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Hypoglycemic Activity of the Root and Stem of *Salacia reticulata* var. *β-diandra* in Alloxan Diabetic Rats

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Abstract

The hypoglycemic activity of the aqueous extract of the root bark of *Salacia reticulata* (known as *kotala himbatu*) has previously been confirmed in the normal healthy rat model. In this investigation, molecules present in the powdered root and stem of *Salacia reticulata* Weight var. β-*diandra* were fractionated according to their polarity into several fractions by sequential extraction. Hypoglycemic potential of each fraction was evaluated, and the effect of active fraction was investigated in alloxan diabetic rats given long-term oral treatment. A significant hypoglycemic activity was established in the precipitate of methanol fraction, and it was used for the long-term oral treatment. Chronic oral administration of the precipitate from methanol fraction to alloxan diabetic rats twice a day for 120 days improved glucose tolerance and significantly reduced fasting blood glucose, fructosamine, and glycosylated hemoglobin levels. The polydypsia, hyperphagia, and weight loss of the alloxan diabetic rats were also reduced by the treatment. The results suggest that the hypoglycemic effect of *Salacia reticulata* in alloxan diabetic rats may involve an extrapancreatic effect on glucose production or clearance.

Keywords: Alloxan diabetic rats, fructosamine, glycosylated hemoglobin, hypoglycemic activity, *Salacia reticulata* Weight var. β-*diandra*, Sprague-Dawley rats.

Introduction

Traditional medicinal practices remain culturally strong in Asia, especially in the Orient (WHO Report, 1980). In Sri Lanka and India, aqueous extracts of several plant species are administered orally to control glycemia and other diabetic complications (Attygale, 1952; Jayaweera, 1981). Approximately 40 plants available locally are reputed to have oral hypolgycemic activity. However, very few of these plants have been investigated and the majority await adequate scientific and medical evaluation to assess their efficacy. Some of these plant treatments have been shown to assist glycemic control in animal models such as normal and diabetic rats and in non-insulin-dependent diabetic patients.

Salacia reticulata var. β-*diandra* Weight (Celestraceae) (known as *Kotala himbatu*) is a woody climber growing in the low-country rain forests of the southern region of Sri Lanka. Regular consumption of aqueous decoction of the root bark of the plant is recommended by Ayruvedic and traditional medical practitioners in Sri Lanka in the treatment of diabetes mellitus (Attygalle, 1952; Jayaweera, 1981). In the course of our study on natural antidiabetic principles and foodstuffs, the antidiabetic activity of *Salacia reticulata* (Ruvin Kumara et al., 1994, 1997, 2000[a], 2000[b], 2003) was established in animal models and non-insulin dependent diabetic patients. As a continuation of screening natural products for antidiabetic activity, some of the active principles were isolated (Ruvin Kumara et al., 1994, 2003).

The current study has been undertaken to establish the active fraction and evaluate the acute and cumulative effects of prolonged oral treatment (120 days) with the active fraction of *Salacia reticulata* in alloxan diabetic rats.

Materials and Methods

Experimental animals

Male Sprague-Dawley rats, 150 to 200 g, were used and maintained on a standard laboratory diet and tap water

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and had free access to food and water. Ethical approval for the study was given by the ethics committee of the Faculty of Medicine, University of Ruhuna, Sri Lanka. Diabetes was induced in the rats by the injection of 150 mg kg^{-1} of alloxan monohydrate (Sigma Chemical, St. Louis, Mo, USA) intraperitoneally. Fasting blood-glucose was determined in each rat prior to and +2, +4, and +6 days after alloxan administration. After a stabilization period, only those animals showing serum glucose concentrations between 140 and 200 mg dl^{-1} were employed in the study. Two groups of diabetic rats of 12 each were used for the investigation of long-term effect of active fraction in diabetic rats. A third group of healthy normal rats matched for age and sex, receiving the same amount of distilled water, served as normal control group.

