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Original Article

Effect of *Helicteres isora* Bark Extract on Protein Metabolism and Marker Enzymes in Streptozotocin-Induced Diabetic Rats

Ganesan Kumar[a], Gani Sharmila Banu[b], Arunachalam Ganesan Murugesan[a] and Moses Rajasekara Pandian[b*]

[a]*Manonmaniam Sundaranar University, Sri Paramakalyani Centre for Environmental Science, Alwarkurichi, Tamilnadu, India.* [b]*Centre for Biotechnology, Muthayammal College of Arts and Sciences, Kakkaveri, Rasipuram, Namakkal, Tamilnadu, India.*

Abstract

The present study investigated the possible protective effect of *Helicteres isora* (Sterculiaceae) bark extracts on certain biochemical markers in streptozotocin (STZ)-induced diabetes in rats. STZ treatment (60 mg/kg/i.p) caused a hyperglycemic state that led to various physiological and biochemical alterations. Blood levels of glucose, urea, uric acid and creatinine, plasma levels of albumin and albumin/globulin ratio and the activities of diagnostic marker enzymes including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and γ-glutamyl transpeptidase (γ -GT) in plasma, liver and kidney were markedly altered in STZ diabetic rats. Oral administration of *H. isora* (100, 200 mg/kg/p.o) for 21 days restored all these biochemical parameters to near normal levels. Thus, the present results have shown that *H. isora* bark extract has the antihyperglycemic effect and consequently may alleviate liver and renal damage associated with STZ-induced diabetes in rats.

Keywords: *Helicteres isora*; Sterculiaceae; Streptozotocin; Diabetes mellitus; Protein metabolism.

Introduction

Oxidative stress is currently suggested as one of the mechanism underlying diabetes mellitus, which affects carbohydrate, lipid and protein metabolism. Several alterations in diabetic individuals are oxidative in nature or may depend on increased oxidative stress (1). Glycation (2) and hyperglycemic pseudohypoxia (3) can generate a redox imbalance inside the cells, especially in the liver (4). In a recent paper, we reported the elevated levels of plasma thiobarbituric acid reactive substance (TBARS) and hydroperoxide (an index of tissue injury) in streptozotocin (STZ) diabetic rats. The higher levels of these substances suggest an increased rate of tissue injury in STZ diabetic rats. Diabetes mellitus (DM) is also grossly reflected by profound changes in protein metabolism and by a negative nitrogen (N) balance and loss of nitrogen from most organs (5). Increased urea nitrogen production in diabetes may be accounted for by enhanced catabolism of both liver and plasma proteins (6, 7). Indian traditional medicine has used different herbs for the treatment of a broad spectrum of ailments such as inflammation, DM and the management of various hepatic and renal disorders. Ayurveda, the ancient system of Indian medicine, has identified hepatic and

* Corresponding author:
E-mail: rajamoses@yahoo.com

renal diseases quite early and recommended a number of herbal drugs, which are a good source of natural antioxidants believed to exert their effects by reducing the formation of the final active metabolite of the drug-induced systems or by scavenging the reactive molecular species to prevent their reaching a target site (8-10). In addition, Bopanna et al. (11) and Eskander et al. (12) demonstrated that the administration of several herb extracts could restore the changes in the activities of serum enzymes like alkaline phosphatase (ALP), acid phosphatase and transaminases: aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

The *Helicteres isora* Linn. (Sterculiaceae) is a shrub or small tree available in forests throughout Central and Western India. In traditional Medicine the root juice is claimed to be useful in diabetes, empyema and a favorite cure for snakebite (13, 14). The root and bark are expectorant, demulcent, constipating and are useful in colic, scabies, gastropathy, diarrhoea and dysentery (15). The fruits are astringent, refrigerant, stomachic, vermifuge, vulnerary and useful in griping of bowels, flatulence of children (16) and have antispasmodic effect (17). 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, bow of violin, planking, tool-handles, beams and fence posts (18). The root of *H.isora* was undertaken to verify the claim and evaluate the anti-diabetic property (19).

