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Effect of aqueous *Enicostemma littorale* Blume extract on key carbohydrate metabolic enzymes, lipid peroxides and antioxidants in alloxan-induced diabetic rats

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Abstract

The present study investigates the effect of oral administration of an aqueous *Enicostemma littorale* whole plant extract on some key carbohydrate metabolic enzymes and antioxidant defence in alloxan-induced diabetes in rats. Rats were rendered diabetic by alloxan (150 mg kg^{-1} body weight) administration. Oral administration of *E. littorale* extract for 45 days increased the activity of hexokinase and decreased the activities of glucose 6-phosphatase and fructose 1,6-bisphosphatase significantly in the serum, liver and kidney of diabetic rats. The extract lowered the concentration of thiobarbituric acid reactive substances and lipid hydroperoxides significantly in brain and increased it significantly in heart in diabetic rats. *E. littorale* administration increased the concentration of reduced glutathione and the activity of glutathione peroxidase in diabetic rats. The activities of superoxide dismutase and catalase were increased significantly by *E. littorale* treatment in diabetic rats. The effect of a 2 g kg^{-1} dose was greater than that of a 1 g kg^{-1} dose. Insulin (6 units kg^{-1}) normalized all the parameters in diabetic rats. Our study has provided evidence for the antidiabetic activity of *E. littorale* aqueous extract. This study can also be extrapolated to clinical studies in future.

Introduction

Diabetes mellitus is characterized by defects in insulin action, insulin secretion or both, and involves high levels of blood glucose, which contribute to an increase in free radical production (Kehrer 1993; Baynes & Thorpe 1999). Hexokinase is the key enzyme in the glycolytic pathway, which is insulin dependent and plays a major role in the maintenance of glucose homeostasis. All the cells that metabolize glucose by ATP produce glucose 6-phosphate. Glucose 6-phosphatase and fructose 1,6-bisphosphatase are regulatory enzymes of the gluconeogenic pathway. The activities of hexokinase, glucose 6-phosphatase and fructose 1,6-bisphosphatase are altered in diabetes mellitus.

Free radicals are implicated in the causation of several diseases, such as liver cirrhosis, atherosclerosis, cancer, diabetes, etc., and compounds that can scavenge free radicals have great potential in ameliorating these disease processes (Wilson et al 1988). Antioxidants thus play an important role in protecting the human body against the damage caused by reactive oxygen species. Increased oxidative stress has been postulated in the diabetic state. Oxygen free radicals can initiate peroxidation of lipids, which in turn stimulate glycation of proteins, inactivation of enzymes and alteration in the structure and function of collagen, basement and other membranes, and play a role in the long-term complications of diabetes mellitus (Baynes 1991).

Oxidative stress in diabetes coexists with a reduction in the antioxidant status, which can increase the deleterious effects of free radicals. Several different endogenous antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)) protect cells against the damaging effects of reactive oxygen species (ROS). SOD has been described as one of the most important enzymes in the enzymatic antioxidant defence system. It scavenges the superoxide anion to form hydrogen peroxide, thereby lowering the toxic effects caused by this radical. CAT, which is involved in the detoxification of hydrogen peroxide, is inactivated by superoxide

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anion (Soon & Tan 2002). GPx plays an important role in the detoxification of H_2O_2. It has been reported to reduce hydroperoxides by reducing glutathione (GSH) (Pereira et al 1995).

Enicostemma littorale Blume is a glabrous perennial herb belonging to the family Gentianaceae. It is a 2–5 inch tall herb that grows throughout India. It is more common in the plains and near the sea. Preliminary reports have shown the hypoglycaemic effect of the extract in alloxan-induced diabetes in rabbits (Vyas et al 1979). There has also been a report showing that aqueous extract of *E. littorale* lowers blood glucose and glycosylated haemoglobin in alloxan-induced diabetic rats (Vijayvargia et al 2000). The blood glucose lowering effect of the extract has also been reported in streptozotocin-treated rats (Murali et al 2002). The insulin secretory effect of an aqueous extract of *E. littorale* has been reported by Maroo et al (2002).

