Anti-diabetic activity of *Celosia argentea* root in streptozotocin-induced diabetic rats

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This study was designed to investigate the anti-diabetic hypoglycaemic properties of an ethanolic extract of the root of *Celosia argentea* which is widely used in India as a traditional treatment for diabetes mellitus. An ethanolic extract of *C. argentea* root was found to lower blood glucose in basal conditions and after a heavy glucose load in normal rats. Maximum reduction in serum glucose was observed after 90 minutes at a dose of 500 mg/kg (63.28%) of body weight, but petroleum ether and chloroform extracts (8.52% and 9.81%, respectively) did not reduce the serum glucose. Ethanolic extract of *C. argentea* was also found to reduce the increase of blood sugar found in streptozotocin-induced diabetic rats (73.43% at 250 mg/kg and 80.20% at 500 mg/kg body weight on 15th day). Chronic administration of the extract significantly reduced the blood sugar in streptozotocin-induced diabetic rats for several days (15 days). The ethanolic extract was also found to reduce the increased levels of cholesterol, triglycerides and urea. The extract also restored the decreased level of proteins and liver glycogen in streptozotocin-induced diabetic animals and inhibited the body weight reduction induced by streptozotocin administration. These results indicate that *C. argentea* root extracts are able to ameliorate biochemical damages induced by streptozotocin in diabetic rats.

Key words: Anti-diabetic activity, *Celosia argentea*, streptozotocin

INTRODUCTION

Diabetes mellitus, a disease of metabolic disorders, is associated with a number of chronic complications like nephropathy, neuropathy, retinopathy and cardiovascular diseases.[1] It is recognised as one of the leading causes of morbidity and mortality and considered as one of the five leading causes of death in the world. About 150 million or 1.3% people are suffering from diabetes worldwide, which is almost five times more than the estimates 10 years ago and this may double by the year 2030.[2] Diabetes mellitus is characterised by impaired glucose utilisation and is the underlying factor for both hypoglycaemia and hyperglycaemia. Hyperglycaemia is a condition in which blood glucose level is high and there is diminished action of insulin either because of decrease in the circulatory concentration of insulin or due to decrease in the response of peripheral tissue to insulin. These abnormalities give rise to altered metabolism of lipids, carbohydrates and amino acids. All these effects produce hyperglycaemia. Chronic hyperglycaemia results in impaired function or failure of various organs, especially eyes, kidneys, nerves, heart, and blood vessels.[3]

Celosia argentea (Family: Amaranthaceae) seeds possess aphrodisiac, anti-pyretic, anti-spasmodic, anti-cancer, diuretic, and anti-bacterial properties. Also, they are reported to be useful in jaundice, inflammation, gonorrhea, healing of wounds and injuries.[4] In folklore practice, the decoction of *C. argentea* seeds has been reported to be useful in diabetes mellitus.[5] Scientific investigation has been reported on *C. argentea* regarding immunostimulating activity,[6] anti-inflammatory and diuretic activity,[7] anti-diabetic activity,[8] and the flavonoid was isolated from alcoholic extract of the leaves of *C. argentea* and investigated for its anti-inflammatory activity in animal models.[9] There is no investigation on its roots for anti-diabetic activity. Hence, this study was conducted to evaluate the anti-diabetic activity of the root of *C. argentea*.

MATERIALS AND METHODS

Plant

Fresh roots of *C. argentea* were collected from the Pune district of Maharashtra, India. The plant was identified and authenticated by Dr. P.S.N. Rao, Joint Director of Botanical Survey of India, Pune, and a voucher specimen (No. BSI/WC/Tech/2007/217) has been kept in the Department of Pharmacology of Botanical Survey of India.

Preparation of Extract

Roots were shade dried, powdered, Soxhlet-extracted successively with petroleum ether, chloroform and ethanol at 60–80°C, for 24 hours. The solution when

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evaporated gave a waxy reddish residue, and petroleum ether extract (yield**:** 4.7%), chloroform (yield: 3.78%) and ethanol extract (yield: 9.27%) were stored in desiccators. A preliminary phytochemical screening of the petroleum ether and chloroform extracts showed the presence of fixed oil and carbohydrates and the ethanolic extract showed the presence of glycosides (saponin glycosides), alkaloids and carbohydrates and absence of alkaloids and steroids.