In the current literature, there is no much data concerning the effect of the *H.isora* on the biochemical parameters and the activities of enzymes which are abnormally altered due to DM. Therefore, the present study aims to examine the influence of oral administration of the bark extract of *H. isora* on the levels of some biochemical parameters and the activities of some enzymes in serum, liver, and kidney in STZ-induced diabetic rats.

Experimental

Animals

Male Wistar albino rats (160-200g) 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 for the Care and Use of Laboratory Animals' (20).

Preparation of plant extract

The dried bark of *H.isora* was ground into fine powder with auto-mix blender. Then, the fine powder was suspended in 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) was served as the aqueous extract of *H. isora* for experimentation.

Induction of diabetes

Rats were made diabetic by single administration of STZ (60 mg/kg/i.p) obtained from Sigma Chemical Co. (St. Louis, MO, USA) dissolved in 0.1-M citrate buffer, pH 4.5. After 48 h, blood sample 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.

Experimental design

Diabetes was induced in animals 2 weeks before starting the treatment. After the induction of diabetes, rats were divided into 4 groups of 6 animals each (group II-V). Group I received the vehicle alone and was served as the control. Group II received STZ (60 mg/kg/i.p) dissolved in 0.1-M citrate buffer. Group III & Group IV received the bark extract of *H. 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.

Animals described as fasting were deprived of food for 12 h but allowed free access to drinking water. After 21 days of treatment, the animals were killed by cervical dislocation. Blood was collected into heparinized tubes, and the plasma and serum were separated by centrifugation. The liver and kidney were

Table 1. Effect of bark extract of *H. isora* on liver and kidney weight in control and experimental animals.

Group	Treatment (mg/kg/p.o)	Liver wt (g)	Liver wt/100 g body wt	Kidney wt (g)	Kidney wt/100g body wt
I	Control (2% gum acacia)	6.02±0.31[b]	3.31±0.20[b]	1.12±0.03[b]	0.51±0.02[b]
II	Diabetic control	4.33±0.12[a]	2.64±0.13[a]	1.56±0.1[a]	0.96±0.30[a]
III	Diabetic+*H. isora* 100	5.73±0.31[b]	3.13±0.37[b]	1.35±0.05[b]	0.78±0.02[a]
IV	Diabetic+*H. isora* 200	3.92±0.15[a]	3.60±0.15[b]	1.10±0.05[b]	0.63±0.04[b]
V	Diabetic+Tolbutamide 250	6.13±0.07[b]	3.63±0.14[b]	1.12±0.07[b]	0.66±0.04[b]

Values are given as means±SD of six animals in each group. Values not sharing a common superscript (a, b, c and d) differ significantly at p<0.05, Duncan's Multiple Range Test (DMRT).

quickly removed, washed in ice cold, isotonic saline and blotted individually on ash-free filter paper and weighed were measured. The tissues were then homogenized in 0.1 M Tris-HCl buffer, pH 7.4. The homogenate was used for the estimation of proteins, enzymes, and other parameters.

Estimation of biochemical parameters

Plasma glucose level was measured by the method of Sasaki and Sonae (21) and serum concentration of urea, uric acid and creatinine were determined by Autoanalyser using reagent kit obtained from Boehringer (Mannheim, Germany). The protein content in plasma, liver and kidney was measured by the method of Lowry et al. (22). The albumin and globulin content in plasma were determined by the method described by Reinhold (23). The enzymes (AST, ALT and ALP) were assayed by the method of King and Armstrong (24) and γ-glutamyl transpeptidase (γ -GT) was assayed by the method of Rosalki and Rau (25).

Statistical analysis

Values were presented as Means±SD. Data were analyzed using analysis of variance (ANOVA) and group means were compared with Duncan's multiple range test (DMRT) using SPSS (Statistical Package for Social Science).

