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Effect of *Helicteres isora* Bark Extracts on Brain Antioxidant Status and Lipid Peroxidation in Streptozotocin Diabetic Rats

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Abstract

The current study investigated the effect of the aqueous extract of *Helicteres isora* L (Sterculiaceae) bark on oxidative stress in the brains of rats during diabetes. The aqueous extract of *H. isora* bark was administered orally (100, 200 mg/kg b.w.) and the effect of the extract on blood glucose, plasma insulin, and the levels of thiobarbituric acid reactive substances (TBARS), hydroperoxides, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione *S*-transferase (GST), and reduced glutathione (GSH) were estimated in streptozotocin (STZ) diabetic rats. Tolbutamide was used as the standard reference drug. A significant increase in the activities of plasma insulin, SOD, CAT, GPx, GST, and GSH were observed in the brain on treatment with 100 and 200 mg/kg b.w. of *H. isora* bark extract (HIBe) and tolbutamide for 5 weeks. Both treated groups (bark extract and drug) showed a significant decrease in TBARS and hydroperoxides formation in brain, suggesting its role in protection against lipid peroxidation–induced membrane damage. These findings suggest a possible antiperoxidative role of *H. isora* bark extract that may be used for therapeutic purposes.

Keywords: Brain antioxidant, *Helicteres isora*, lipid peroxidation, streptozotocin.

Introduction

The neurologic consequences of diabetes mellitus in the central nervous system (CNS) are now receiving greater attention. Cognitive deficits, along with morphologic and neurochemical alterations, illustrate that the neurologic complications of diabetes are not limited to peripheral neuropathies (Biessels et al., 1994). The central complications of hyperglycemia also include the potentiation of neuronal damage observed after hypoxic/ischemic events, as well as stroke (McCall, 1992). Glucose utilization is decreased in the brain during diabetes (McCall, 1992), providing a potential mechanism for increased vulnerability to acute pathologic events.

Oxidative stress, leading to an increased production of reactive oxygen species (ROS), as well as lipid peroxidation, is increased in diabetes (Wolff, 1993) and also by stress in euglycemic animals (Liu et al., 1996). Similarly, oxidative damage in rat brain is increased by experimentally induced hyperglycemia (Aragno et al., 1997). Under experimental conditions, hyperglycemia dramatically increases neuronal alterations and glial cell damage caused by temporary ischemia (Li, et al., 1998). Several lines of evidence indicate that the modified oxidative state induced by chronic hyperglycemia (Aragno et al., 2000) may contribute to nervous tissue damage; free radical species impair the central nervous system, attacking neurons and Schwann cells (Kumar & Menon, 1993) and the peripheral nerves (Kawai et al., 1998). Because of their high polyunsaturated lipid content, Schwann cells and axons are particularly sensitive to oxygen free radical damage; lipid peroxidation may increase cell membrane rigidity and impair cell function.

Increases in superoxide production are observed in the serum of type 1 diabetic patients and are reduced with improved glycemic control (Ceriello et al., 1991). Lipid peroxidation products are also increased in the brains of type 1 diabetic rats (Makar et al., 1995) and type 2 diabetic mice (Kumar & Menon, 1993). Diabetes and

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stress-mediated increases in oxidative stress, as well as decreases in antioxidant activity, may make the brain more vulnerable to subsequent pathologic events.

Currently, the use of complementary/alternative medicine and especially the consumption of botanicals have been increasing rapidly worldwide, mostly because of the supposedly less frequent side effects when compared with modern Western medicines (Hu et al., 2003).

The bark of *Helicteres isora* Linn. (Sterculiaceae) has been used in the 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, demulcent, and are useful in colic, scabies, gastropathy, diabetes, diarrhea, and dysentery (Kirtikar & Basu, 1995). The roots have a significant hyperglycemic effect (Venkatesh et al., 2003). The fruits are astringent, refrigerant, stomachic, vermifugal, vulnerary, and useful in griping of bowels, flatulence of children (Chopra et al., 1956), and antispasmodic effects (Pohocha & Grampurohit, 2001). From the roots, cucurbitacin B and isocucurbitacin B were isolated and reported to possess cytotoxic activity (Bean et al., 1985). The aqueous extract of the bark showed a significant hypoglycemic effect (Kumar et al., 2006a) and lowering effect of hepatic enzymes (Kumar et al., 2006b).

The purpose of the current investigation was to assess the brain antioxidant and antiperoxidative efficacy of *H. isora* in streptozotocin (STZ) diabetic rats.

Materials and Methods

Animals

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

Collection and processing of plant material

The bark of *Helicteres isora* was collected during May 2003 from Solakkadu, Kollimalai, Namakkal District, Tamil Nadu, India, and authenticated by Fr. K.M. Matthew, Director, Rapinat Herbarium, St. Joseph's College, Tiruchirapalli. Voucher herbarium specimens have been deposited at the herbarium (collection no. 23644, 27406) for future reference.