Animals

Wister albino rats (150–200 g) of both sexes were maintained under standard environmental laboratory conditions and fed with laboratory diet and water *ad libitum.* All the protocols were performed in accordance with the Institutional Animal Ethical committee (Ref: IAEC/PP/17/2007-08) as per the directions of the CPCSEA (Reg No: 997/c/06/CPCSEA).

Chemicals

Trichloroacetic acid was from Hi-media laboratories (Mumbai, India). Streptozotocin was purchased from Sigma Chemical Company (St. Louis, MO, USA). Glucose, urea, total protein, triglyceride and cholesterol kits were procured from Span diagnostic Pvt. Ltd. (Surat, India). All the chemicals and reagents were of analytical grade.

Acute Toxicity Study

Healthy adult female Swiss albino mice weighing between 20 and 25 g body weights were selected for the acute toxicity study with the extracts of *C. argentea*. Doses of 5, 50, 300 and 2000 mg/kg were selected based on the fixed dose (OCED Guideline No. 401) method of CPCSEA.[10] Animals were divided into four groups of three animals each and fasted overnight. The doses of 5, 50, 300 and 2000 mg/kg b.wt. were administered to the animals of Groups I, II, III, and IV, respectively. The extracts were administered orally. The animals were continuously observed for 24 hours to detect changes in autonomic or behavioural responses. Mortality in each group was observed for 7 days.

Oral Glucose Tolerance Test

The rats were divided into five groups consisting of six animals in each and overnight fasted animals were used in each group. Group I served as control and received vehicle (1 ml/100 g) orally, Groups II and III received petroleum ether and chloroform extracts at a dose of 500 mg/kg orally and Groups IV and V received ethanolic extract orally at doses of 250 and 500 mg/kg, respectively. Rats of all the groups were loaded with glucose (3 g/kg, p.o.), 30 minutes after drug administration. Blood samples were collected via retro-orbital puncture at 30, 90, 150 minutes after glucose loading. Serum glucose level was measured immediately.[11]

Anti-diabetic Activity

Induction of diabetes

Streptozotocin (40 mg/kg) dissolved in citrate buffer, 0.1 M, pH 4.5, was injected by a single intravenous injection in rats previously fasted for 16 hours. Animals with post-prandial glycaemia over 300 mg/kg, confirmed by Diastrix strips, 3 days after streptozotocin administration, were considered diabetic. Non-diabetic control animals received citrate buffer injection.

Sub-chronic experiments

Three days after streptozotocin (diabetic rats) or citrate buffer (non-diabetic rats) injection, the animals were divided into five groups: Group I – non-diabetic control rats; Group II – diabetic control rats; Group III – diabetic rats treated with 250 mg/kg of ethanolic extract; Group IV – diabetic rats treated with 500 mg/kg of ethanolic extract; and Group V – diabetic rats treated with 10 mg/kg of glibenclamide. Both diabetic and non-diabetic groups received extract or water (controls) orally by gavages once a day for 15 days. Body weight was determined on 2nd (2 days after injection of streptozotocin) and 15th days (15 days after administration of plant extract). Serum glucose was measured on day 1, 4, 7, 10 and 15 after the extract treatment. Blood samples were collected by retro-orbital sinus puncture under mild ether anaesthesia. At the end of the experimental period, rats were anaesthetised and sacrificed, and samples of free running blood were collected for the measurement of serum glucose,[12] cholesterol,[13] triglycerides,[14] urea[15] and protein[16] levels, using commercial kits. The livers were removed and weighed immediately and kept in 5% trichloroacetic acid solution for liver glycogen estimation.

Statistical Analysis

The data were represented as mean ± SEM, and statistical significance between extract-treated and diabetic control groups was analysed using one-way analysis of variance (ANOVA), followed by Tukey–Kramer multiple comparison test. $P < 0.05$ was considered statistically significant.