Results

Table 1 shows the liver and kidney weights in control and STZ diabetic rats. *H. isora* restored the liver weight to near normal. The kidney weight was increased in diabetic rats and *H. isora* normalized the kidney weight in STZ diabetes. Table 2 shows the blood levels of urea, uric acid, and creatinine in plasma of normal and STZ diabetic rats. These biochemical variables were significantly elevated in STZ diabetic rats (p<0.05) when compared to control animals. Oral administration of *H. isora* bark extract (100, 200 mg/kg/p.o) for 21 days significantly lowered urea, uric acid and creatinine levels in STZ diabetic rats.

The levels of protein, plasma albumin and albumin/globulin ratio in normal and STZ diabetic rats are shown in Table 2. The level of protein in plasma was found to be reducing in

Table 2. Effect of bark extract of *H. isora* on blood levels of urea, uric acid, creatinine, serum protein, albumin and A/G ratio in control

Group	Treatment (mg/kg/p.o)	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)	Protein (g/dl)	Albumin (g/dl)	A/G ratio
I	Control (2% gum acacia)	23.66±2.6[a]	1.3±0.1[a]	0.93±0.09[a]	6.67±0.81[a]	3.70±0.35[a]	1.26±0.15[b]
II	Diabetic Control	37.0±2.76[b]	1.9±0.1[b]	2.16±0.24[b]	4.15±0.81[b]	1.70±0.15[b]	0.69±0.15[b]
III	Diabetic+*H. isora* 100	29.0±2.8[a]	1.6±0.07[a]	1.76±0.15[a]	5.69±0.55[a]	2.80±0.16[a]	0.96±0.42[a]
IV	Diabetic+*H. isora* 200	23.0±0.9[a]	1.33±0.07[a]	1.35±0.04[a]	6.12±0.59[a]	3.23±0.29[a]	1.11±0.31[a]
V	Diabetic+Tolbutamide 250	21.7±1.9[a]	1.19±0.05[a]	1.26±0.07[a]	6.23±0.5[a]	3.30±0.28[a]	1.12±0.31[a]

Values are given as means±SD of six animals in each group. Values not sharing a common superscript (a, b, c and d) differ significantly at p < 0.05, Duncan's Multiple Range Test (DMRT).

Table 3. Effect of bark extract of *H. isora* on serum diagnostic marker enzymes in control and experimental animals.

Group	Treatment (mg/kg/p.o)	AST (Ux/l)	ALT (Ux/l)	ALP(Uy/l)	γ -GT (Uz/l)
I	Control (2% gum acacia)	76.33±7.39[a]	21.16±1.40[a]	76.33±2.25[a]	12.64±1.70[a]
II	Diabetic control	118±5.00[b]	56.66±6.03[b]	134.16±3.94[b]	24.83±2.28[b]
III	Diabetic+*H. isora* 100	94.83±3.71[b]	41.16±2.71[a]	116±3.28[a]	18.64±1.89[a]
IV	Diabetic+*H. isora* 200	84.16±3.83[a]	31.83±3.12[a]	98.33±3.72[a]	16.80±1.65[a]
V	Diabetic+Tolbutamide 250	84.8±3.14[a]	27.66±2.23[a]	87.30±5.24[a]	16.40±1.22[a]

Values are given as means±SD of six animals in each group. Values not sharing a common superscript (a, b, c and d) differ significantly at p<0.05, Duncan's Multiple Range Test (DMRT).
Ux=mmol of pyruvate liberated/h;
Uy= mmol of phenol liberated/min;
Uz= mol of p-nitroanilide liberated/min

diabetic animals (p<0.05) when compared to control animals. The lowered level of protein, after bark extract treatment, increased to near normal. The levels of albumin and albumin/ globulin ratio in plasma were decreased in diabetic animals. These lowered levels of plasma albumin and albumin/globulin ratio level were reverted back significantly in *H. isora* treated diabetic rats.