The dried bark of *Helicteres isora* was ground into fine powder with an automix 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 185 g/kg of powdered bark) served as aqueous extract of *Helicteres isora* for experimentation.

Drugs and chemicals

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

Induction of experimental diabetes

Rats were made diabetic by single intraperitoneal administration of streptozotocin (60 mg/kg) dissolved in 0.1 M citrate buffer, pH 4.5 (Siddique et al., 1987). After 48 h, blood samples were collected, and glucose levels were determined to confirm the development of diabetes. Only those animals showing hyperglycemia (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 I: Normal rats.
Group II: Normal rats given *H. isora* bark extract (HIBe) (100 mg/kg b.w.) in aqueous solution daily using an intragastric tube for 5 weeks.
Group III: Normal rats given *H. isora* bark extract (HIBe) (200 mg/kg b.w.) in aqueous solution daily using an intragastric tube for 5 weeks.
Group IV: Diabetic control rats.
Group V: Diabetic rats given HIBe (100 mg/kg b.w.) (Kumar et al., 2006a) in aqueous solution daily using an intragastric tube for 5 weeks.
Group VI: Diabetic rats given HIBe (200 mg/kg b.w.) in aqueous solution daily using an intragastric tube for 5 weeks.
Group VII: 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 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 until the end of the study (i.e., 5 weeks). At the end of the fifth week, all the rats were sacrificed by decapitation (pentobarbitone sodium) anesthesia (60 mg/kg). Blood was collected in two different tubes, one with anticoagulant (sodium citrate) for

plasma and another without anticoagulant for serum separation. Plasma and serum were separated by centrifugation. Whole brain was immediately dissected out and washed in ice-cold saline to remove the blood. The brains were weighed, and 10% tissue homogenate was prepared with 0.025 M 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 blood glucose and plasma insulin

Blood glucose was determined by the *o*-toluidine method (Sasaki et al., 1972). Plasma insulin was assayed by ELISA, using a Boeheringer-Mannheim Kit with a Boeheringer analyzer ES300.

Estimation of lipid peroxidation

Lipid peroxidation in brain 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 dismotase (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). Reduced 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).

Estimation of protein

Protein was determined by the method of Lowry et al. (1951) using bovine serum albumin (BSA) as standard.

Statistical analysis

All data were expressed as mean ± 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 comparisons were 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

Table 1 shows the level of blood glucose and plasma insulin in normal and experimental groups. The level of blood glucose was significantly increased, whereas the level of plasma insulin was significantly decreased in diabetic control rats. Oral administration of 100 mg, 200 mg/kg of HIBe and 250 mg/kg of tolbutamide to diabetic rats significantly reversed all these changes to near-normal levels.

Table 2 illustrates markers of lipid peroxidation, namely, TBARS and hydroperoxides from brains of normal and experimental rats. The levels of TBARS and hydroperoxides were significantly increased in diabetic control rats. Administration of HIBe to diabetic rats significantly decreased the levels of lipid-peroxidative markers. Treatment of normal rats with HIBe did not show significant changes in lipid peroxidation. The effect produced by HIBe was more significant than that of tolbutamide.

The effect of HIBe on antioxidant status, the activities of enzymatic antioxidants SOD, CAT, GPx, GST, and nonenzymatic antioxidant GSH were measured (Tables 3 and 4). The activities of enzymatic and the levels of nonenzymatic antioxidant were significantly decreased in diabetic control rats. They presented significant increases in diabetic rats treated with HIBe. Administration of HIBe to normal rats increased the antioxidants levels with no significant differences. The effect produced by HIBe was comparable with that of tolbutamide. The

Table 1. Effect of aqueous extracts of the bark of *Helicteres isora* on blood glucose and plasma insulin in normal and experimental rats.

Group (n = 6)	Treatment	Fasting blood glucose (mg/dL)	Plasma insulin (μunit/mL)
I	Normal	85 ± 5^{a}	13 ± 0.8^{a}
II	Normal + HIBe (100 mg/kg)	79 ± 4^{a}	15 ± 1^{b}
III	Normal + HIBe (200 mg/kg)	74 ± 5^{b}	16 ± 3^{b}
IV	Diabetic control	270 ± 15^{c}	4 ± 0.3^{c}
V	Diabetic + HIBe (100 mg/kg)	115 ± 3^{d}	11 ± 4^{d}
VI	Diabetic + HIBe (200 mg/kg)	96 ± 3^{e}	9 ± 3^{e}
VII	Diabetic + tolbutamide (250 mg/kg)	98 ± 6^{e}	9 ± 1^{e}

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

Table 2. Effect of aqueous extracts of bark of *Helicteres isora* on brain TBARS and hydroperoxides in normal and experimental rats.

