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Effect of *Helicteres isora* bark extract on blood glucose and hepatic enzymes in experimental diabetes

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The effect of oral administration of an aqueous extract of the bark of *Helicteres isora* was investigated on blood glucose and plasma antioxidant status in streptozotocin (STZ) induced diabetic rats. The study was also undertaken to evaluate the role of hepatic enzymes in experimental diabetes. Oral administration of a bark extract of *Helicteres isora* (100, 200 mg/kg) in STZ diabetic rats caused a significant increase in body weight, hepatic hexokinase activity and significant decrease in hepatic glucose-6-phosphatase, serum acid phosphatase (ACP), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH). Based on these findings, we suggest that *Helicteres isora* possesses hypoglycemic and hepatoprotective activity and is able to ameliorate biochemical damage in STZ induced diabetic rats.

1. Introduction

By the year 2010, the total number of people worldwide suffering from Diabeters mellitus (DM) is projected to reach 2.39 millions. Regions with the greatest potential for increase are Asia and Africa, where DM rates could rise to 2–3 fold the present rates (ADA 1997). The recommendation of the WHO committee on DM encouraged research on hypoglycemic agents of plant origin used in traditional medicine and it has greatly motivated research in this area (WHO 1980).

Helicteres isora L. (Sterculiaceae) is a shrub or small tree found in forests throughout India. Traditionally the juice of the root is claimed to be useful in diabetes and empyema and is a favorite cure for snakebite (Kiritikar and Basu 1995; Singh et al. 1984). The roots and bark have expectorant, demulcent and constipating properties and are useful in colic, scabies, gastropathy, diarrhoea and dysentery (Prajapati et al. 2003). The fruits are astringent, refrigerant, stomachic, vermifuge, vulnerary and useful in griping of the bowels and flatulence of children (Chopra et al. 1956) and have an antispasmodic effect (Pohocha and Grampurohit 2001). The bark and leaves are used as tan and the wood, which is twice as hard as teak, is extensively used for boat-building, carts, carriages, firewood, violin, bows planking, tool-handles, beams and fence posts (Nadkarni and Nadkarni 1976).

Our preliminary experimental results were highly encouraging as they revealed that blood glucose level was significantly lowered after oral administration of *Helicteres isora* bark in STZ induced diabetes. Thus the present investigation confirms the hypoglycemic effect of *Helicteres isora* and its effect on hepatic and serum enzymes is STZ induced diabetic rats.

2. Investigations and results

Changes in blood glucose and urine glucose on treatment of diabetic rats with bark extract of *Helicteres isora* or tolbutamide are presented in Table 1. The blood glucose and urine glucose increased in STZ diabetic rats as compared to normal rats. Administration of bark extract of *Helicteres isora* (100 mg, 200 mg/kg/p.o) or tolbutamide (250 mg/kg/p.o) decreased the blood and urine glucose.

The effects of the administration of bark extract of *Helicteres isora* or tolbutamide on hepatic hexokinase and glucose-6-phosphatase are illustrated in Table 2. The activity of hepatic hexokinase decreased while the activity of hepatic glucose-6-phosphatase increased in STZ treated diabetic rats as compared to normal rats. Administration of

Table 1: Effect of bark extract of *Helicteres isora* on blood glucose and urine sugar in STZ-diabetic rats

Group	Blood glucose (mg per 100 ml)		Urine sugar
	Initial	Final	
Control	68.1 ± 2.4	72.5 ± 3.5	Nil
Diabetic control	255.6 ± 6.2	272.5 ± 4.9**	+++
Helicteres isora (100 mg/kg/p.o)	253.3 ± 4.4	152.4 ± 7.9*	++
Helicteres isora (200 mg/kg/p.o)	259.4 ± 5.2	129.4 ± 8.2*	+
Tolbutamide (250 mg/kg/p.o)	254.5 ± 4.8	117.5 ± 8.5*	+

Values are mean ± S.D. from 6 rats in each group; diabetic control was compared with normal control; experimental groups are compared with diabetic control; values are statistically significant at **$P < 0.001$ as compared with normal; *$P < 0.001$ as compared with the diabetic control; + indicates 0.25% sugar; +++ indicates 2% sugar

Table 2: Effect of bark extract of *Helicteres isora* on hepatic hexokinase and glucose-6-phosphatase in STZ diabetic rats