RESULTS

The results of the acute toxicity studies showed that the extracts of root of *C. argentea* were non-lethal up to a dose of 2000 mg/kg b.wt., so that 1/8th and 1/4th of 2000 mg/kg dose (i.e. 250 and 500 mg/kg orally) was selected for anti-diabetic activity.

The effect of *C. argentea* root extracts on glucose tolerance is shown in Figure 1. The petroleum ether and chloroform extracts, administered at a dose of 500 mg/kg body weight, did not show significant glucose tolerance effect in glucose fed rats. The ethanolic extract at a dose of 500 mg/kg showed significantly increased (63.28%) tolerance for glucose ($P<0.01$). Maximum glucose tolerance was noted for the tested dose levels, 90 minutes after glucose loading.

Effects of *C. argentea* root extract on fasting blood glucose levels of streptozotocin-diabetic rats are shown in Figure 2. At 72-hour post streptozotocin injection, all the diabetic rats exhibited hyperglycaemia with blood glucose ranging between 330 and 500 mg/dl, while the normal control rats showed normal blood sugar level of 90.50 mg/dl. After 2 weeks of treatment with the extracts, the glycaemic levels of 250 mg/kg in *C. argentea* extract-treated diabetic rats dropped significantly from 391.16±11.98 mg/dl on day 4 to 133.33±2.29 mg/dl (P<0.001) on day 15 and from 397.83±9.67 mg/dl to 99.33±1.84 mg/dl (P<0.001) for 500 mg/kg dose, corresponding to 66 and 75% reductions, respectively.

The effects of ethanolic *e*xtract of the *C. argentea* root on the body weight of diabetic rats are shown in Table 1. During 2 weeks of observation of the extract-treated diabetic rats, there was significant reduction in body weight relative to day 2. The diabetic rats treated with glibenclamide reference drug also showed significant (P<0.01) reduction in body weight compared to rats on day 2 after injection of streptozotocin. But in the diabetic control rats, there was a decrease in the body weight relative to day 2 after injection of streptozotocin.

In *C. argentea* extract-treated rats, a significant decrease (P<0.001) in serum triglycerides was observed, 26.52% for 250 mg/kg and 33.85% for 500 mg/kg, after the treatment period when compared to the diabetic control group. Treatment with *C. argentea* and glibenclamide caused significant decrease (P<0.001) in total cholesterol by 38.24 and 44.78% for 500 and 10 mg/kg, respectively. Total cholesterol concentrations in normal and diabetic control rats were not affected significantly after the treatment period. Moreover, there were significant changes in the serum urea level at 250 mg/kg (39%) and 500 mg/kg (67.8%) extract-treated group compared to that of diabetic control group. Serum protein level was significantly decreased in diabetic group as compared to that of normal rats. Extract-treated animals showed increase in protein levels as compared to diabetic group [Table 2].

There was a marked reduction in liver glycogen level in streptozotocin diabetic animals. The ethanolic extract

Table 1: Effects of ethanolic extract of *C. argentea* root on the body weight of streptozotocin-induced diabetic rats

Treatment	Dose (mg/kg)	Body weight (g)		
		2 days after injection of streptozotocin	15 days after administration of ethanolic extract	Relative weight gain (%)
Vehicle control	Vehicle (1 ml/100 g)	221.16±5.79	237.16±5.11	+7.23
Diabetic control (streptozotocin)	40	222.16±4.90	202.16±4.25	−9.00
Ethanolic extract	250	212.5±3.16	202.16±5.20*	−4.86
Ethanolic extract	500	173.33±6.90	170.61±9.09**	1.56
Glibenclamide	10	206.16±2.64	200.5±3.97**	−2.74

Values are expressed as means±SEM (n = 6); + increase; − decrease, *P < 0.05; **P < 0.01, significantly different compared to the values on day 2 after injection of streptozotocin

