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Hypoglycemic activity of *Lagerstroemia speciosa* L. extract on streptozotocin-induced diabetic rat: Underlying mechanism of action

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Abstract

The hypoglycemic effect of *Lagerstroemia speciosa* L. leaves hot water extract on chemically induced diabetes in rat was investigated. Experimental result showed that, streptozotocin significantly ($p<0.001$) elevated the normal blood sugar level whereas treatment with hot water extract depressed the streptozotocin-induced high blood sugar level about 43.2% as compare to diabetic controls. Treatment with hot water extract increased the activity of shunt enzyme glucose–6-phosphate dehydrogenase (33.8%) and glutathione level (31.3%) and depression of the activity of hepatic gluconeogenic enzymes glucose-6-phasphatase (31.6%) and fructose-1,6-bisphosphatase (27.4%). These studies thus strongly suggest that the hot water extract of *L. speciosa* leave attributed its prominent hypoglycemic activity on experimental diabetic rats through suppression of gluconeogenesis and stimulation of glucose oxidation using the pentose phosphate pathway.

Introduction

Diabetes is a disorder of mechanism due to absolute deficiency or diminished effectiveness of insulin. Due to lack of insulin, hyperglycemia and glycosuria almost invariably occur. The search for a curative agent against this disease resulted in the introduction of several hypoglycemic agents. Some of which are used therapeutically. However, various harmful side effects and weak effectiveness of them made their use limited and the search to find more effective agents continues. Investigation in the plant kingdom culminated in the discovery of many herbal hypoglycemic agents. One of them is taken for investigation in this study.

Lagerstroemia speciosa Linn. (locally known as Jarul) belonging to the natural order- Lythraceae is widely distributed in most part of the world including Bangladesh. The hot water leaves extract of the plant has reported to reduce diabetic symptoms in genetically diabetic KK-AY mice (Kakuda et al., 1996), and have also been shown to slow weight gain in genetically obese mice (Suzuki et al., 1999). Recently, it has been reported that various active ingredients isolated from *L. speciosa* leaves shows their hypoglycemic properties through increase the rate of glucose uptake or inducing glucose transport like insulin and decrease the isoproterenol-induced glycerol release (Liu et al., 2001; Hattori et al., 2003; Klein et al., 2007). Although critical evaluation for clinical use has not been reported, a recent study showed that standard extract from *L. speciosa* leaves exerts its hypoglycemic effect on type II diabetes (Judy et al., 2003).

It have been reported that ethanol extract of *L. speciosa* leaves posses the hypoglycemic activity (Mishra et al., 1990). In our previous communication, we have shown (Saha et al., 2006) the hypoglycemic

activity of various leaves extracts of *L. speciosa* on experimental diabetic rats. However, enough evidence is lacking to evaluate how these extracts of *L. speciosa* leaves exhibit their hypoglycemic activity on experimental diabetic animals. In the present study, attempts have been made to evaluate the mechanisms of action of hot water extract of *L. speciosa* on experimental diabetic rats through suppression of gluconeogenesis and stimulation of glucose oxidation using the pentose phosphate pathway.

Materials and Methods

Animals and diet

Male/female Albino rats obtained from the animal house of BCSIR-Laboratory, Chittagong, weighing 180-200 g was used in the entire study. The animals were acclimatized to standard laboratory conditions (temperature 24 ± 1°C, relative humidity 55 ± 5% and a 12 hours photoperiod) in suspended wire-meshed galvanized cages (4-6 rats/cage) for one week before the commencement of the experiment. During the entire period of study, the rats were supplied with a semipurified basal diet and water *ad libitum*. All animals were maintained according to the published criteria of Saha et al., 2001.

Preparation of hot water extract

The leaves of *L. speciosa* were collected from the experimental plantation area of BCSIR-Laboratory campus, Chittagong. The fresh leaves of *L. speciosa* were crushed in a blender and blended leaves were allowed to ferment at room temperature in thick layer for 20 hours. After fermentation, the crushed leaves were dried at room temperature in thin layer using exhaust fan. The partially dried leaves were then crushed again to small particles and dried in hot air drier. The dried leaves was extracted with hot water between 75-90°C for 20-30 min.

Estimation of blood sugar and glutathione

Blood sugar estimation was estimated by using standard glucose kit essentially followed by glucose oxidase-peroxidase (GOD-POD) methods (Trinder, 1969). Glutathione level in blood was determined by the methods of Patterson et al., 1949.

Determination of enzymes activities and total proteins

Glucose-6-phosphatase (EC 3.1.3.9) was assayed as described by Baginski et al. (1974), fructose-1,6-bis phos -phatase (EC 3.1.3.11) as described by McGilvery (1955). For these determinations, 1 g of fresh liver was chopped and homogenized in ice-cold sucrose (15 mL, 250 mM) with a Potter-Elvehjem homogenizer for 2 min, centrifuged at 10,000 rpm for 30 min, and the pellet was discarded and the supernatant was used as the source of the above mentioned enzymes. Protein content of the supernatant was determined by the method of Lowry et al., 1951.