E. littorale Blume is a plant containing a number of antioxidative phytochemicals, including alkaloids, sterols, catechins, phenolic acids and p-coumaric acid. It also contains minerals and amino acids (Murali et al 2002). There are no reports pertaining to the effect of *E. littorale* on carbohydrate metabolic enzymes, lipid peroxides and antioxidants in diabetes mellitus. In view of these facts, the present study was designed to evaluate the effect of aqueous *E. littorale* plant extract on hexokinase, glucose 6-phosphatase, fructose 1,6-bisphosphatase, thiobarbituric acid reactive substances (TBARS), lipid hydroperoxides (HP), GSH, GPx, SOD and CAT in alloxan-induced diabetic rats.

Materials and Methods

Plant extract

E. littorale growing at various places in Cuddalore, Tamil Nadu, India were collected. The collection was made by the uprooting method, taking care to collect the roots as well. The plant was identified at the Herbarium of Botany Directorate in Annamalai University, Annamalai Nagar, Tamil Nadu. A voucher specimen (No. 641) was also deposited there. The plant was cleaned and dried in the shade and packed into loose masses in plastic bags.

Preparation of aqueous *E. littorale* extract

The *E. littorale* aqueous extract was prepared as described by Murali et al (2002). A total of 3 kg of the shade-dried herb containing all vegetative and reproductive parts of *E. littorale* was mixed with 24 L of water and heated until it was reduced to half (12 L). The whole mass was filtered and concentrated further twice (6 L) by heating. The marc obtained from this was again mixed with fresh 12 L of water and heated till approximately 6 L of water was left. The whole mass was filtered and mixed again, and the filtrate was concentrated to 3 L by heating. The marc remaining was discarded. Both the filtrates were mixed and further concentrated at low temperature. The concentration of dried *E. littorale* was $2\,g\,mL^{-1}$ in the final extract. A 2% sodium benzoate solution was added as a preservative. The extract was stored at a temperature of 15–20°C in a well-closed glass container.

Experimental animals

All the animal experiments carried out by us were approved by the Ethics Committee of Annamalai University, Tamil Nadu, India. Female albino Wistar rats of 160–190 g body weight were procured from the Central Animal House, Department of Experimental Medicine, Rajah Muthiah Medical College and Hospital, Annamalai University. They were housed in polypropylene cages lined with husk, renewed every 24 h under a 12/12 h light/dark cycle at around 22°C and had free access to tap water and food. The rats were fed on a standard pellet diet (Kamadhenu Agencies, Bangalore, India).

Induction of experimental diabetes

The rats were injected with alloxan in saline (0.9% NaCl) at a dose of $150\,mg\,kg^{-1}$ body weight intraperitoneally. Control rats were injected with saline only. Prior to this, the rats were fasted for 12 h. After a fortnight, urine sugar determination was done by Benedict's method. Blood glucose levels in rats were estimated using the o-toluidine method (Sasaki et al 1972). The rats with high urine sugar and blood glucose ($230–280\,mg\,dL^{-1}$) were selected for the diabetic group.

Experimental design

In our study, a total of 36 rats (12 normal; 24 alloxan-diabetic surviving) were used. The rats were divided into six groups of six rats each: Group I, normal untreated rats given saline orally using an intragastric tube for 45 days; Group II, normal untreated rats orally administered with *E. littorale* extract ($2\,g\,kg^{-1}$) for 45 days by an intragastric tube; Group III, alloxan ($150\,mg\,kg^{-1}$)-induced diabetic rats given saline orally for 45 days; Groups IV and V, diabetic rats treated orally with *E. littorale* extract (1 and $2\,g\,kg^{-1}$) for 45 days; Group VI, diabetic rats administered protamine zinc insulin ($6\,units\,kg^{-1}$) intraperitoneally for 45 days.

After 45 days of treatment, all the rats were decapitated after an overnight fast. Blood was collected in potassium oxalate- and sodium fluoride-containing tubes for the estimation of fasting blood glucose. Serum was separated by centrifugation. Liver, kidney, heart and brain were excised immediately, rinsed in ice-chilled normal saline and stored for further biochemical estimations.

Preparation of tissue homogenate

A known weight of the tissue was homogenized in 5 mL of appropriate buffer solution. The homogenate was centrifuged at low speed for 5 min. The supernatant was used for the estimation of various biochemical parameters.

