

ORIGINAL ARTICLE

Effect of *Helicteres isora* bark extracts on heart antioxidant status and lipid peroxidation in streptozotocin diabetic rats

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Summary

The present study investigated the effect of the aqueous extract of *Helicteres isora* L. (Sterculiaceae) bark on oxidative stress in the heart of rats during diabetes. The aqueous extract of *Helicteres isora* bark (100 mg, 200 mg/kg body weight, b.w.) was screened for its antioxidant effect in streptozotocin (STZ) induced diabetic rats. An appreciable decrease in peroxidation products, thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), and hydroperoxides (HP) was observed in the heart tissues of *Helicteres isora* (HI) treated diabetic rats. The decreased activities of key antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and glutathione (GSH) in diabetic rats were brought back to near normal range upon HI treatment. Tolbutamide was used as the standard reference drug. These results suggest that HI possesses promising antioxidative activity in STZ diabetic rats.

Key words: *Helicteres isora* – Sterculiaceae – diabetes – lipid peroxidation – antioxidant enzymes

INTRODUCTION

Diabetes is associated with significant oxidative stress, and oxidative damage to tissues may be a contributory factor in several diabetic complications (Kakkar et al. 1995). Reactive oxygen species are an important part of the defense mechanisms against

infection, but the excessive generation of free radicals in unsaturated fatty acids has been implicated in the pathogenesis of vascular disease (Halliwell and Gutteridge 1984, Neville et al. 1996, Manonmani et al. 2005). Diabetic patients have an increased incidence of vascular disease and it has been shown that free radical activity is elevated during diabetes (Oberley 1988, Garg et al. 1996). Normal levels of the antioxidant defense mechanism are not sufficient for the eradication of free radical induced injury. Therefore, the administration of antioxidants from a natural origin has a promising role to play. Several antioxidants of plant material are experimentally proved and widely used as more effective agents against oxidative stress (Battacharya et al. 1997,

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Ilavarasan et al. 2001, Manonmani et al. 2005).

The bark of *Helicteres isora* Linn. (Sterculiaceae) has been used in indigenous systems of medicine in India for the treatment of diabetes mellitus since time immemorial. The plant is a shrub or small tree available in forests throughout the Central and Western India. The roots and the bark are expectorant and demulcent and are useful in colic, scabies, gastropathy, diabetes, diarrhoea and dysentery (Kirtikar and Basu 1995). The fruits are astringent, refrigerant, stomachic, vermifugal, vulnerary and useful in griping of bowels and flatulence in children (Chopra et al. 1956), and possess an antispasmodic effect (Pohocha and Grampurohit 2001). From the roots, cucurbitacin B and isocucurbitacin B were isolated and reported to possess cytotoxic activity (Bean et al. 1985). The roots have a significant hyperglycaemic effect (Venkatesh et al. 2003). The aqueous extract of the bark showed a significant hypoglycaemic effect (Kumar et al. 2006a), hypolipidaemic activity (Kumar and Murugesan, 2008), lowering effect of hepatic enzymes (Kumar et al. 2006b), and glycoprotein levels (Kumar and Murugesan 2007) and an antiperoxidative effect (Kumar et al. 2007).

The present investigation was to assess the heart antioxidant and antiperoxidative efficacy of *H. isora* in STZ diabetic rats.

MATERIALS AND METHODS

Animals

Male Wistar albino rats (weighing 160–200 g) were procured from the Animal house, Bharathidasan University, Tiruchirapalli and maintained under standard environmental conditions (12 h light/dark cycles at 25–28 °C, 60–80% relative humidity). They were fed with a standard diet (Hindustan Lever, India) and water ad libitum and allowed to acclimatize for 14 days before the procedure. All the studies were conducted in accordance with the National Institute of Health guide (1985).

Collection and processing of plant material

The bark of *Helicteres isora* Linn. was collected during May 2003 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 have been deposited in the (collection number 23644, 27406) herbarium for future reference.