G6PDH (EC 1.1.1.49) was assayed by the method of Lohr and Waller (1974). Liver (0.2 g) was chopped and homogenized in 5 mL of ice cold EDTA/saline (66 mM EDTA in 0.9% saline), and centrifuged at 1,000 rpm at 2°C for 30 min. The pellet was discarded and the supernatant was used as the source of hepatic G6PDH.

Acute toxicity test

Acute toxicity of the *L. speciosa* leaves extracts has been carried out on 10 rats and 10 mice orally at the rate of 400-800 mg/kg body weight and was closely observed for 24 hours after treatment of extracts and next ten days for any delayed effect.

Experimental protocol (for each study)

Thirty two Albino rats (same sex) were randomly divided into four (A-D) different experimental groups (eight rats per group). Three groups (B-D) were treated with streptozotocin at the rate of 65 mg/kg body weight intraperitoneally. Group A rats were not administered any drug and serve as normal control. Group B rats considered as diabetic control. Group C rats were dosed standard drug for diabetic treatment (4 mg/kg body weight, orally) and serve as positive control and Group D rats were treated with *L. speciosa* leaves hot water extracts at this experimental regimen.

L. speciosa leaves extracts or standard drug were given orally to streptozotocin-induced 24 hours fasted rats. After 2 hours of drug treatment all the animals were anesthetized with diethyl ether and blood was collected from tail vein. Liver was isolated from the body and kept it for enzyme assay.

Statistical analysis

All data from each treated and control group was analyzed by using Student's t-test.

Results and Discussion

The oral administration of hot water extract of *L. speciosa* leaves to 18 hours-fasted rats significantly lowered (43.2%, $p<0.001$) the blood sugar level of streptozotocin-induced diabetic rats as compared with normal control group (Table I). This is consistent our previous result (Saha et al., 2006). Recently it has been shown that *L. speciosa* Leaves contain corosolic acid, the active ingredient, which exhibits anti-oxidant and antidiabetic activity as like as vitamin E, which can protect cell membranes from lipid peroxidation by scavenging free radicals. This anti-oxidant effect results from elevated serum glucose. Corosolic acid helps to reduced blood glucose level by activating the transport

Table I

Effect of *Lagerstroemia speciosa* leaves hot water extract on streptozotocin-induced diabetic rats blood sugar level

Group	Treatment	Blood sugar level (mg/dL)	Percent increase/ decrease
A	Normal control	60.0 ± 3.2[a]	
B	Diabetic control	124.8 ± 4.2[b]	108.1 (-)
C	Drug treatment (standard)	61.5 ± 2.9[c]	50.2 (-)
D	Sample treatment	70.9 ± 2.5[c]	43.2 (-)

[a]Values are mean ± S.E. (n = 5); [b]p<0.001 when compared with Group A; [c]p<0.001 when compared with Group B

Table II

Effect of *Lagerstroemia speciosa* leaves hot water extract on streptozotocin-induced diabetic rats blood glutathione level

Group	Treatment	Blood glutathione level (mg/dL)	Percent increase/ decrease
A	Normal control	14.3 ± 0.8[a]	
B	Diabetic control	8.0 ± 0.3[b]	43.9 (-)
C	Drug treatment (standard)	11.1 ± 0.5[c]	37.6 (-)
D	Sample treatment	10.5 ± 0.7[d]	31.3 (-)

[a]Values are mean ± S.E. (n = 5); [b]p<0.001 when compared with Group A; [c]p<0.002 and [d]p<0.02 when compared with Group B

of glucose across the cell membranes, in short, corosolic acid has insulin-like effect (Liu et al., 2001; Miura et al., 2006; Fukushima et al., 2006).

Table II showed that oral administration of extract significantly increased (31.3%, p<0.02) the depressed blood glutathione (a tripeptide of glycine, cystein and glutamic acid, GSH) level of chemically induced diabetic rats. The elevation of glutathione level by extract has important implications because of the fact that the tripeptide, in addition to many of its important physiological/biochemical functions such as maintenance of the integrity of the red cell (Fujii et al., 1984), detoxification of hepatic xenobiotics, transport of amino acids in the γ-glutamyl cycle or Alton Meister's cycle (Meister and Anderson, 1983), plays a very important protective role against the damaging effect of toxic oxides radicals such as super oxide (O^{-}_{2}), hydroxyl radical (OH) and toxic peroxides such as hydrogen peroxide (H_2O_2), and other peroxides (R-OOH). These highly reactive species are thought to be partly responsible for the destruction of the β-cells of the pancreas in diabetes (Oberley, 1988). The diabetogenic drug alloxan has been reported to give rise to superoxide (O^{-}_{2}) (Fisher and Hamberger, 1980) whereby the drug destroys the islet of Langerhans of the pancreas and precipitate diabetes mellitus. Alloxan's diabetogenic effects is rendered void if the enzyme superoxide dismutase (SOD), a well known scavenger of the toxic oxide radical (O^{-}_{2}), is administered along with the drug, which gives strong evidence to the suggestion made above. Streptozotocin may be acting in a pattern similar to alloxan. Red cell glutathione level has been reported to be diminished in human diabetic patients. Liver and kidney glutathione levels have been reported to decrease in experimental diabetic rats (Loven et al., 1986; Saha et al., 1997).