Biochemical analysis

Fasting blood glucose (Sasaki et al 1972), hexokinase (Brandstrup et al 1957), glucose 6-phosphatase (Koide & Oda 1959), fructose 1,6-bisphosphatase (Gancedo & Gancedo 1971), TBARS (Fraga et al 1988), HP (Jiang et al 1992), GSH (Ellman 1959), GPx (Rotruck et al 1984), SOD (Kakkar et al 1984) and CAT (Sinha 1972) were estimated.

Statistical analysis

Statistical analysis was performed using SPSS software package, version 6.0. The values were analysed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) (Duncan 1957). All the results were expressed as mean ± s.d. for six rats in each group. P values < 0.05 were considered to be significant.

Results

The levels of blood glucose of normal and diabetic rats are presented in Figure 1. A significant ($P < 0.05$) increase in blood glucose was observed in diabetic rats as compared to normal rats. Oral administration of *E. littorale* extract (1 and 2 g kg^{-1}) for 45 days to diabetic rats decreased the levels of blood glucose significantly ($P < 0.05$).

Table 1 shows the activities of serum hexokinase, glucose 6-phosphatase and fructose 1,6-bisphosphatase for normal and diabetic rats. The activity of hexokinase decreased while glucose 6-phosphatase and fructose 1,6-bisphosphatase activities increased significantly ($P < 0.05$) in diabetic rats as compared with normal rats. Oral administration of *E. littorale* (1 and 2 g kg^{-1}) to diabetic rats for 45 days exerted a significant ($P < 0.05$) effect on these enzymes.

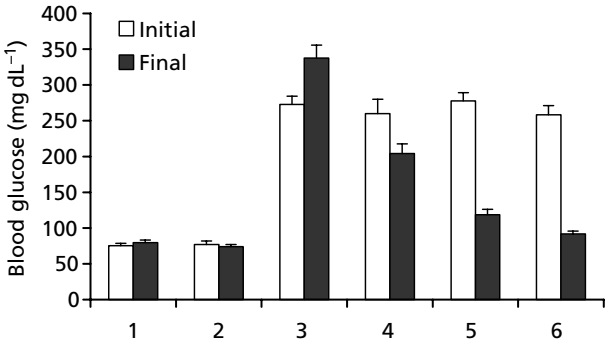

Figure 1 Effect of *E. littorale* on the levels of blood glucose in normal and alloxan-induced diabetic rats. Group 1: normal untreated rats given saline orally using an intragastric tube for 45 days. Group 2: normal rats untreated, orally administered with *E. littorale* extract (2 g kg^{-1}) for 45 days by an intragastric tube. Group 3: alloxan-induced (150 mg kg^{-1}) diabetic rats given saline orally for 45 days. Groups 4 and 5: diabetic rats treated orally with *E. littorale* extract (1 and 2 g kg^{-1}) for 45 days. Group 6: diabetic rats administered protamine zinc insulin (6 units kg^{-1}) intraperitoneally for 45 days.

Table 2 shows the activities of hepatic and renal hexokinase, glucose 6-phosphatase and fructose 1,6-bisphosphatase for normal and diabetic rats. The activity of hexokinase decreased while glucose 6-phosphatase and fructose 1,6-bisphosphatase activities increased significantly ($P < 0.05$) in diabetic rats as compared to normal rats. Oral administration of *E. littorale* (1 and 2 g kg^{-1}) to diabetic rats for 45 days exerted a significant ($P < 0.05$) effect on these enzymes in the liver and kidney.

Table 3 shows the concentration of TBARS and HP in the heart and brain of normal and alloxan diabetic rats. The concentration of TBARS and HP increased significantly in the brain, but significantly ($P < 0.05$) decreased in the heart of alloxan-induced diabetic rats as compared with normal rats. *E. littorale* extract administered at doses of 1 and 2 g kg^{-1} to diabetic rats for a period of 45 days exerted a significant ($P < 0.05$) effect on TBARS and HP in heart and brain.