Preparation of the drug

The plant material was collected from Elpitiya in the southern region of Sri Lanka. The botanical identity of the plant was confirmed by comparison with authentic samples from the herbarium of the Royal Botanical Garden (Peradeniya, Sri Lanka) and the Ayurvedic Research Institute of Sri Lanka. Air-dried root and stem of *Salacia reticulata* plant (500 g) was powdered, macerated, and subjected to sequential extraction with different solvents in order of increasing their polarity. Sequential extraction was performed for 6 h for each solvent (3 l) in a Soxhlet apparatus using pet ether, ethyl acetate, methanol, and water, successively. Each fraction (3 l) was evaporated under reduced pressure in a rotary evaporator to yield solid dry mass. Relevant dosages of each solid fraction were suspended in 2% methylcellulose in distilled water to make soluble adjuvant. Powdered tolbutamide (150 mg) was suspended in 2% methylcellulose in distilled water (100 ml) and used as an inducer of the hypoglycemic activity.

Investigation of different fractions

Diabetic rats were randomly divided into six groups of six each after collection of blood samples for fasting serum glucose levels. Relevant dosage of pet ether fraction (233 mg kg^{-1}), ethyl acetate fraction (29 mg kg^{-1}), precipitate from methanol fraction (350 mg kg^{-1}), water fraction (500 mg kg^{-1}), and tolbutamide (15 mg kg^{-1}) were administrated via stomach tube to five groups of diabetic rats, respectively. The control group received an equivalent volume (10 ml kg^{-1}) of adjuvant prepared from 2% methylcellulose in distilled water. Serum glucose levels were monitored at 1-h intervals for 4 h. Blood samples were collected by venosection of the tail vein, serum was separated immediately, and 10 µl was used for the estimation of blood glucose levels by the glucose oxidase method.

The procedure was repeated as an oral glucose tolerance test by giving an oral dose of glucose (10 ml kg^{-1}, 50% w/v) 15 min after the administration of each fraction.

Investigation of active fraction in long-term treatment

The powdered methanol fraction (175 mg kg^{-1}) was dissolved in water and administered via a stomach tube twice a day for 120 days to one group of 12 diabetic rats. Control diabetic animals received an equivalent volume (10 ml kg^{-1}) of distilled water. A third group of 12 normal healthy rats matched for age and sex received the same volume of distilled water and served as the normal control group.

Blood samples were drawn from the tail vein of fasted conscious animals before and after 14, 35, 50, 75, and 120 days of treatment with *Salacia reticulata* for the estimation of serum fasting glucose, and samples drawn on days 50, 75, and 120 were also used for the estimation of fructosamine levels. Samples for the determination of glycosylated hemoglobin (HbA_{1c}) and insulin concentrations were also taken on day 120 of treatment.

A glucose tolerance test was performed on the 75th day of treatment. After taking the fasting blood samples, animals were given an oral dose of glucose (10 ml kg^{-1}, 50% w/v), and blood samples were collected at 30-min intervals for 3 h. Body weight, food intake, and fluid intake of the animals were monitored daily for 120 days.

Analysis

Serum glucose concentrations were measured based on the glucose oxidase method (Hugget et al., 1957) using glucose oxidase kit (Roch, GmbH, Germany), glycosylated hemoglobin by the ion capture method (Middle, 1983) using Multigent HbA_{1c} assay kit in Abbot-IMX analyzer (USA), fructosamine by the nitroblue tetrazolium reduction method (Johnson et al., 1983) using fructosamine kit (Boehringer, GmbH, Germany) and insulin by the method of Clark and Hales (1991) using ELISA insulin assay kit (Boehringer, GmbH, Germany), respectively.

Statistical analysis

Groups of data were compared using Student's *t*-test. Differences were considered to be significant for $p < 0.05$.