The peroxides arise from metabolism of toxic radicals (McCord, 1993). All these reports indicate that glutathione plays a significant role in tissue defense in various pathological states including diabetes. In context the elevation of rat blood glutathione levels by extract presents an interesting aspect of the antidiabetic property of the herb. The results of this study thus strongly suggested that extract has an anti-oxidant property. This is consistent with the results published by Unno et al., 1997. The antidiabetic and the anti-oxidant properties of the extract might be acting in a synergistic fashion. Report shows that oxidative stress is produced under diabetic conditions and possibly causes various forms of tissue damage in diabetes and

Table III

Effect of *Lagerstroemia speciosa* leaves hot water extract on streptozotocin-induced diabetic rats hepatic glucose-6-phosphatase, fructose-1,6-bis-phosphatase and glucose-6-phosphate dehydrogenase activity

Group	Treatment	Glucose-6-phosphatase (units/mg of protein)	Percent increase/ decrease	Fructose-1,6-bis-phosphatase (units/mg of protein)	Percent increase/ decrease	Glucose-6-phosphate dehydrogenase (units/mg of protein)	Percent increase/ decrease
A	Normal control	0.2 ± 0.01[a]		0.5 ± 0.01[a]		435.5 ± 3.8[a]	
B	Diabetic control	0.3 ± 0.01[b]	36.7 (-)	0.7 ± 0.01[b]	35.2 (-)	303.3 ± 4.0[b]	30.4 (-)
C	Drug treatment (standard)	0.2 ± 0.02[c]	37.4 (-)	0.5 ± 0.02[d]	30.1 (-)	417.3 ± 4.5[d]	37.6(-)
D	Sample treatment	0.2 ± 0.01[c]	31.6(-)	0.5 ± 0.01[d]	27.4 (-)	405.9 ± 5.2[d]	33.8 (-)

[a]Values are mean ± S.E. (n = 5); [b]p<0.001 when compared with Group A; [c]p<0.005 when compared with Group B; [d]p<0.001 when compared with Group B

anti-oxidants can exert beneficial effects on pancreatic β -cell function in diabetes. Thus sufficient supply of antioxidants may prevent or delay β-cell dysfunction in diabetes by providing protection against glucose toxicity (Kaneto, 1999).

Table III clearly indicate that the extract significantly depressed glucose-6-phosphatase (31.6%, $p<0.005$) and fructose-1,6-bisphosphatase (27.4%, $p<0.001$) activities in streptozotocin-induced diabetic rats. In addition the depressing effect on these key hepatic enzymes, the extract had significantly elevated hepatic cell shunt enzyme glucose-6-phosphate dehydrogenase (G6PDH) activity (33.8%, $p<0.001$) in streptozotocin-induced diabetic rats. Although the hypoglycemic effect of extract has been subjected of extensive investigations, to the best of our knowledge the enzymatic effects observed in this study are the first reported.

The data presented suggest that hot water extract of *L. speciosa* leaves exhibits its hypoglycemic effect mediated through suppression of the key hepatic gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase and an accelerated rate of glucose metabolism through the pentose phosphate pathway. The way of depressing the activity of the hepatic gluconeogenic enzymes or elevate that of the shunt enzyme is not understood at present. The shunt enzyme G6PDH is regulated by the $NADP^+/NADPH$ ratio, a high ratio favoring activation of the enzyme, whereas a low ratio is deactivating. It is now well established that the glucogenic effect of pituitary hormones (somatotropin and corticotrophin) is mediated through an elevation of intracellular effect of extract could be due to a lowering of intracellular cyclic AMP, which could be effected in a variety of ways. That activation of the enzymes fructose-1,6-bisphosphatase by the gluconeogenic pancreatic hormone glucagon is linked to an elevation of intracellular cyclic AMP is now well established (Shibib et al., 1993). The depressing effect of extract on fructose-1,6-bisphosphatase reported here is secondary to a lowering of intracellular cyclic AMP. However, no study has reported the mechanisms of action by the extract at the cellular and molecular levels, and it is not known how the extract exerts these effects remains to be further investigation.

Conclusion

Hot water extract of *L. speciosa* leaves attributed its prominent hypoglycemic activity on experimental diabetic rats through suppression of gluconeogenesis and stimulation of glucose oxidation using the pentose phosphate pathway.

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