Table 4 shows the concentration of GSH and the activity of GPx in the heart and brain of diabetic rats. The concentration of GSH and the activity of GPx decreased significantly ($P < 0.05$) in the heart and brain of alloxan-induced diabetic rats as compared with normal rats. Administration of *E. littorale* extract orally to diabetic rats at doses of 1 and 2 g kg^{-1} for 45 days significantly ($P < 0.05$) increased GSH concentration and GPx activity in heart and brain.

Table 5 shows the activities of SOD and CAT in the heart and brain of normal and alloxan diabetic rats. The activities of these enzymes decreased significantly in brain, but there was no significant ($P < 0.05$) change in the heart of diabetic rats as compared with normal rats. Oral administration with *E. littorale* extract (1 and 2 g kg^{-1}) for 45 days to diabetic rats increased the activities of these enzymes significantly ($P < 0.05$).

In all the parameters studied, the dose of *E. littorale* (2 g kg^{-1}) was found to be more effective than 1 g kg^{-1}. Insulin (6 units kg^{-1}) administration normalized all these parameters in diabetic rats. Treatment of normal rats with *E. littorale* (2 g kg^{-1}) did not show any significant effect in all the parameters studied.

Discussion

Effect of aqueous *E. littorale* extract on key carbohydrate metabolic enzymes

Alloxan causes a massive reduction in insulin release through the destruction of the β-cells of the islets of Langerhans and induces hyperglycaemia (Goldner & Gomori 1943). In diabetes mellitus, enzymes of glucose metabolism such as hexokinase, glucose 6-phosphatase and fructose 1,6-bisphosphatase are markedly altered and produce hyperglycaemia, which leads to pathogenesis of diabetic complications (Sockhar et al 1985).

Alloxan-induced diabetes causes changes in the properties and functions of the cell, resulting in increased release of some enzymes due to cell necrosis, which can result in the leakage of these enzymes out of the tissues and into the

Table 1 Effect of *E. littorale* on the activities of serum hexokinase, glucose 6-phosphatase and fructose 1,6-bisphosphatase in normal and diabetic rats

Group	Serum		
	Hexokinase (mmole of glucose phosphorylated h^{-1} mL^{-1})	Glucose 6-phosphatase (μmole of Pi liberated min^{-1} mL^{-1})	Fructose 1,6-bisphosphatase (μmole of Pi liberated min^{-1} mL^{-1})
1	0.17 ± 0.004^{a}	97.1 ± 3.9^{a}	66.5 ± 2.3^{a}
2	0.16 ± 0.006^{a}	95.2 ± 3.3^{a}	64.8 ± 1.8^{a}
3	0.09 ± 0.009^{b}	149.4 ± 2.0^{b}	81.0 ± 2.8^{b}
4	$0.12 \pm 0.004^{b,c}$	122.6 ± 2.9^{c}	$78.4 \pm 2.1^{b,c}$
5	0.15 ± 0.004^{d}	105.8 ± 4.5^{d}	71.3 ± 2.7^{d}
6	$0.16 \pm 0.003^{a,d}$	98.0 ± 2.7^{a}	$68.6 \pm 1.8^{a,d}$

See Figure 1 for details of groups. Each value is mean ± s.d. for six rats in each group. Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

Table 2 Effect of *E. littorale* on the activities of hepatic and renal hexokinase, glucose 6-phosphatase and fructose 1,6-bisphosphatase in normal and diabetic rats