Results

Induction of diabetes in rats with alloxan impaired their body weight gain and produced hyperglycemia, hypoinsulinemia, hyperphagia, and polydypsia (see Fig. 3). The effect of different fractions obtained from sequential

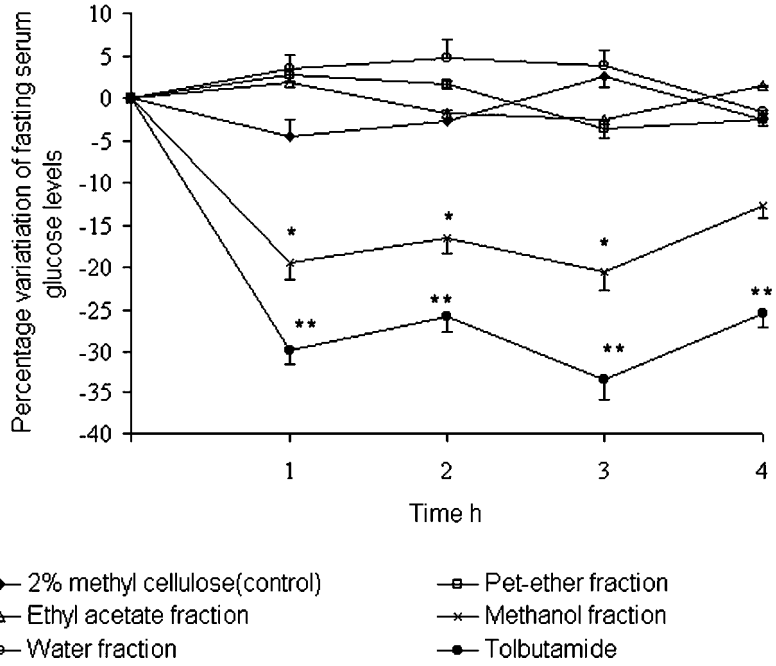

Figure 1. Effect of different fractions of *S. reticulata* on % variation of fasting blood glucose levels of alloxan diabetic rats. Rats were administered a relevant dosage of fractions dissolved in 2% methyl cellulose or just 2% methyl cellulose for the control. Serum glucose levels were monitored at 1 h intervals for Each point is a mean of six determinants ± SEM. When examined by Student's *t*-test, significantly different from control values. $^{*}p < 0.01$, $^{**}p < 0.001$.

extraction of *S. reticulata* on percentage variation of fasting blood glucose levels are shown in Figure 1. The group treated with methanol fraction showed a significant reduction ($p < 0.01$) in the fasting blood glucose levels compared with the control group, whereas groups treated with other fractions did not show significant hypoglycemic activity.

The effect of different fractions on oral glucose tolerance is shown as area under-the-curve in Figure 2. The group treated with the methanol fraction showed significant improvement ($p < 0.01$) in oral glucose tolerance in diabetic rats compared to the control group.

Oral administration of the methanol fraction of *S. reticulata* twice daily to the diabetic animals reduced the fluid and food intake. The gain in body weight was also improved in the drug-treated group (Fig. 3). Percentage increase in blood glucose concentration was consistently less in the drug-treated group than in the control group throughout the experiment (Fig. 3), the values showing significant differences from the control diabetic group from day 50 ($p < 0.05$) through day 75 ($p < 0.01$) to day 120 ($p < 0.001$).

Serum concentration of fructosamine in the treatment, control diabetic, and normal control groups again showed a pattern very similar to variations in fasting serum glucose concentration. Significant reductions in the serum fructosamine concentration in the treatment diabetic group, compared with the diabetic control group, were observed on the 50th ($p < 0.01$), 75th ($p < 0.01$) and 120th days ($p < 0.05$) of treatment (Fig. 3).

Results of the glucose tolerance test performed on the 75th day after commencement of the experiment expressed as area under-the-curves of serum glucose levels. The results show that there is a highly significant reduction ($p < 0.01$) in the area under-the-curve in the diabetic-treated group (536.6 ± 9.91) relative to the diabetic control group (1045.05 ± 153.07), and the value was comparable to the normal control group (401.23 ± 23.5).

The estimation of glycosylated hemoglobin as a parameter of metabolic control in diabetes is now well established (Goldstein et al., 1982), particularly as it reflects the overall metabolic control present over the preceding 4 weeks. In the current investigation, the treatment of alloxan diabetic rats with *S. reticulata* commenced on the sixth day after induction of diabetes. The glycosylated hemoglobin measured 120 days after commencement of the experiment shows a high HbA_{lc} concentration in the control diabetic group (Table 1). The HbA_{lc} concentration in the treatment group showed a significant reduction when compared to the control diabetic group, clearly showing that the plant extract exerts a control on the homeostasis of blood glucose.