Tables 3, 4 and 5 show the activities of AST, ALT, ALP and γ -GT in plasma, liver and kidney of the control and STZ diabetic rats, respectively. The activities of these enzymes were found to be significantly increased (p<0.05) in plasma and liver of diabetic rats. In the kidney of diabetic animals, the activities of ALP and γ-GT were increased while the activities of AST and ALT were not altered. Oral administration of *H. isora* (100, 200 mg/kg/p.o) for 21 days resulted in the near normalization of the activities of AST, ALT, ALP and γ -GT in plasma, liver and kidney of diabetic rats.

Discussion

The present investigation indicates the hypoglycemic and protective effects of *H. isora* in the liver and kidney of STZ diabetic rats. We have observed a significant weight gain in *H. isora* treated diabetic rats when compared with untreated animals. This observation shows the anabolic effect of *H. isora* on body weight of the diabetic animals. A decrease in the liver weight observed in diabetic animals might be due to an increased breakdown of glycogen and/or pronounced gluconeogenesis. After 21 days of treatment with *H. isora* in diabetic animals, an increase in the liver weight was observed. This result agrees well with the result of Jefferson et al. (26) who has reported that insulin therapy can increase the accumulation of glycogen in diabetic liver. Seyer-Hansen (27) and Esterby and Gundersen (28) reported 15% rise in whole kidney weight within 72 h of induction of STZ in experimental diabetic rats. We have also observed an increased whole kidney weight

Table 4. Effect of bark extract of *H. isora* on liver and kidney transaminases in control and experimental animals.

Group	Treatment (mg/kg/p.o)	AST (Ux/mg protein)		ALT (Ux/mg protein)	
		Liver	Kidney	Liver	Kidney
I	Control (2% gum acacia)	644.0±17.48[a]	780.0±14.54	927.73±19.46[a]	881.36±20.82
II	Diabetic control	830.2±16.06[b]	740.2±12.6	1250.36±16.27[b]	851.63±20.28
III	Diabetic+*H. isora* 100	748.8±22.33[a]	749.15±13.5	1041.62±13.09[a]	861.66±20.96
IV	Diabetic+*H. isora* 200	673±14.78[a]	751.97±8.51	1002.6±19.70[a]	864.33±9.16
V	Diabetic+Tolbutamide 250	644.7±12.94[a]	761.2±8.80	945.40±15.10[a]	870.7±16.48

Values are given as means±SD of six animals in each group. Values not sharing a common superscript (a, b, c and d) differ significantly at p<0.05, Duncan's Multiple Range Test (DMRT).
Ux=mol of pyruvate liberated/h

Table 5. Effect of bark extract of H. isora on liver and kidney ALP and γ -GT in control and experimental animals.

Group	Treatment (mg/kg b.w)	ALP (Uy /mg protein)		γ -GT (U/mg protein)	
		Liver	Kidney	Liver	Kidney
I	Control (2% gum acacia)	0.18±0.01[a]	0.24±0.02[a]	3.33±0.23[a]	2.63±0.16[a]
II	Diabetic control	0.38±0.03[b]	0.47±0.05[b]	5.46±0.44[b]	3.36±0.38[b]
III	Diabetic+*H. isora* 100	0.22±0.01[a]	0.36±0.02[a]	4.33±0.38[a]	3.83±0.24[a]
IV	Diabetic+*H. isora* 200	0.22±0.02[a]	0.34±0.02[a]	3.53±0.36[a]	3.36±0.38[a]
V	Diabetic+Tolbutamide 250	0.21±0.01[a]	0.28±0.02[a]	3.54±0.34[a]	3.06±0.26[a]

Values are given as means ± SD of six animals in each group. Values not sharing a common superscript (a, b, c and d) differ significantly at $p < 0.05$, Duncan's Multiple Range Test (DMRT).
Uy= mmol of phenol liberated/min;
Uz= mmol of p-nitroanilide liberated/min

in diabetic animals when compared with normal control animals. This is due to the glomerular cell proliferation accompanying glomerular enlargement in the early phase of STZ-induced diabetes in rats. In our present study, oral administration of *H. isora* significantly decreases the kidney weight to near normal value. This might be due to the protective effect of *H. isora* on glomerular cells in STZ-induced diabetic rats.