Group	Liver			Kidney		
	Hexokinase (mmole of glucose phosphorylated h^{-1} mg^{-1} protein)	Glucose 6-phosphatase (μmole of Pi liberated min^{-1} mg^{-1} protein)	Fructose 1, 6-bisphosphatase (μmole of Pi liberated min^{-1} mg^{-1} protein)	Hexokinase (mmole of glucose phosphorylated h^{-1} mg^{-1} protein)	Glucose 6-phosphatase (μmole of Pi liberated min^{-1} mg^{-1} protein)	Fructose 1, 6-bisphosphatase (μmole of Pi liberated min^{-1} mg^{-1} protein)
1	0.29 ± 0.03^{a}	21.5 ± 0.6^{a}	17.6 ± 0.8^{a}	0.43 ± 0.02^{a}	5.7 ± 0.2^{a}	7.6 ± 0.3^{a}
2	0.29 ± 0.02^{a}	22.2 ± 0.9^{a}	18.1 ± 0.9^{a}	0.44 ± 0.02^{a}	5.2 ± 0.2^{a}	7.9 ± 0.4^{a}
3	0.13 ± 0.04^{b}	43.1 ± 1.1^{b}	29.0 ± 0.7^{b}	0.29 ± 0.04^{b}	17.1 ± 0.3^{b}	17.8 ± 0.9^{b}
4	0.18 ± 0.02^{c}	36.0 ± 2.0^{c}	25.8 ± 1.0^{c}	0.33 ± 0.03^{c}	12.6 ± 0.1^{c}	14.6 ± 0.4^{c}
5	0.26 ± 0.03^{d}	27.6 ± 1.6^{d}	21.0 ± 0.8^{d}	0.39 ± 0.03^{d}	8.7 ± 0.1^{d}	9.6 ± 0.5^{d}
6	0.28 ± 0.02^{a}	23.31 ± 1.0^{a}	$19.6 \pm 1.0^{a,d}$	0.41 ± 0.02^{a}	6.8 ± 0.1^{a}	$8.5 \pm 0.4^{a,d}$

See Figure 1 for details of groups. Each value is mean ± s.d. for six rats in each group. Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

Table 3 Effect of *E. littorale* on the concentration of TBARS and HP in the heart and brain of normal and alloxan diabetic rats

Group	TBARS (mM 100 g^{-1} wet tissue)		HP (mM 100 g^{-1} wet tissue)	
	Heart	Brain	Heart	Brain
1	0.39 ± 0.01^{a}	1.73 ± 0.03^{a}	21.6 ± 1.3^{a}	7.11 ± 0.34^{a}
2	0.38 ± 0.01^{a}	1.71 ± 0.03^{a}	21.9 ± 1.8^{a}	7.07 ± 0.22^{a}
3	0.28 ± 0.02^{b}	2.84 ± 0.05^{b}	16.4 ± 2.0^{b}	13.69 ± 0.50^{b}
4	$0.31 \pm 0.02^{b,c}$	2.44 ± 0.10^{c}	18.1 ± 1.6^{c}	11.18 ± 0.60^{c}
5	0.35 ± 0.01^{d}	1.83 ± 0.03^{d}	20.2 ± 1.3^{d}	8.51 ± 0.46^{d}
6	0.39 ± 0.01^{a}	$1.79 \pm 0.04^{a,d}$	21.0 ± 1.6^{a}	$7.42 \pm 0.36^{a,d}$

See Figure 1 for details of groups. Each value is mean ± s.d. for six rats in each group. Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

Table 4 Effect of *E. littorale* on the concentration of GSH and the activity of GPx in the heart and brain of diabetic rats

Group	GSH (mM g^{-1} wet tissue)		GPx (μg of GSH consumed min^{-1} mg^{-1} protein)	
	Heart	Brain	Heart	Brain
1	12.0 ± 0.4^{a}	22.5 ± 1.3^{a}	0.88 ± 0.03^{a}	1.12 ± 0.06^{a}
2	12.3 ± 0.6^{a}	22.8 ± 1.7^{a}	0.90 ± 0.03^{a}	1.12 ± 0.04^{a}
3	7.4 ± 0.6^{b}	17.2 ± 1.4^{b}	0.70 ± 0.05^{b}	0.88 ± 0.06^{b}
4	8.8 ± 0.5^{c}	18.1 ± 1.1^{c}	$0.74 \pm 0.06^{b,c}$	0.94 ± 0.07^{c}
5	10.8 ± 0.9^{d}	19.7 ± 0.8^{d}	0.82 ± 0.05^{d}	1.00 ± 0.06^{d}
6	11.4 ± 0.4^{a}	22.0 ± 1.0^{a}	0.85 ± 0.04^{a}	1.10 ± 0.04^{a}

See Figure 1 for details of groups. Each value is mean ± s.d. for six rats in each group. Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

Table 5 Effect of *E. littorale* on the activities of SOD and CAT in the heart and brain of normal and alloxan diabetic rats