As shown in Table 1, long-term treatment with *S. reticulata* did not show any significant differences in the insulin concentrations between the treated diabetic

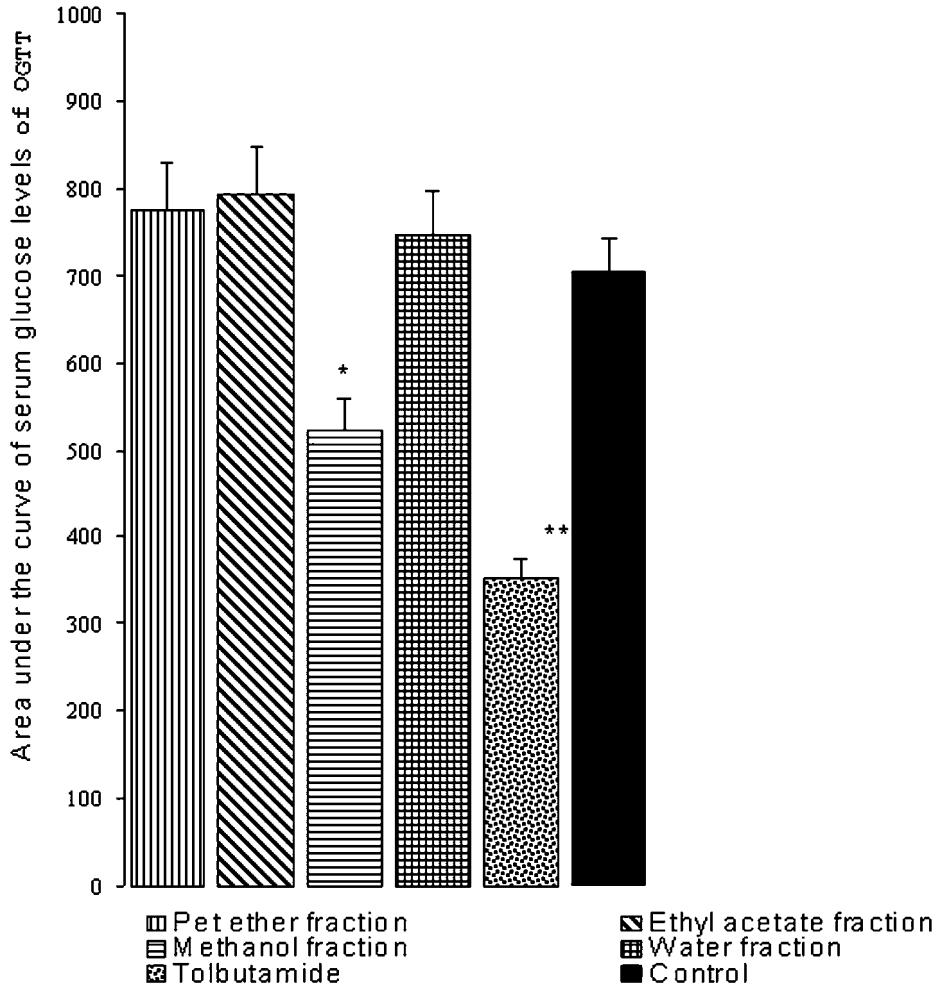

Figure 2. Effects of different fractions of *S. reticulata* on an oral glucose tolerance test in alloxan diabetic rats. The bars represent the area under-the-curve of blood glucose levels in oral glucose tolerance tests (OGTT). Rats were administered a relevant dosage of fractions dissolved in 2% methyl cellulose or 2% methyl cellulose. Oral glucose load (10 ml/kg, 50% w/v) was given 15 min after the administration of each fraction. Serum glucose levels were monitored at 1 h intervals for 4 h. Each point is a mean of six determinants $\pm$ SEM. When examined by Student's *t*-test, significantly different from control values. $^{*}p < 0.01$, $^{**}p < 0.001$.

and control diabetic groups, although the insulin concentration in the treated group was higher.