The diabetic hyperglycemia induces the elevation of plasma levels of urea, uric acid and creatinine, which are considered as the significant markers of renal dysfunction (5). The results in Table 2 show significant ($p<0.05$) increase in the level of plasma urea and creatinine in the diabetic rats when compared with respective control rats, while after the treatment of STZ diabetic rats with the bark extract of *H. isora* (100, 200 mg/kg), the levels of urea, uric acid and creatinine were significantly ($p<0.05$) decreased. These results are in agreement with the previous studies on the mesocarp extract of *B. aegyptiaca* (29, 30) and root extract of *Panax ginseng* (31).

As illustrated in Table 2, a marked ($p<0.05$) reduction in plasma total protein (TP) and albumin (A) levels was observed in diabetic rats and this is consistent with the results obtained by Bakris (32) and Tuvemo et al. (33). The decrease in TP and A may be due to microproteinuria and albuminuria, which are important clinical markers of diabetic nephropathy (34), and/or may be due to increased protein catabolism (35). The results of the present study demonstrated that the treatment of diabetic rats with the bark extract of *H. isora* caused a noticeable elevation in the plasma TP and A levels as compared with their normal levels. Such improvement of serum protein and albumin was previously observed after the oral administration of *B. aegyptiaca* to experimentally diabetic rats (29). It has been established that insulin stimulates the incorporation of amino acids into proteins (5). The enzymes directly associated with the conversion of amino acids to ketoacids are aspartate transferase and alanine transferase, and these enzymes are increased in diabetic condition. Begum and Shanmugasundaram (36) also reported an increase in the activities of aspartate transaminase and alanine transaminase in the liver of diabetic animals. The rise in the activity of alanine transaminase is due to hepatocellular damage and is usually accompanied with aspartate transaminase (37). Treatment with *H. isora* (100 and 200 mg/kg) or tolbutamide (250 mg/kg) normalized these enzymes activities. Similarly, increased activities of aspartate and alanine transaminase in diabetic liver were also reported by Jorda et al. (7).

Elevated activity of ALP was observed in STZ diabetic rats. Prince et al. (38) have also reported increased ALP activity in experimentally diabetic rats. The increased activity of this enzyme in plasma may be a result of diabetes-induced damage to the tissues. *H. isora* (100, 200 mg/kg) treatment restored the activity of these enzymes to near normal by reducing their induction in diabetes. γ -GT catalyzes the transfer of the γ -glutamyl group from γ -glutamyl peptides to another peptide or L-amino acids or to water. The assay of γ-GT is a helpful adjunct in detecting hepatic damage (39). A highly significant

elevation in the activity of γ-GT was observed in plasma, liver and kidney of STZ induced diabetic rats. This is in accordance with earlier investigations (40), wherein there has been shown a dramatic increase in γ-GT expression in the liver of diabetic rats. In addition, hepatocellular damage or cholestasis may also contribute to the elevation of this activity. Increased activity of γ-GT in STZ-induced diabetic rats was lowered to near normal by *H. isora* (100, 200 mg/kg/p.o) treatment that indicates the possible prevention of necrosis by *H. isora* treatment.

From the roots of *H. isora*, cucurbitacin B and isocucurbitacin B have been isolated and reported (41). Neolignans, helisterculins A, B and helisorin have been separated from the fruits, while, betulic acid, daucosterol, sitosterols, isorin (42) have been isolated from the roots and barks of *H. isora*. It is interesting to note that many plant sitosterols have been reported to exhibit hypoglycemic effects (43-46). However, the full potential of sitosterol as the hypoglycaemic agent can only be realized after further comprehensive pharmacological and toxicological investigations.

In conclusion, the present study demonstrated that the bark extract of *H. isora* could influence the protein metabolism and marker enzymes in STZ induced diabetic rats. Further, bark extract of *H. isora* ameliorated the impaired renal function and inhibited the liver damage associated with STZ diabetes in rats.

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