Group	SOD (units mg^{-1} protein)		Catalase (μmoles of H_2O_2 consumed min^{-1} mg^{-1} protein)	
	Heart	Brain	Heart	Brain
1	10.6 ± 0.3^{a}	7.3 ± 0.2^{a}	8.1 ± 0.4^{a}	2.01 ± 0.02^{a}
2	10.7 ± 0.3^{a}	7.7 ± 0.2^{a}	8.0 ± 0.6^{a}	2.12 ± 0.06^{a}
3	9.8 ± 0.7^{a}	4.9 ± 0.2^{b}	7.6 ± 0.8^{a}	0.83 ± 0.04^{b}
4	10.1 ± 0.8^{a}	$5.8 \pm 0.2^{b,c}$	7.8 ± 0.4^{a}	$1.14 \pm 0.06^{b,c}$
5	10.3 ± 0.8^{a}	6.5 ± 0.2^{d}	7.9 ± 0.6^{a}	1.78 ± 0.08^{d}
6	10.4 ± 0.8^{a}	7.0 ± 0.3^{a}	8.0 ± 0.4^{a}	1.91 ± 0.06^{a}

See Figure 1 for details of groups. SOD: one unit is defined as the enzyme concentration required to inhibit the OD at 560 nm of chromogen production by 50% in 1 min. Each value is mean ± s.d. for six rats in each group. Values not sharing a common superscript differ significantly at $P < 0.05$ (DMRT).

blood. An increase in hexokinase, glucose 6-phosphatase and fructose 1,6-bisphosphatase in diabetic rats correlates with damage to liver and kidneys.

Hexokinase, one of the important key enzymes in the catabolism of glucose, was lowered in the serum, liver and kidneys of alloxan diabetic rats in this study. Similar results were reported by other researchers in experimental diabetes (Grover et al 2000). Lowered activity of hexokinase can result in decreased glycolysis and thus decreased utilization of glucose for energy production (Mahapatra et al 1985). Oral administration of *E. littorale* extract to alloxan diabetic rats increased the activity of hexokinase and this may be due to increased uptake of glucose and ultimately increased glycolysis. The decreased levels of blood glucose after treatment with *E. littorale* extract in alloxan diabetic rats may be due to increased glycolysis and hence increased hexokinase activity.

The activities of gluconeogenic enzymes, glucose 6-phosphatase and fructose 1,6-bisphosphatase were elevated in serum, liver and kidney (Grover et al 2000). Glucose 6-phosphatase is an important enzyme in the homeostasis of blood glucose as it catalyses the terminal step both in gluconeogenesis and glycogenolysis. This enzyme is present in liver and kidney and is an important regulatory enzyme involved in the release of glucose from the liver and kidney (Minassian et al 1996). We have noted an increase in the activity of glucose 6-phosphatase in serum, liver and kidney in diabetic rats. The increased activity of this enzyme lowered the concentration of glucose 6-phosphate and inhibited glycogen synthesis and the glycolytic pathway of glucose 6-phosphate, which in turn caused elevated levels of blood glucose (Aiston et al 1999). Increased activity of glucose 6-phosphatase has been reported in experimental diabetes by other workers (Baquer et al 1998).

Fructose 1,6-bisphosphatase is one of the key enzymes of the gluconeogenic pathway. It is present in liver and kidney (Murray et al 2000). This enzyme catalyses one of the irreversible steps in gluconeogenesis and serves as a site for the regulation of this process (Tillmann et al 2002). A deficiency of insulin in the diabetic state increases the activity of fructose 1,6-bisphosphatase.

E. littorale aqueous extract administered orally for 45 days to alloxan-induced diabetic rats significantly reduced the activities of glucose 6-phosphatase and fructose 1,6-bisphosphatase. This might be due to a state of increased insulin concentration, which reflects the insulin secretory effect of the extract. In this context Maroo et al (2002) reported the insulin stimulatory effect of aqueous *E. littorale* in diabetic rats.