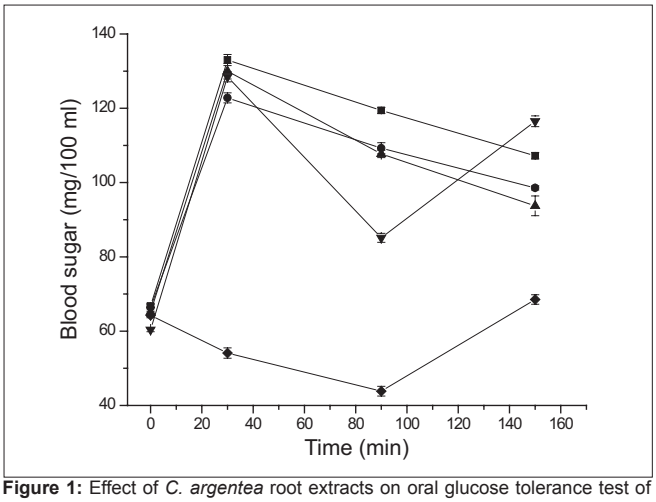

Figure 1: Effect of *C. argentea* root extracts on oral glucose tolerance test of normal rats. (■) after administration of 3 g/kg glucose treated, (●) Petroleum ether extract 500 mg/kg, (▲) Chloroform extract 500 mg/kg, (▼) Ethanolic extract 250 mg/kg, (♦) Ethanolic extract 500 mg/kg. Each value represent the mean±S.E.M. (n = 6).

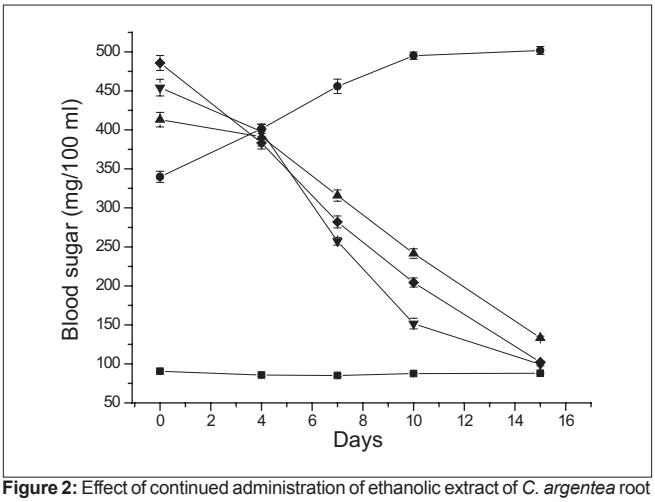

Figure 2: Effect of continued administration of ethanolic extract of *C. argentea* root on blood glucose levels in rats treated with streptozotocin. (■) Normal control, (●) Diabetic control, (▲) Ethanolic extract 250 mg/kg, (♦) Ethanolic extract 500 mg/kg, (▼) Glibenclamide 10 mg/kg. Each value represent the mean±S.E.M. (n = 6).

Table 2: Effects of ethanolic extract of *C. argentea* root on serum lipid levels of streptozotocin-induced diabetic rats

Treatment	Dose (mg/kg)	Urea (mg/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	Protein (g/dl)
Vehicle control	Vehicle (1 ml/100 g)	20.56±0.57	97.57±1.14	146.07±1.44	7.80±0.13
Diabetic control (streptozotocin)	40	83.17±1.80	127.80±2.20	218.17±2.78	4.61±0.13
Ethanolic extract	250	50.68±1.80*** (39.0%)	92.79±0.71*** (27.39%)	160.31±1.48*** (26.52%)	5.76±0.08*** (24.94%)
Ethanolic extract	500	26.78±1.05*** (67.8%)	78.92±1.82*** (38.24%)	144.32±2.81*** (33.85%)	6.41±0.08*** (39.04%)
Glibenclamide	10	31.77±0.83*** (61.8%)	70.57±0.84*** (44.78%)	151.30±1.44*** (30.65%)	7.24±0.23*** (57.05%)

Each value represents the mean ± SEM (n = 6), ***P < 0.001 as compared to diabetic control group; values in parenthesis represent % protection compared to diabetic control

treatment remarkably attenuated this reduction in glycogen content [Figure 3].