The dried bark of HI was ground into a fine powder with an auto-mix blender. Then the fine

powder was suspended in an equal amount of water, 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 185 g/kg of powdered bark) served as aqueous extract of HI for experimentation.

Drugs and chemicals

All the drugs and biochemicals used in this experiment were purchased from Sigma Chemical Company Inc., St Louis, Mo, USA. The chemicals were of analytical grade.

Induction of experimental diabetes

Rats were made diabetic by a single intraperitoneal administration of streptozotocin (60 mg/kg) dissolved in 0.1M-citrate buffer, pH 4.5 (Siddique et al. 1987). Blood samples were collected 48 h later and glucose levels were determined to confirm the development of diabetes. Only those animals which showed hyperglycaemia (blood glucose levels > 240 mg/dl) were used in the experiment.

Experimental design

In the experiment, a total of 42 rats (24 diabetic surviving rats, 18 normal rats) were used. The rats were divided into 7 groups of 6 rats each.

Group 1: Normal rats.

Group 2: Normal rats given HI bark extract (100 mg/kg b.w.) in aqueous solution daily using an intragastric tube for 5 weeks.

Group 3: Normal rats given HI bark extract (200 mg/kg b.w.) in aqueous solution daily using an intragastric tube for 5 weeks.

Group 4: Diabetic control rats.

Group 5: Diabetic rats given HI bark extract (100 mg/kg b.w.) (Kumar et al. 2006a) in aqueous solution daily using an intragastric tube for 5 weeks.

Group 6: Diabetic rats given HI bark extract (200 mg/kg b.w.) in aqueous solution daily using an intragastric tube for 5 weeks.

Group 7: Diabetic rats given tolbutamide (250 mg/kg b.w.) in aqueous solution daily using an intragastric tube for 5 weeks (Kumar et al. 2006a).

All the doses were started 48 h after STZ injection. No detectable irritation or restlessness was observed after each drug or vehicle administration. Blood samples were drawn at weekly intervals till the end of the study (i.e. 5 weeks). At the end of 5th week, all the rats were killed by decapitation (Pentobarbitone sodium anaesthesia – 60 mg/kg). The whole heart was immediately dissected out, and washed in ice cold saline water to remove the blood.

The hearts were weighed and a 10% tissue homogenate was prepared with 0.025M Tris HCl buffer, pH 7.5. After centrifugation at 2000 rpm for 10 min, the clear supernatant was used to measure the assay of enzyme activities.

Biochemical analysis

Estimation of lipid peroxidation

Lipid peroxidation in the heart was estimated calorimetrically by thiobarbituric acid reactive substances (TBARS) and hydroperoxides by the method of Nichans and Samuelson (1968) and Jiang et al. (1992) respectively.

Assay of antioxidant enzymes

Catalase (CAT) was assayed by Sinha (1972). Superoxide dismutase (SOD) was assayed utilizing the technique of Kakkar et al. (1978). Glutathione peroxidase (GPx) activity was measured by the method described by Rotruck et al. (1973). Glutathione (GSH) was determined by the method of Ellman (1959). The glutathione-S-transferase (GST) activity was determined spectrophotometrically by the method of Habig et al. (1974) and lipid peroxidation products (conjugated dienes) (Recknagel and Glende, 1984).

Statistical analysis

All the data are expressed as mean \pm SD of number of experiments ($n = 6$). The statistical significance was evaluated by one-way analysis of variance (ANOVA) using SPSS version 7.5 (SPSS, Cary, NC, USA) and the individual comparison was obtained by Duncan's Multiple Range Test (DMRT). A value of $p < 0.05$ was considered to indicate a significant difference between groups (Duncan 1957).

RESULTS

Levels of cardiac lipid peroxidation products and glutathione content

Table 1 illustrates markers of lipid peroxidation; namely, TBARS, hydroperoxides, conjugated dienes and GSH from the hearts of normal and experimental rats. The levels of TBARS, hydroperoxides, conjugated dienes were significantly increased and decreased the GSH content in diabetic control rats. Administration of HI to diabetic rats significantly decreased the levels of lipidperoxidative markers. Treatment of the normal rats with HI did not show significant changes in lipid peroxidation. The effect produced by HI was significant than by tolbutamide.