Effect of aqueous *E. littorale* extract on lipid peroxidation

In our study, we observed an increased concentration of TBARS and HP in diabetic rat brain. This indicates the activation of the lipid peroxidation system. Brain tissue, because of its high rate of oxygen consumption and high phospholipid content with polyunsaturated fatty acid, is particularly susceptible to peroxidative agents, free radical generating compounds and increased lipid peroxidation (Suresh Kumar & Menon 1993). Meydani et al (1988) have shown that brain is more prone to lipid peroxidation since it contains low levels of vitamin E and selenium-dependent glutathione peroxidase activity. In this context, Suresh Kumar & Menon (1993) also reported an increase in lipid peroxides in the alloxan diabetic brain. Oral administration of *E. littorale* (1 and $2\,g\,kg^{-1}$) decreased the concentration of TBARS and HP in the diabetic brain.

Decreased levels of TBARS and HP were noted in the hearts of diabetic rats. In cardiac tissue, the level of lipid peroxides decreased in spite of increased fatty acids and decreased activity of SOD and CAT. The fatty acids produced in the myocardial tissue may be utilized by other tissues for energy production in diabetes. It has also been observed that under conditions of stress, the myocardium utilizes glucose in preference to fatty acids for its energy production (Whitmer et al 1978). The decreased utilization of fatty acids by myocardial tissue may therefore be one of the factors for decreased formation of lipid peroxide as well as the observed decreased in the activity of SOD and CAT.

Effect of aqueous *E. littorale* extract on antioxidants

Oxidative stress is an imbalance between ROS and the antioxidant defence mechanisms of a cell or tissue. This leads to lipid peroxidation and inactivation of many enzymes (Halliwell & Gutteridge 1984). Clinical complications in diabetes may be due to the dysfunction of key antioxidant enzymes.

GSH protects tissues from damage caused by diabetes, which impairs the antioxidant system. Depletion of tissue GSH is one of the primary factors that permits lipid peroxidation (Konukoglu et al 1988). It has been proposed that antioxidants that maintain the concentration of GSH may restore the cellular defence mechanisms, block lipid peroxidation and thus protect the tissues against oxidative damage (Chugh et al 1999). The level of GSH decreases in the heart and brain of alloxan-induced diabetic rats. GSH is an important substrate for the enzyme GPx, which is present in myocardial tissue. The decreased level of GSH in diabetic rats may be due to its increased utilization by GPx. Besides this, the mixed valence iron model predicts that in a system containing all Fe(III), lipid peroxidation will be inhibited on the addition of a reductant. The decreased level of GSH should therefore result in decreased peroxidation in the heart and brain. The observed decrease in the levels of lipid peroxides in heart tissue is in agreement with this view.

GPx has been reported to reduce hydroperoxides by reducing GSH (Pereira et al 1995). The observed decreased activity of GPx in this study might be due to decreased concentration of GSH in diabetic tissues. In this context, Suresh Kumar & Menon (1992) have reported decreased activity of GSH in the heart and brain of alloxan-induced diabetic rats.

The decreased activities of SOD and CAT observed by us in diabetic heart and brain can lead to excess availability of superoxide (O^{2-}) and hydrogen peroxide (H_2O_2) in the biological systems, which in turn generate hydroxyl radicals, resulting in the initiation and propagation of lipid peroxides. Oral administration of *E. littorale* (1 and $2\,g\,kg^{-1}$) to diabetic rats for 45 days increased the activities of SOD and CAT in heart and brain.

In our study, we noted that the oral administration of aqueous *E. littorale* extract significantly restored the lipid peroxides and antioxidants to near normal in alloxan-induced diabetic rats. Phytochemical studies reveal the presence of alkaloids, sterols, catechins, saponins, phenolic acids, flavonoids, amino acids, p-coumaric acid and ferulic acid in *E. littorale*. A variety of flavonoids, alkaloids, coumarins and phenolic acids isolated from various plants were tested for their antioxidant activity as reflected in their ability to inhibit lipid peroxidation (Ng et al 2000). The antioxidant property of *E. littorale* might be due to the presence of these constituents.

Conclusion

Our findings show that oral administration of *E. littorale* extract controls the increase in the levels of glucose in diabetes by increasing glycolysis and decreasing gluconeogenesis. This is possible as it controls the activities of key enzymes of glycolysis. This could be due to the insulin stimulatory action of *E. littorale* extract. The antioxidant phytoconstituents present in the plant extract scavenge free radicals and prevent the depletion of endogenous antioxidants. Our study provides considerable evidence for the antidiabetic activity of the extract. The results can be extrapolated to clinical studies.

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