Discussion

Traditional herbal preparations for the treatment of diabetes mellitus continue to receive widespread use in many countries (Said 1969). Several such plant preparations have been shown to possess hypoglycemic properties in animal models of diabetes and in human non-insulin-dependent diabetes mellitus patients (Hale et al., 1989; Ruvin Kumara, 1998).

Administration of aqueous extract of *S. reticulata* stem (5 g kg^{-1}) to normal, diabetic rats and non-insulin-dependent diabetes mellitus patients showed gastrointestinal side effects, abdominal discomfort, and diarrhea due to soluble short-chain hydrocarbons. Sequential extraction removed soluble short-chain hydrocarbons and other unwanted compounds to yield precipitate from methanol fraction that eliminate diarrhea, discomfort, and other gastrointestinal side effects (Ruvin Kumara, 1998; Ruvin Kumara et al., 2000a, 2000b, 2003). In the current investigation, chronic administration of the methanol fraction of *S. reticulata* to alloxan diabetic rats significantly reduced hyperglycemia and other symptoms of the diabetic condition within 50 days of treatment and continued until the end of the experimental period. The basal insulin concentrations measured 120 days after continuous treatment were not significantly different in the less hyperglycemic treatment group. Whether the observed small increment in circulating insulin is responsible for the control of hyperglycemia and all other effects on glucose homeostasis observed in the study is doubtful. Because the insulin response is insignificant, improvement of