Activities of cardiac antioxidant enzymes

The effect of HI on antioxidant status, the activities of enzymatic antioxidants SOD, CAT, GPx, GST were measured (Table 2). The activities of cardiac antioxidant enzymes were significantly decreased in diabetic control rats. They presented significant increases in the diabetic rats treated with HI. Administration of HI to normal rats increased the antioxidant levels with no other significant differences. The effect produced by HI was comparable with that of tolbutamide. The results show that the antioxidant effect of aqueous extract of HI (200 mg/kg/p.o.) was significantly higher than that seen in the tolbutamide treated rats.

DISCUSSION

Diabetes mellitus arises from the irreversible destruction of the pancreatic β -cells causing degranulation and reduction of insulin secretion (Junod et al. 1969). The present study demonstrated that the aqueous extract of bark of HI (100, 200 mg/kg b.w.) had an antiperoxidative effect in the STZ induced diabetic rats.

Hypoinsulinemia in diabetes increases the activity of the enzyme fatty acyl coenzyme A oxidase which initiates beta oxidation of fatty acids, resulting in lipid peroxidation (Horie et al. 1981). Increased lipid peroxidation impairs membrane function by decreasing membrane fluidity and changing the activity of membrane bound enzymes and receptors (Acworth et al. 1997). Its products are harmful to most of the cells in the body and are associated with a variety of diseases (Xing and Tan 2000). Our present study showed a significant elevation of heart TBARS, CD and HP content in diabetic rats. The increased TBARS content of diabetic rats suggests that peroxidative injury may be involved in the development of diabetic complications. The bark extract of HI could significantly reduce the heart lipid peroxidation product levels in diabetic rats (Table 1). This indicates that HI is a potent inhibitor of the oxidative damage of cardiac tissues.

GSH is mainly involved in the synthesis of important macromolecules and in protection against reactive oxygen compounds (Burk 1983, Kaplowitz et al. 1985). A marked decrease in cardiac GSH was observed in the STZ diabetic rats (Helen and Vijayammal 1997). The decreased GSH content contributes to the pathogenesis of complications associated with a chronic diabetic state. The present study showed a decreased content of GSH in cardiac tissue which was significantly increased upon bark extract treatment. This may be attributed to the

presence of the antioxidant compounds in the bark of *H. isora*.

SOD and CAT are the two scavenging enzymes that remove toxic free radicals (Wohaieb and Godin 1987). In the enzymatic antioxidant defense system, SOD is one of the most important enzymes and scavenges $O_2^{\cdot -}$ anion (which is the first product of O_2 radicals) to form H_2O_2 and hence diminishes the toxic effects due to this radical or other free radicals derived from secondary reactions (Arunabh et al. 1999). The $O_2^{\cdot -}$ anion is known to inactivate CAT and GPx (Halliwell and Gutteridge 1984). Catalase has been regarded as a major determinant of hepatic and

cardiac antioxidant status (Wohaieb and Godin 1987). It is known to be involved in detoxification of H_2O_2 concentrations (Yoshikawa et al. 1993, Manonmani et al. 2002), whereas GPx is sensitive to lower concentrations of H_2O_2 . These enzyme activities were inactivated by ROS during diabetes (Ahmed et al. 2000). In the present study, it was observed that the bark extract could increase the SOD, CAT, GPx and GST activities in the cardiac tissues of diabetic rats. This indicates that bark extract of HI could inhibit or reduce the oxidative stress in diabetes.

