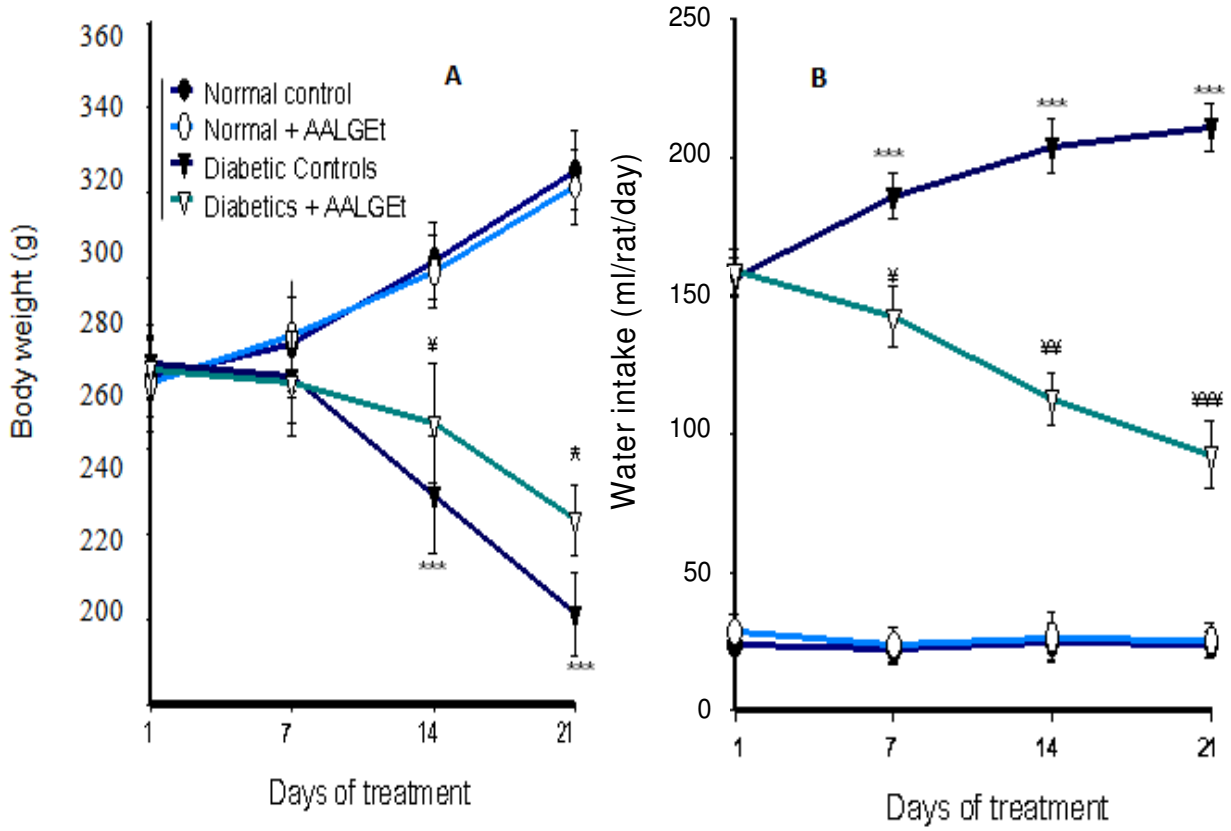

Figure 2. Effects of 3 weeks treatment with AALGEt (300 mg/kg body weight) on body weight (A) and water intake (B) of normal and alloxan-induced diabetic rats. Mean values were significantly different compared to normal control rats, $^{*}p < 0.05$, $^{***}p < 0.001$. Mean values were significantly different compared to diabetic control rats, $^{¥}p < 0.05$, $^{¥¥}p < 0.01$, $^{¥¥¥}p < 0.001$.

Table 2. Effects of treatment with AALGEt (300 mg/kg body weight) on the activities of hepatic glucokinase and glucose-6-phosphatase

Rat groups	Glucokinase (U/g protein)	Glucose 6- phosphatase (U/mg protein)
Normal control rats	298.01 ± 11.4	0.198 ± 0.05
Normal + AALGEt	304.56 ± 8.34	0.213 ± 0.06
Diabetic control rats	282.82 ± 6.18	0.521 ± 0.02*
Diabetics + AALGEt	288.13 ± 9.81	0.282 ± 0.06¥

Each value represents ± SD, n = 8. Mean value was significantly different from those of normal control rats. *p < 0.05. Mean value was significantly different as compared to diabetic control rats, ¥p < 0.05.

in this respect, AALGEt probably act like plant ex-tracts of *Gymnema sylverstre* (Chattopadhyay, 1998) and *Ipomea batatas* L. (Kusano and Abe, 2000) which are reported to repair pancreatic islet cells damaged by either alloxan or streptozotocin (Bnouham et al., 2006).