Group	Hexokinase (μmol glucose phosphorylated/mg protein/h)	Glucose-6-phosphatase (μmol phosphate/mg protein/min)
Control	0.170 ± 0.03	0.176 ± 0.004
Diabetic control	$0.097 \pm 0.03^{*}$	$0.355 \pm 0.003^{*}$
Helicteres isora (100 mg/kg/p.o)	$0.140 \pm 0.015^{**}$	$0.189 \pm 0.004^{**}$
Helicteres isora (200 mg/kg/p.o)	$0.162 \pm 0.008^{**}$	$0.180 \pm 0.001^{**}$
Tolbutamide (250 mg/kg/p.o)	$0.163 \pm 0.016^{**}$	$0.175 \pm 0.008^{**}$

Values are mean ± S.D. from 6 rats in each group; diabetic control was compared with normal control; experimental groups are compared with diabetic control; values are statistically significant at $^{*}P < 0.001$ as compared with normal; $^{**}P < 0.001$ as compared with the diabetic control

Table 3: Effect of bark extract of *Helicteres isora* on serum ACP, ALP, and LDH in STZ diabetic rats

Group	Acid phosphatase (K.A unit/dl)	Alkaline phosphatase (K.A unit/dl)	Lactate dehydrogenase (μmol pyruvate/g protein/min)
Control	3.4 ± 0.6	10.4 ± 1.5	119.2 ± 3.5
Diabetic control	$7.7 \pm 1.5^{*}$	$23.5 \pm 2.8^{*}$	$172.2 \pm 5.4^{*}$
Helicteres isora (100 mg/kg/p.o)	$4.2 \pm 1.2^{**}$	$15.7 \pm 2.4^{**}$	$134.6 \pm 5.8^{**}$
Helicteres isora (200 mg/kg/p.o)	$4.0 \pm 1.4^{**}$	$12.6 \pm 2.5^{**}$	$122.6 \pm 4.2^{**}$
Tolbutamide (250 mg/kg/p.o)	$3.8 \pm 1.0^{**}$	$11.2 \pm 2.2^{**}$	$120.3 \pm 3.8^{**}$

Values are mean ± S.D. from 6 rats in each group; diabetic control was compared with normal control; experimental groups are compared with diabetic control; values are statistically significant at $^{*}P < 0.001$ as compared with normal; $^{**}P < 0.001$ as compared with the diabetic control

bark extract of *Helicteres isora* (100 mg, 200 mg/kg/p.o) or tolbutamide (250 mg/kg/p.o) increased the activity of hexokinase and decreased the activity of glucose-6-phosphatase as compared to diabetic rats.

The effects of the administration of bark extract of *Helicteres isora* or tolbutamide on serum ACP, ALP and LDH are shown in Table 3. The activity of these enzymes was elevated in STZ diabetic rats as compared to normal rats. Administration of bark extract of *Helicteres isora* (100 mg, 200 mg/kg/p.o) or tolbutamide (250 mg/kg/p.o) decreased the enzymes as compared to diabetic rats.

The body weight, weight changes, food and water intake in control and experimental animals are shown in Table 4. A reduction in body weight was observed in STZ diabetic animals, but when the animals were treated with *Helicteres isora* bark extract (100 mg, 200 mg/kg), the decrease in body weight was reduced to almost nil and an improvement in body weight was observed afterwards.

3. Discussion

STZ is a diabetogenic agent (Dulin and Wyse 1969) and β cell cytotoxic and it can be used to induce experimental diabetes in rodents (Rerup 1970). When animals are injected with STZ (60 mg/kg/i.p.), it induces conditions of hyperglycemia (Hoftiezer and Carpenter 1973). In our study we found that *Helicteres isora* decreases blood glucose in STZ diabetic rats. The possible mechanism by which *Helicteres isora* brings about its hypoglycemic action may be potentiation of the insulin effect of plasma by increasing either pancreatic secretion of insulin from the β cells of islets of Langerhans or its release from bound insulin.

The activity of hexokinase enzymes decreased in the liver of diabetic rats (Sheela and Augusti 1992; Stanely Mainzen Prince et al. 2000). Administration of *Helicteres isora* bark to STZ treated rats resulted in an increased activity of hexokinase in the liver. The increased activity of hexokinase can cause the increase in glycolysis and utilization of glucose for energy production. Administration of bark extract of *Helicteres isora* was observed to decrease the concentration of glucose in the blood. The decrease in the concentration of blood glucose in STZ treated rats given *Helicteres isora* may be as a result of increased glycolysis (increased liver hexokinase activity).

The activity of glucose-6-phosphatase increased in the liver of diabetic rats (Sheela and Augusti 1992; Stanely Mainzen Prince et al. 2000). Administration of bark extract of *Helicteres isora* reduced the activity of glucose-6-phosphatase in the liver. A reduction in glucose-6-phosphatase can result in a decreased concentration of blood glucose.