Group (n = 6)	Treatment	TBARS (mM/100 g tissue)	Hydroperoxides (mM/100 g tissue)
I	Normal	1.12 ± 0.06^{a}	114.15 ± 3.20^{a}
II	Normal + HIBe (100 mg/kg)	1.04 ± 0.05^{a}	110.50 ± 2.70^{a}
III	Normal + HIBe (200 mg/kg)	0.98 ± 0.03^{a}	98.70 ± 3.05^{b}
IV	Diabetic control	1.98 ± 0.07^{b}	135.68 ± 2.45^{c}
V	Diabetic + HIBe (100 mg/kg)	1.25 ± 0.06^{c}	113.22 ± 3.26^{d}
VI	Diabetic + HIBe (200 mg/kg)	1.20 ± 0.03^{c}	110.42 ± 3.08^{d}
VII	Diabetic + tolbutamide (250 mg/kg)	1.36 ± 0.06^{d}	120.40 ± 4.05^{e}

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

result shows that the antioxidant effect of aqueous extract of HIBe (200 mg/kg, p.o.) was significantly higher than that of seen in the tolbutamide-treated rats.

Discussion

This work is one of a series of studies showing that chronic hyperglycemia causes an imbalance in the oxidative status of the nervous tissue and that the resulting free radicals damage the brain through a peroxidative mechanism. The STZ diabetic rat serves as an excellent model to study the molecular, cellular, and morphologic changes in brain induced by stress during diabetes (Aragno et al., 2000). Under normal conditions, the generation of free radicals or of active species in the brain, as in other tissues, is maintained at extremely low levels (Liu et al., 1996). Diabetes also contributes to cerebrovascular complications, reductions in cerebral blood flow, disruption of the blood-brain barrier, and cerebral edema (Aragno et al., 1997). All of these neurochemical and neurophysiologic changes ultimately contribute to the long-term complications associated with diabetes, including morphologic abnormalities, cognitive impairments, and increased vulnerability to pathophysiologic event (Li et al., 1998).

In the current study, treatment with aqueous extract of bark of *Helicteres isora* showed significant antihyperglycemic activity. The antihyperglycemic activity of this plant may be, at least in part, through release of insulin from the pancreas in view of the measured increase in the plasma insulin concentrations. Earlier studies in this laboratory have demonstrated a defective metabolism of lipid peroxides in other tissues of diabetic animal (Kumar et al., 2006b). This may be because the brain contains relatively high concentration of easily peroxidizable fatty acids (Carney et al., 1991). In addition, it is known that certain regions of the brain are highly enriched in iron, a metal that, in its free form, is catalytically involved in production of damaging oxygen free radical species (Nistico et al., 1992). In this process, the ferric iron is reduced by superoxide, with subsequent oxidation of ferrous iron by H_2O_2 forming hydroxyl radical:

$$Fe^{3+} + O_2^{*-} \longrightarrow Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^{*} + OH^{-}$$

The destruction of superoxide radical or H_2O_2 by SOD or CAT would ameliorate STZ toxicity, as would substances able to scavenge the hydroxyl radical (Walling, 1975; Lubec et al., 1996). Vulnerability of brain to oxidative stress induced by oxygen free radicals seems

Table 3. Effect of aqueous extract of bark of *Helicteres isora* on brain CAT and SOD in normal and experimental rats.

Group (n = 6)	Treatment	CAT (Units[A]/mg protein)	SOD (Units[B]/mg protein)
I	Normal	3.25 ± 0.29^{a}	7.80 ± 0.40^{a}
II	Normal + HIBe (100 mg/kg)	3.85 ± 0.20^{a}	7.55 ± 0.32^{a}
III	Normal + HIBe (200 mg/kg)	4.05 ± 0.20^{b}	7.42 ± 0.35^{a}
IV	Diabetic control	0.76 ± 0.15^{c}	5.24 ± 0.15^{b}
V	Diabetic + HIBe (100 mg/kg)	2.95 ± 0.20^{d}	7.15 ± 0.38^{c}
VI	Diabetic + HIBe (200 mg/kg)	3.05 ± 0.45^{d}	7.48 ± 0.20^{d}
VII	Diabetic + tolbutamide (250 mg/kg)	2.35 ± 1.35^{e}	7.12 ± 0.30^{c}

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

[A]μmole of H_2O_2 consumed/minute.

[B]One unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in 1 min.

Table 4. Effect of aqueous extract of bark of *Helicteres isora* on brain GPx, GST, and GSH in normal and experimental rats.