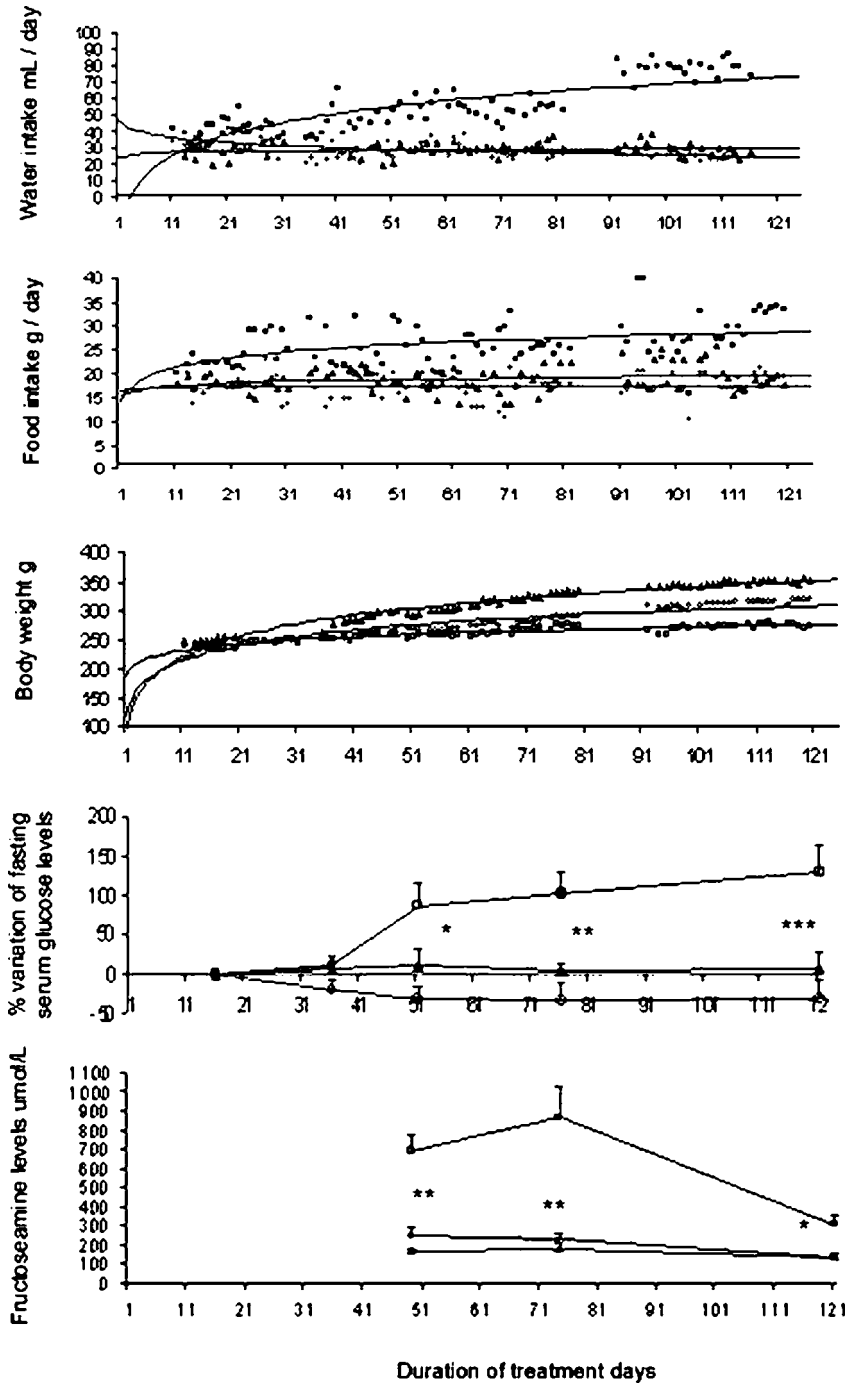

Figure 3. Effect of long-term treatment (120 days) with precipitate from methanol fraction of *S. reticulata* on body weight, water and food intake, fructoseamine levels, and percentage variation of fasting blood glucose levels in alloxan diabetic rats. Precipitate from methanol fraction (175 mg kg^{-1}) was administered twice a day to diabetic treated group (-O-) and the same volume of distilled water to the diabetic untreated group (-□-) for 120 days. A third group of healthy rats (-▲-) matched for age and sex receiving same amount of distilled water served as control group. The body weight and food and water intake of rats were monitored daily. Fasting blood glucose levels were measured on the 14th, 75th, 35th, 50th, and 120th days while fructoseamine levels were measured on the 50th, 75th, and 120th days after commencement of the experiment. Each line is a power regression and each point is a mean of 10 determinations ± SEM. $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$ compared with diabetic control group.

glycemic control in diabetic rats observed in the current investigation could involve an extrapancreatic effect on glucose disposal.

Some extracts of *Salacia macrosperma*, a species related to *S. reticulata*, have been shown to correct all metabolic aberrations of diabetes mellitus in severe

Table 1. Effect of long-term treatment with methanol extracts (350 mg kg^{-1}) of *Salacia reticulata* on serum insulin and glycosylated hemoglobin levels.

Group	Serum insulin (μU ml^{-1})	HbA_{1c} (percentage)
Diabetic rats treated with the extract	2.41 ± 0.19 (NS)	$5.72 \pm 0.35^*$
Diabetic rats untreated (given distilled water)	1.895 ± 0.714	9.72 ± 0.98
Normal rats untreated (given distilled water)	3.54 ± 0.63	5.26 ± 0.37

Effect of long-term treatment with methanol extracts (350 mg kg^{-1}) of *Salacia reticulata* on serum insulin and glycosylated hemoglobin levels performed at the end of the experiment in diabetic rats relative to diabetic control and normal rats given distilled water (10 ml kg^{-1}). Blood samples were taken from the three groups of rats, serum was separated immediately, and 25 μl was assayed for insulin. EDTA was added to blood, and 100 μl were assayed for glycosylated hemoglobin (HbA_{1c}). Data are expressed as mean ± SEM. When examined by Student's *t*-test, no significant difference was observed in serum insulin concentration, whereas the mean glycosylated hemoglobin concentration in the diabetic treated group was significantly different from the diabetic untreated group. $^*p < 0.05$.

alloxan diabetic rats within a period of 8 days (Venkateswarlu et al., 1993). An insulin-like activity has been suggested for the active principles present in these extracts. Metabolic aberrations such as impaired body weight gain, hyperglycemia, hyperphagia, and polydypsia of alloxan diabetic rats in the treated group were significantly corrected (Fig. 3) by the long-term treatment with the methanol extract of *S. reticulata* twice a day for 120 days. Thus, *S. reticulata* extract may contain active principles mimicking insulin actions at the cellular level as suggested by Venkateswarlu et al. (1993).