Our results suggest that the ethanolic extract of root of *C. argentea* has dose-dependent anti-diabetic activities on streptozotocin-induced diabetes. The metabolic disturbances were corrected after the plant extracts were administered for 2 weeks, as shown by the normalisation of fasting blood glucose levels and reduction in the elevated lipid levels by diabetic-treated rats. *C. argentea* appeared to have greater potency in reducing the body weight.

Some hypothesis may be raised to explain the delayed increase in serum glucose in normal rats treated with extract, observed in oral glucose tolerance test (OGTT) [Figure 1]. Among them are lowered intestinal glucose absorption, inhibition of renal glucose re-absorption with increased elimination of glucose in urine, improved tissue glucose uptake or even neoglycogenesis inhibition (highly activated by 16 hour fasting before glucose load) when the glucose load was administered.

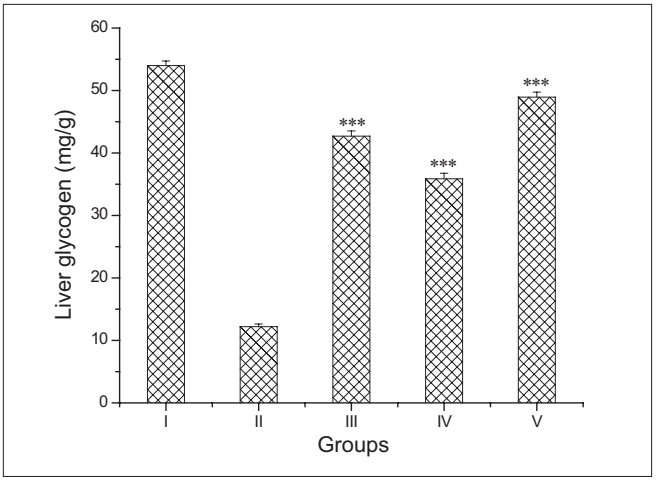

Figure 3: Effect of ethanolic extract of *C. argentea* on liver glycogen in streptozotocin-induced diabetic rats (values are expressed as mean ± SEM from six animals in each group): (I) vehicle control; (II) diabetic control; (III) glibenclamide 10 mg/kg; (IV) ethanolic extract 250 mg/kg; (V) ethanolic extract 500 mg/kg. ***P < 0.001 as compared to diabetic control group

DISCUSSION

The mechanisms by which streptozotocin brings about its diabetic state include selective destruction of pancreatic insulin secreting beta cells, which makes the cells less active[17] and leads to poor glucose utilisation by tissues.[18] This suggests that the extract may possess as insulin-like effect on peripheral tissues by either promoting glucose uptake or metabolism, by inhibiting hepatic gluconeogenesis[19,20] or absorption of glucose into the muscles and adipose tissues[21] by stimulation of regeneration process and revitalisation of the remaining beta cells.[22,23]

The levels of serum lipids are usually elevated in diabetes mellitus and such an elevation represents a risk factor for coronary heart disease.[24] This abnormal high level of serum lipids is mainly due to the uninhibited actions of lipolytic hormones on the fat depots. We have an earlier report saying that the hypercholesterolaemia and hypertriglyceridemia occurs in streptozotocin-induced diabetic rats.[25,26] Under normal circumstances, insulin activates the enzyme lipoprotein lipase which hydrolyses triglycerides.[27] However, in the diabetic state, lipoprotein lipase is not activated due to insulin deficiency, resulting in hypertriglyceridemia. In addition, treatment of animals with *C. argentea* caused a decrease in cholesterol levels. It indicates that the extract of *C. argentea* was more useful in the treatment of diabetes as it has hypolipidaemic effect since the diabetes is always associated with hyperlipidaemia. Moreover, its hypolipidaemic effect could represent a protective mechanism against the development of atherosclerosis which is usually associated with diabetes. Lowering of serum lipid levels through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease.