Increased activity of serum ALP, ACP, and LDH have been observed in diabetic rats (Stanely Mainzen Prince et al. 1997). The increase in the levels of these enzymes in diabetes may be as a result of their leaking out from tissue and migrating into the blood stream. Administration of *Helicteres isora* bark extract brings about a reduction in the activity of these enzymes.

Body weight was decreased in STZ diabetic rats (Prakasam et al. 2003). Administration of *Helicteres isora* bark increased the body weight in STZ diabetes. The ability of *Helicteres isora* to prevent weight loss seems to be the result of its ability to reduce hyperglycemia.

Thus our findings show that *Helicteres isora* has a hypoglycemic effect. Our findings also show that *Helicteres isora* controls the increase in the concentration of glucose by increasing glycolysis and decreasing glucose formation. This is possible because it controls the activities of the two key enzymes of glycolysis. Our study also shows that administration of the *Helicteres isora* extract decreased the degree of tissue damage in diabetes as is evident from the activities of ALP, ACP, and LDH.

Table 4: Body weight, body weight changes, food intake and water intake in control and experimental animals

Group	Treatment mg/kg po	Body weight (g)		Body weight changes (g)	Food intake (g/week)	Water intake (L/week)
		Initial	Final			
I	Control (2% gum acacia)	162.5 ± 2.7	202.5 ± 6.9	$+40.0 \pm 7.7$	82.1 ± 3.20	4.52 ± 0.38
II	Diabetic control	165.8 ± 3.8	$150.8 \pm 3.8^{**}$	-15.0 ± 5.5	$76.8 \pm 1.75^{*}$	$7.32 \pm 0.33^{**}$
III	*Helicteres isora* 100	160.8 ± 2.0	$163.3 \pm 2.6^{*}$	$+ 3.3 \pm 3.4$	$81.4 \pm 2.7^{*}$	$6.66 \pm 0.36^{*}$
IV	*Helicteres isora* 200	159.2 ± 2.1	$165.0 \pm 2.6^{**}$	$+ 5.8 \pm 2.6$	$87.6 \pm 2.7^{**}$	$6.52 \pm 0.22^{*}$
V	Tolbutamide 250	161.7 ± 2.6	$167.5 \pm 5.2^{**}$	$+ 5.8 \pm 4.1$	$87.2 \pm 3.56^{**}$	$5.85 \pm 0.33^{**}$

Values are mean ± S.D. from 6 rats in each group; diabetic control was compared with normal control; experimental groups are compared with diabetic control; values are statistically significant at $^{*}P < 0.001$ as compared with normal; $^{**}P < 0.001$ as compared with the diabetic control

4. Experimental

4.1. Animals

Male Wistar albino rats (weighing 160–200 g) were procured from the animal house, Bharathidasan University, Tiruchirapalli under standard environmental conditions (12 h light/dark cycles at 25–28 °C, 60–80% relative humidity), and fed with a standard diet (Hindustan Lever, India) and water *ad libitum*. All the studies were conducted in accordance with the National Institute of Health Guide (1985).

4.2. Plant material

The bark of *Helicteres isora* L. was collected from Solakkadu, Kollimalai, Namakkal District, Tamilnadu, India and authenticated by Fr. K. M. Matthew, Director, Rapinat Herbarium, St. Joseph's College, Tiruchirapalli. Voucher Herbarium specimens were deposited in the herbarium (collection number 23644, 27406) for future reference.

4.3. Preparation of plant extract

The dried bark of *Helicteres isora* L. was ground into a fine powder with an auto-mix blender. Then the fine powder was suspended in an equal amount of water and stirred intermittently and left overnight. The macerated pulp was then filtered through a coarse sieve and the filtrate was dried at reduced temperature. This dry mass (yield 85 g/kg of powdered bark) served as the aqueous extract of *Helicteres isora* L. for experimentation.

4.4. Induction of diabetes

Rats were made diabetic by a single i.p. administration of STZ (60mg/kg) obtained from Sigma Chemical Co, (St. Louis, MO, USA) dissolved in 0.1 M-citrate buffer, pH 4.5. After 48 h, blood samples were collected and glucose levels were determined to confirm the development of diabetes. Only those animals which showed hyperglycemia (blood glucose levels $>$ 240 mg/dl) were used in the experiment.