Group (n = 6)	Treatment	GPx (Units[A]/mg protein)	GST (Units[B]/mg protein)	GSH (mg/100 g tissue)
I	Normal	3.48 ± 0.15^{a}	5.74 ± 0.15^{a}	35.25 ± 3.21^{a}
II	Normal + HIBe (100 mg/kg)	3.59 ± 0.18^{a}	5.75 ± 0.28^{a}	38.12 ± 2.14^{a}
III	Normal + HIBe (200 mg/kg)	3.66 ± 0.20^{a}	5.84 ± 0.20^{a}	39.18 ± 3.20^{a}
IV	Diabetic control	1.10 ± 0.25^{b}	1.24 ± 0.02^{b}	14.82 ± 1.39^{b}
V	Diabetic + HIBe (100 mg/kg)	2.85 ± 0.15^{c}	2.65 ± 0.13^{c}	25.16 ± 2.01^{c}
VI	Diabetic + HIBe (200 mg/kg)	3.24 ± 0.20^{d}	3.25 ± 0.20^{d}	32.08 ± 2.20^{d}
VII	Diabetic + tolbutamide (250 mg/kg)	2.47 ± 0.12^{e}	2.15 ± 0.16^{e}	25.50 ± 2.10^{c}

Values are given as means ± SD of six animals in each group. Values not sharing a common superscript (a, b, c, d, and e) differ significantly at $p < 0.05$, Duncan's multiple range test (DMRT).
[A]μg of GSH consumed/min.
[B]μmoles of CDNB-GSH conjugate formed/min.

to be due to the fact that, on one hand, the brain utilizes about one fifth of the total oxygen demand of the body, and on the other hand, that it is not particularly enriched, when compared with other organs, in any of the antioxidant enzymes. Relatively low levels of these enzymes may be responsible in part for the vulnerability of this tissue (Baynes & Thrope, 1999).

The altered balance of the antioxidant enzymes caused by a decrease in CAT, SOD, GPx, GST, and GSH activities may be responsible for the inadequacy of the antioxidant defenses in combating ROS-mediated damage. The decreased activities of CAT and SOD may be a response to increased production of H_2O_2 and O_2 by the autoxidation of glucose and nonenzymatic glycation (Aragno et al., 1997; Pari & Latha, 2004). These enzymes have been suggested to play an important role in maintaining physiologic levels of oxygen and hydrogen peroxide by hastening the dismutation of oxygen radicals and eliminating organic peroxides and hydroperoxides generated from inadvertent exposure to STZ (Bolzan & Bianchi, 2002). Treatment with HIBe increased the activity of enzymes and may help to control free radicals, as *Helicteres isora* has been reported to be rich in secondary metabolites (Bean et al., 1985; Qu et al., 1991; Yasuhiro et al., 1999), well-known antioxidants, which scavenge the free radicals generated during diabetes.

Under *in vivo* conditions, GSH acts as an antioxidant, and its decrease was reported in diabetes mellitus (Rotruck et al., 1973). We have observed significant decrease in GSH levels in brain during diabetes. The decrease in GSH levels represents increased utilization due to oxidative stress (Matcovis et al., 1982). The depletion of GSH content may also lower the GST and GPx activity (Yu, 1994). GPx has been shown to be an important adaptive response to condition of increased peroxidative stress (Rotruck et al., 1973). The increased GSH content in the brain of the rats treated with HIBe and tolbutamide may be a factor responsible for inhibition of lipid peroxidation. The elevated level of GSH protects cellular proteins against oxidation through glutathione redox cycle and also directly detoxifies reactive oxygen species generated from exposure to STZ (Yu, 1994). The significant increase in GSH content and GSH-dependent enzymes GPx and GST in diabetic rats treated with HIBe indicates an adaptive mechanism in response to oxidative stress.

Significantly lower levels of lipid peroxides in brains of HIBe-treated diabetic rats and increased activities of enzymatic and nonenzymatic antioxidants in brain suggest that the extract reduces oxidative stress by quenching free radicals.

Secondary metabolites were reported to have free radical scavenging activity and antioxidant capacity in diabetes (Jang et al., 2000; Singh et al., 2000). Any compound, natural or synthetic, with antioxidant properties that might contribute toward the partial or total alleviation of this damage may have a significant role in the treatment of diabetes mellitus. The antioxidant responsiveness mediated by *Helicteres isora* may be anticipated to have biological significance in eliminating reactive free radicals that may otherwise affect the normal cell functioning. The dysfunction of these antioxidant enzymes has been implicated in several disorders including rheumatoid arthritis, reperfusion injury, cardiovascular diseases, immune injury, as well as diabetes mellitus (Singh et al., 2000).

It may be concluded that in diabetes, brain tissue was more vulnerable to oxidative stress and showed increased lipid peroxidation. The above observation shows that the aqueous extract of bark of *Helicteres isora* plant possesses antioxidant activity, which could exert a beneficial action against pathologic alterations caused by the presence of free radicals in STZ diabetes.

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