Blood urea levels were significantly increased in diabetic group compared to that of normal control due to excessive breakdown of body protein. The extract treatment reduced the elevated levels of serum urea.

Protein levels were significantly decreased in diabetic group as compared to that of normal rats. Excessive breakdown of body protein in conjunction with either inadequate supply or defective utilisation observed in uncontrolled diabetes may be accompanied by hypoalbuminemia.[28] *C. argentea* seems to resort this effect due to hypoglycaemic status. Glibenclamide is often used as a standard anti-diabetic drug in streptozotocin-induced moderate diabetes to compare the efficacy of variety of hypoglycaemic compounds.

Glycogen is the primary intracellular storable form of glucose and its levels in various tissues, especially hepatic and skeletal muscle, are a direct reflection of insulin activity as insulin promotes intracellular glycogen deposition by stimulating glycogen synthetase and inhibiting glycogen phosphorylase. Since destruction of β-cells of islets of Langerhans results in marked decrease in insulin levels, it is rational that glycogen levels in tissues (skeletal muscle and liver) decrease as they depend on insulin for the influx of glucose.[29] Moreover, alteration in muscle and hepatic glycogen content is normalised by insulin treatment.[30] A normal level of glycogen reflects the normalisation of insulin levels.

Improvement of body weight of the extract-treated animals further supports the anti-diabetic effect of ethanolic extract of *C. argentea* as diabetic condition is associated with loss of body weight.

However, the present study further supports the folk practice of using *C. argentea* for routine treatment of diabetes mellitus. So, the present investigation reveals that ethanolic extract of *C. argentea* root has significant hypoglycaemic action in streptozotocin-induced diabetic rats.