Uncontrolled diabetes mellitus is often associated with abnormal plasma lipid levels, in particular, elevated plasma triglyceride levels and reduced plasma HDL-cholesterol levels (Sniderman et al., 2002; Mooradian, 2009). Hypertriglyceridemia represents an independent risk factor for the development of coronary heart disease in people with type 2 diabetes (Ooi and Ooi, 1998; Sniderman et al., 2002). We have observed in this study, that continuous treatment of alloxan-induced diabetic rats with AALGEt (300 mg/kg body weight) for a period of 3 weeks significantly reduced their plasma triglyceride levels when compared with diabetic control rats. Under normal circumstances, insulin inhibits the enzyme hormone sensitive lipase (HSL), which mobilizes free fatty acids (FFAs) from adipose tissues (Kwiterovich, 2000; Smith et al., 2005). FFAs released by the action of HSL are the major substrates for the hepatic triglyceride

synthesis (Kwiterovich, 2000). Insulin also activates the enzyme lipoprotein lipase (LPL), which clears triglyceride rich proteins from blood plasma (Kwiterovich, 2000; Smith et al., 2005). However, in a diabetic state, HSL is not inhibited and LPL is not activated due to insulin deficiency (Kwiterovich, 2000; Mlinar et al., 2007). Thus, the effect of AALGEt on plasma triglyceride levels could be attributed to its effects on plasma insulin levels.

Hepatic glycogenolysis and gluconeogenesis are major causes of fasting hyperglycemia seen in both type 1 and type 2 diabetes mellitus (Smith et al., 2005). Inhibition of enzymes involved in gluconeogenesis and/or glycolgenolysis therefore constitutes an alternative approach to suppress hepatic glucose production and lower fasting plasma glucose (Agius, 2007). G6Pase catalyses the final reaction in hepatic glucose production by both gluconeogenesis and glycogenolysis, and has been proposed as a potential target for antihyperglycaemic drugs for type-2 diabetes (Pari and Satheesh, 2006; Agius, 2007). A significant increase in the activity of the hepatic G6Pase of untreated alloxan-induced diabetic rats as compared to normal rats was observed in the current study. Treatment of diabetic rats with AALGEt (300 mg/kg body weight) for 3 weeks resulted in a significant reduction of GPAase activity when compared to diabetic controls. Thus, AALGEt could contain substances that act like the oral hypoglycaemic agent, metformin (Bastaki, 2005; Cheng and Fantus, 2005) and plant extracts of *Boerhaavia diffusa* L. (Pari and Satheesh, 2004) and *Pterocarpus marssupium* (Pari and Satheesh, 2006) which have been shown to lower blood sugar levels by inhibiting hepatic production of glucose.

Glucokinase is a rate limiting enzyme involved in the hepatic storage and utilization of glucose (Smith et al., 2005). Following a carbohydrate rich meal, hepatic GK clears a significant amount of glucose from the blood circulation and facilitates its conversion into glycogen and fatty acids (Smith et al., 2005; Agius, 2007). Thus, hepatic GK play a significant role in the prevention of postprandial hyperglycemia. The activity of hepatic GK is reported to be reduced in alloxan-induced experimental diabetes (Zhang et al., 2007) and some antidiabetic plant extracts are reported to exert their hypoglycemic activity, in part by increasing the activity of this enzyme (Pari and Satheesh, 2006). In agreement with the known effects of alloxan on the activity of GK (Zhang et al., 2007), we have observed in this study that the hepatic GK activity was significantly reduced when compared to normal controls. However, oral administration of AALGEt (300 mg/kg body weight) to both normal and alloxan-induced diabetic rats for 3 weeks did not significantly alter the activity of hepatic GK in these animals.

Uncontrolled diabetes mellitus is also associated with body weight loss, polyurea, polydypsia and polyphagia (Bastaki, 2005). Weight loss in diabetes mellitus results from a combination of dehydration (caused by frequent urination), increased breakdown of muscle proteins (for provision of gluconeogenic amino acids) and enhanced mobilization of fat stores (provision of FFAs to be used as fuel) (Bastaki, 2005; Smith et al., 2005). These events are directly or indirectly related to insulin deficiency or lack of insulin actions (Smith et al., 2005). We have observed a significant decrease in the body weight of untreated alloxan-induced diabetic rats as compared to normal control rats. The loss of weight in alloxan-induced diabetic rats was accompanied by increased water intake (polydypsia) by these rats, suggesting that dehydration was a contributing factor towards weight loss. Continuous treatment of alloxan-induced diabetic rats with AALGEt for 3 weeks significantly prevented body weight loss. However, the mean body weight of AALGEt treated rats was still significantly reduced when compared to that of the normal control rats. The marked improvement in body weight loss of treated diabetic rats as compared to untreated alloxan induced diabetic rats could also be attributed to the effect of AALGEt on plasma insulin levels and hence on improved glucose metabolism in these rats.

The findings of this study suggest that oral administration of an aqueous extract of the leaf gel of *A. arborescens* (AALGEt) (300 mg/kg body weight) for 3 weeks brings about significant beneficial effects in various physiological/biochemical parameters altered by the diabetic state. Although, the exact chemical compound(s) responsible for the antidiabetic activity of AALGEt are currently unknown, AALGEt appears to exert its blood glucose lowering effect in alloxan-induced diabetic rats by protecting insulin secreting pancreatic beta cell from further damage by alloxan. Further studies, in particular, histological studies of pancreatic beta cells of AALGEt treated alloxan-induced diabetic rats are needed in order to confirm this hypothesis. Furthermore, the isolation and identification of active compounds AALGEt will go a long way in promoting the use and acceptance of *A. arborescens* leaf as an antidiabetic remedy. Although, lower doses may also have led to a positive effect, the dosage that was used in these experiments (equivalent to 2.4 g/day per 80 kg human) were probably higher than the dosage used by traditional healers. In general, these results strongly support the traditional use and preparation of AALGEt for treating diabetes in humans.

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