4.5. Experimental design

Diabetes was induced in the animals 2 weeks before starting the treatment. After the induction of diabetes rats were divided into 5 groups of 6 animals each. Group I received vehicle alone and served as control. Group II received STZ (60 mg/kg/i.p.) dissolved in 0.1 M-citrate buffer. Group III and Group IV received the bark extract of *Helicteres isora* (100 mg, 200 mg/kg/p.o) once daily for 21 days. Group V received tolbutamide (250 mg/kg/p.o) once daily for 21 days.
During the first, second and third weeks of treatment, the urine sugar and blood glucose of all the rats were determined. Animals described as fasting were deprived of food for 12 h but allowed free access to drinking water. After 21 of treatment, the animals were killed by cervical dislocation.

4.6. Collection of blood

Blood was collected in two separate tubes. One tube containing heparinized blood was used for estimation of glucose. The blood in the other tube was allowed to clot at room temperature and the serum obtained after centrifugation was used for enzyme assays. The liver was also taken immediately and kept in ice-cold containers for enzyme assay.

4.7. Estimation of biochemical parameters

Blood glucose level was measured by the O-toludine method (Sasaki et al. 1972). The activity of hexokinase in liver was determined by the method of Brandstrup et al. (1957). Liver glucose-6-phosphatase was determined according to the procedure of Koide and Oda (1959). Acid phosphatase (ACP), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were determined by the methods of King (1959a, b).

4.8. Statistical analysis

All experimental data were expressed as mean ± S.D, and statistically assessed by one-way analysis of variance (ANOVA). The difference between test animals and controls were evaluated by Student's t-test (Scheff'e 1953).

References

American Diabetes Association (1997) Clinical practice recommendations. Diabetes Care 20 (Suppl): S1–S70.

Brandstrup N, Kirk JE, Bruni C (1957) Determination of hexokinase in tissues. J Gerontol 12: 166–171.

Chopra RN, Nayar SL, Chopra JC (1956) Glossary of Indian Medicinal Plants, CSIR, New Delhi.

Dulin WE, Wyse BB (1969) Reversal of streptozotocin induced diabetes in the rat. Diabetes 16: 512–513.

Hoftiezer V, Carpenter AM (1973) Comparison of streptozotocin- and alloxan induced diabetes in the rat. Diabetologia 9: 178–184.

King J (1959a) Determination of serum alkaline and acid phosphatase. In: Practical Clinical Enzymology. D van Nostrand, London.

King J (1959b) A routine method for the estimation of lactic dehydrogenase activity. J Med Lab Tech 16: 265–272.

Kirtikar KR, Basu BD (1995) Indian Medicinal plants. Vol. 1, International Book Distributors, Dehradun, India.

Koide H, Oda T (1959) Pathological occurrence of glucose-6-phosphatase in serum in liver diseases. Clin Chem Acta 4: 554–561.

Nadkarni KM, Nadkarni AK (1976) Indian Materia Medica, Vol. I, Popular Prakashan, Bombay, India, pp. 615–616.

National Institute of Health: Guide for the Care and Use of Laboratory Animals, DHEW Publication (NIH), revised (1985), Office of Science and Health Reports, DRR/NIH, Bethesda, USA.

Pohocha N, Grampurohit ND (2001) Antispasmodic activity of the fruit of *Heliteres isora* Linn. Phytother Res 15(1): 49–52.

Prajapati ND, Purohit SS, Sharma AK, Kumar T (2003) A handbook of medicinal plants (ed.) Updesh Purohit for Agrobios (India), Jodhpur p. 265.

Prakasam A, Sethupathy S, Pugalendi KV (2003) Effect of *Casearia esculenta* root extract on blood glucose and plasma antioxidant status in streptozotocin diabetic rats. Pol J Pharmacol 55: 43–49.

Rerup CC (1970) Drugs producing diabetes through damage of the insulin secreting cells. Pharmacol Rev 22: 485–518.

Scheff'e HA (1953) A method for judging all contrasts in the analysis of variance. Biometrika 40: 87–104.

Sheela CG, Augusti KT (1992) Antidiabetic effects of S-allyl cysteine sulphoxide isolated from garlic *Allium sativum* Linn. Ind J Exp Biol 30: 523–526.

Singh SB, Singh AK, Thakur RS (1984) Chemical constituents of the leaves of *Helicteres isora.* Ind J Pharma Sci 46(4): 148–149.

Stanely Mainzen Prince P, Venugopal Menon P, Pari L (1997) Effect of *Syzigium cumini* extracts on hepatic hexokinase and glucose-6-phosphatase in experimental diabetes. Phytother Res 11: 529–531.

Stanely Mainzen Prince P, Venugopal Menon P (2000) Hypoglycemic and other related actions of *Tinospora cordifolia* roots in alloxan-induced diabetic rats. J Ethanopharmacol 70: 9–15.

WHO (1980) The WHO Expert Committee on Diabetes Mellitus. Technical Report Series, WHO, Geneva.