Masayuki et al. (1997) have shown that a single dose (25–100 mg kg^{-1}) of a crude extract of *S. reticulata* reduced blood glucose levels by reducing the glucose absorption from the gut, in a sucrose tolerance test in normal rats. Hence, inhibition of sucrase and maltase by the extract in the gut has been suggested. Salicinol and kotalanol isolated from root of *Salacia oblonga* have been shown to possess inhibitory effect on sucrase and moltase enzymes (Hisashi et al., 1998).

In the current investigation, chronic administration of the precipitate from methanol fraction (350 mg kg^{-1} day^{-1}) gradually reduced hyperglycemia and other symptoms of the diabetic condition in alloxan diabetic rats. Inhibition of brush border disaccharides and oligosaccharides could be responsible for this effect as suggested by reduction in area under-the-curve of the oral sucrose tolerance test in non-insulin-dependent diabetic patients (Ruvin Kumara et al., 2003). Thus, the findings of the Masayuki et al. (1997) and Hisashi et al. (1998) are partly compatible with our results.

Acarbose, a drug that is used to control hyperglycemia in NIDDM patients in some countries, is a reversible, competitive inhibitor of brush border α-glucosidase (Jenkins et al., 1981). It reduces postprandial glycemia and delays the glucose peak due to inhibition of the digestion of oligosaccharides and disaccharides at the brush border, resulting in slower absorption of monosaccharides. Percentage increase in blood glucose and fructoseamine concentrations were consistently less in the drug-treated group than in the control group throughout the experiment. This proved that *S. reticulata* extract could posses similar enzyme inhibitors that interfere with degradation of carbohydrates to glucose and other monosaccharides, reduce postprandial glycemia, and delay the glucose peak. This may at least explain part of the hypoglycemic effect of *S. reticulata* extract.

In addition to the inhibition of glucose absorption, the methanol fraction of *S. reticulata* reduced fasting serum glucose levels (Fig. 1) and area under-the-curve of the glucose tolerance test (Fig. 2) in acute treatment with the methanol extract. Significant reduction is also observed in fasting blood glucose levels ($p < 0.001$) at the 120th day (Fig. 3) and the area under-the-curve of the glucose tolerance test ($p < 0.01$) performed at the 75th day of the chronic treatment.

The reduction in fasting serum glucose is closely correlated with the decline in basal hepatic glucose production by inhibiting hepatic gluconeogenesis and glycogenolysis. This is due to L-diandrol (unpublished data) present in the methanol fraction, which inhibits hepatic key regulatory enzymes present in gluconeogenesis and glycogenolysis pathways. The reduction in area under-the-curve of the glucose tolerance test may be due to a decline in glucose absorption, enhanced peripheral utilization, or sensitizing insulin induced glucose uptake at the tissue level.

Improvement in body weight gain, reduction in fluid and food intake, and other metabolic aberrations of diabetes mellitus in alloxan diabetic rats suggest that the methanol fraction could increase the peripheral use of blood glucose or sensitizing glucose-induced insulin release from the β-cells of the pancreas or correct the abnormal activity of aldose reductase enzyme. Sorbitol synthesized from freely available glucose by aldose reductase enzyme in the polylol pathway has been implicated in chronic complications such as peripheral neuropathy, retinopathy, and cataracts of diabetes. Hisashi et al. (1999) have shown that triterpenoids and diterpenoids isolated from *Salacia oblonga* possess inhibitory

activity on aldose reductase enzyme. Similar diterpenoids and triterpenoids are present in methanol extract (Ruvin Kumara, 1998), which may be responsible for the inhibitory effect on aldose reductase enzyme and correct the complication related to the enzyme activity. Further experiments are in progress to elucidate the mechanism of action of *S. reticulata* extract and to isolate the active principles responsible for the hypoglycemic effect.

Conclusions

Our studies have confirmed that *S. reticulata* shows both acute and cumulative effects on glucose homeostasis in alloxan diabetic rats. The extract may contain antidiabetic principles that directly influence hepatic or peripheral glucose disposal and those regulating carbohydrate absorption. Thus, *S. reticulata* deserves further consideration as a possible adjunct to conventional antidiabetic treatments and as a source of new hypoglycemic compounds.

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