Table 1. Effect of *H. isora* (HI) on cardiac lipid peroxides like TBARS, CD, HP (mM/100 g wet tissue) and glutathione (mM/100 g wet tissue) in control and experimental groups of rats

Parameters	Normal Control	Bark extract of HI (100 mg/kg) treated control	Bark extract of HI (200 mg/kg) treated control	
TBARS	0.42 ± 0.01^a	0.41 ± 0.03^a	0.40 ± 0.03^a	
CD	14.28 ± 0.50^a	14.29 ± 0.60^a	4.30 ± 0.64^a	
HP	13.25 ± 0.43^a	13.32 ± 0.45^a	13.24 ± 0.45^a	
GSH	409.94 ± 15.70^a	408.52 ± 10.60^a	408.75 ± 10.60^a	

Parameters	Diabetic control	Bark extract of HI (100 mg/kg) treated diabetic	Bark extract of HI (200 mg/kg) treated diabetic	Tolbutamide (250 mg/kg) treated diabetic
TBARS	0.58 ± 0.10^b	0.47 ± 0.01^c	0.43 ± 0.01^d	0.45 ± 0.01^d
CD	20.2 ± 0.80^b	17.85 ± 0.30^c	15.85 ± 0.40^d	15.92 ± 0.40^d
HP	29.25 ± 2.20^b	18.98 ± 0.41^c	15.68 ± 0.45^d	15.74 ± 0.43^d
GSH	66.35 ± 9.30^b	449.95 ± 16.30^c	428.42 ± 15.24^d	427.12 ± 10.60^d

Values are given as means \pm SD of six animals in each group

Values not sharing a common superscript (a, b, c and d) differ significantly

The above results suggest that the effectiveness of the drugs depends, probably, on the accumulative effect of active principles (Peungvicha et al. 1998). From the roots and barks of HI, betulic acid,

daucosterol, sitosterol, isorin (Qu et al. 1991) were isolated. However, the full potential of this antioxidant agent can only be realized after further comprehensive pharmacological and toxicological

investigations.

The LD₅₀ value of the aqueous extract of bark of HI (5 g/kg) was higher than the therapeutic effective dose. We can suggest, therefore, that aqueous extract

of the bark of HI has a low acute toxicity and may be considered relatively free of toxic effects (Kumar et al. 2006a).

Table 2. Effect of *H. isora* (HI) on cardiac antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione -S- transferase (GST) in control and experimental groups of rats

Parameters	Normal Control	Bark extract of HI (100 mg/kg) treated control	Bark extract of HI (200 mg/kg) treated control	
SOD	13.42 ± 0.51 ^a	13.51 ± 0.53 ^a	13.40 ± 0.50 ^a	
CAT	8.28 ± 0.40 ^a	8.29 ± 0.50 ^a	8.26 ± 0.64 ^a	
GPx	1.25 ± 0.04 ^a	1.30 ± 0.05 ^a	1.24 ± 0.05 ^a	
GST	4.92 ± 0.70 ^a	4.87 ± 0.60 ^a	4.89 ± 0.62 ^a	

Parameters	Diabetic control	Bark extract of HI (100 mg/kg) treated diabetic	Bark extract of HI (200 mg/kg) treated diabetic	Tolbutamide (250 mg/kg) treated diabetic
SOD	7.58 ± 0.20 ^b	12.47 ± 0.11 ^c	13.24 ± 0.13 ^d	13.20 ± 0.12 ^d
CAT	5.82 ± 0.80 ^b	7.65 ± 0.28 ^c	8.04 ± 0.30 ^d	8.05 ± 0.12 ^d
GPx	0.75 ± 0.20 ^b	1.08 ± 0.11 ^c	1.20 ± 0.15 ^d	1.18 ± 0.23 ^d
GST	3.35 ± 0.30 ^b	4.25 ± 0.25 ^c	4.48 ± 0.24 ^d	4.46 ± 0.30 ^d

Symbols as in Table 1

The current study provides some useful insight into the cardiac antioxidant and antiperoxidative potency of bark of HI in STZ induced diabetes. However, we suggest that further work should be carried out at the molecular level to find out the absolute mechanism of action of the bark of HI in experimental diabetes.

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