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REFERENCES

1. Mahdi AA, Chandra A, Singh RK, Shukla S, Mishra LC. Effect of herbal hypoglycemic agents on oxidative stress and anti-oxidant status in diabetic rats. Indian J Clin Biochem 2003;18:8-14.
2. Wild S, Sireee R, Roglic G, King H, Green A. Global prevalence of diabetic: Estimation of the year 2000 and projection for 2030. Diabetes Care 2004;27:1047-53.
3. Susheela P, Balaravi TN, Raju J, Theophilus, Reddy PU. Evaluation of hypoglycemic and anti-diabetic effect of *Melia dubia* fruit extract and its effect on pancreas and kidneys of induced diabetes mice model. Int J Pharmacol Biol Sci 2007;1:37-46.
4. Anonymous. The Wealth of India. New Delhi, India: Council Scientific and Industrial Research; 1992. p. 414-5.
5. Shah MB, Patel KN, Chauhan MG. Contribution to indigenous drugs. I: *Celosia argentea.* Int J Pharmocog 1993;31:223-34.
6. Hase K, Kadota S, Basnet P, Nimba T. Immunostimulating activity of *Celosia* antihepatotoxic polysaccharide isolated from *Celosia argentea*. Planta Med 1997;63:216-9.
7. Vetrichelvan T, Jegadeesan M. Evaluation of anti-inflammatory and diuretic activity of *Celosia argentea.* Indian Drugs 2000;37:286-90.
8. Vetrichelvan T, Jegadeesan M, Devi BA. Anti-diabetic activity of alcoholic extract of *Celosia argentea* Linn seeds in rats. Biol Pharm Bull 2002;25:526-8.
9. Bhujbal SS, Chitlange SS, Suralkar AA, Shinde DB, Patil MJ. Anti-inflammatory activity of an isolated flavonoid fraction from *Celosia argentea* Linn. J Med Plant Res 2008;2:52-4.
10. Organization for Economic cooperation and development (OECD). OECD Guidelines for testing of chemicals Acute Oral Toxicity, OECD, No 401; 1993.
11. Babu V, Gangadevi Y, Subramoniam A. Anti-diabetic activity of ethanol extract of *Cassia kleinii* leaf in STZ induced diabetic rats and isolation of an active fraction and toxicity evaluation of the leaf extract. Indian J Pharmacol 2003;36:95-8.
12. Lott JA, Turner K. Evaluation Trinder's glucose oxidase method for measuring glucose in serum and urine. Clin Chem 1975;21:1754-60.
13. Allain CC, Poon LS, Chan CS, Richmond W, Paul C. Enzymatic determation of total serum cholesterol. Clin Chem 1974;20:470-5.
14. Nader R, Paul B, John A. Lipids: Lipoproteins and apolipoproteins. In: Burtis CA, Ashwood ER, editors. Tietz Text book of Clinical Chemistry. 3rd ed. Philadelphia: W.B. Saunders; 1994. p. 809-52.
15. Lawrie H. A simple terbidimetric method of estinating blood urea. Clin Chem 1957;3:691-5.
16. Pomory CM. Color development time of the Lowry protein assay. Anal Biochem 2008;375:216-7.
17. Junod A, Lambert AE, Stauffacher W, Renold AE. Diabetogenic action of streptozotocin of dose to metabolic response. J Clin Invest 1969;48:2129-39.
18. Larissa I, Netchiporouk, Nataliya F, Shram Nicole Jaffrezic-Renault, Claude Martelet, Raymond Cespuglio. In Vivo Brain Glucose Measurements: Differential Normal Pulse Voltammetry with Enzyme-Modified Carbon Fiber Microelectrodes. Anal. Chem., 1996, 68 (24), 4358–4364.
19. Ali L, Azad Khan AK, Mamun MI, Mosihuzzaman M, Nahar N, Nur-E-Alan M, *et al*. Studies on the hypoglycaemic effects of fruits pulp, seed and whole plant of *Momordica charantia* on normal and diabetic model rats. Planta Med 1993;59:408-12.
20. Gray AM, Abdel-Wahab YH, Flatt PR. The traditional plant treatment, *Sabucus nigra* (Elder), exhibits insulin-like and insulin releasing actions in vitro. J Nutr 2000;130:15-20.
21. Kamanyi A, Djamen D, Nkeh B. Hypoglycaemic properties of the aqueous roots extract of *Morinda lucida* (Rubiacea) study in the mouse. Phytother Res 1994;8:369-71.
22. Shanmugasundaram ER, Gopinath KL, Shanmugasundaram KR, Rajendran VM. Possible regeneration of the islets of Langerhans in streptozotocin-diabetes rats given *Gymnema sylvestre* leaf extracts. J Ethnopharmacol 1990;30:265-79.
23. Rokeya B, Nahar N, Ali L, Hassan Z, Nure-E-Alam M, Chowdhury SN, *et al*. Effects of five medicinal plants on blood glucose levels in non-diabetic and diabetic model rats. Diabetes Res 1999;34:219-28.
24. Pushparaj P, Tan CH, Tan BK. Effects of *Averrhoa bilimbi* leaf extract on blood glucose and lipids in streptozotocin-diabetic rats. J Ethnopharmacol 2000;72:69-76.
25. Pushparaj PN, Tan BK, Tan CH. The mechanism of hypoglycemic action of the semi-purified fractions of *Averrhoa bilimbi* in streptozotocindiabetic rats. Life Sci 2001;70:535-47.
26. Tan BK, Tan CH, Pushparaj PN. Anti-diabetic activity of the semi purified fractions of *Averrhoa bilimbi* in high fat diet fed-

streptozotocin induced diabetic rats. Life Sci 2005;76:2827-39.
27. Taskinen MR. Lipoprotein lipase in diabetes. Diabetes Metab Rev 1987;3:551-70.
28. Latner A. Carbohydrate metabolism. Abnormalities of post absorptive blood sugar level. Clinical Biochemistry. 2nd ed. Philadelphia: PA7WB Saunders; 1958. p. 48.
29. Whitton PD, Hems DA. Glycogen synthesis in the perfused liver of strptozotocin-diabetic rats. J Biochem 1975;150:153-65.
30. Weber G, Lea MA, Fisher EA, Stamm NB. Regulatory pattern of liver carbohydrate metabolizing enzymes: Insulin as indicator of key glycolytic enzymes. Enzymol Biol Clin (Basel) 1966;7:11-24.

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