ENZYMATIC ALTERATION IN THE VITAL ORGANS OF STREPTOZOTOCIN DIABETIC RATS TREATED WITH AQUEOUS EXTRACT OF ERYTHRINA VARIEGATA BARK

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ABSTRACT

The effect of aqueous extract of Erythrina variegata bark on the altered functions of some of the brain enzymes under pharmacologically induced diabetic conditions was evaluated. The effect of the extract on blood glucose level, lipid profile, protein level, urea, creatinine and liver enzymes were appraised to find out their potential in controlling diabetes related metabolic modifications. Oral diabetes tolerance and acute toxicity were also studied. Moreover the level of enzymatic and non enzymatic antioxidants and extend of basal lipid peroxidation in three vital organs namely liver, kidney and brain were also monitored in normal, diabetic and treated rats. Also, the liver and pancreas were subjected to histological examination. Finally, in order to figure out the effect of plant extract on brain enzymes in diabetic and normal conditions, degree of Na⁺-K⁺ ATPase, Ca²⁺ ATPase , Mg ²⁺ ATPase and 5’nucleotidase along with proteolytic activity of brain extract were assessed. The experimental findings lent pharmacological support for the use of Erythrina variegata stem-bark in the management of diabetes mellitus

Keywords: Erythrina variegata, Diabetes mellitus, Membrane bound ATPase, Basal lipid peroxidation, Enzymatic and non enzymatic antioxidants

INTRODUCTION

Diabetes mellitus is the world’s largest endocrine disorder. It is a metabolic disorder in which the homeostasis of carbohydrate, protein and lipid metabolism is improperly regulated by hormone insulin, thus resulting in elevation of fasting and post prandial blood glucose levels. Hyperglycemia in diabetes mellitus may be due to abnormal insulin production and secretion, resistance towards insulin or both. Hyperglycemia leads to increased production of reactive oxygen species via at least seven routes namely increased glycolysis, intercellular activation of sorbitol pathway, auto oxidation of glucose, non enzymatic protein glycosylation, disruption of polyol pathway, altered eicosanoid metabolism and decreased antioxidant defenses.

Earlier classified as rich man’s disease, diabetes mellitus has now spread among masses. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and eventual failure of organs especially the eyes, kidney, nerves, heart and blood vessels. Hyperglycemia is the most important factor for the onset and progress of diabetic complications mainly by producing oxidative stress. Brain is the most sensitive organ susceptible to oxidative stress due to its great oxygen consumption, high lipid content and poor antioxidant defenses. High oxidative stress and changes in antioxidant balance due to persistent and chronic hyperglycemia, promote free radical generation; evidence based mainly on increased lipid peroxidation that contributes to alterations in membrane ion transport and permeability, neurochemical, neurophysiological and behavioral modifications as well as cerebrovascular disturbances in brain, impairing its functional and structural integrity.

Diabetes mellitus is known to cause neurological disorders due to impaired glucose metabolism involving decreased utilization of glucose by brain. Since brain is affected by recurrent episodes of hypoglycemia and poor diabetic control, protein metabolism undergoes severe alterations during diabetes. Diabetes induced hyperglycemia enhances the extent of neurological disorders due to enzyme inactivation. However neither insulin nor oral hypoglycemic drugs are ideal in treatment due to toxic side effects and sometimes diminution in responses after prolonged use. The disadvantages of presently available drugs are that they have to be given throughout life and produce side effects. Synthetic oral hypoglycemic agents can produce a series of side effects including hematological, gastro intestinal reactions, hypoglycemic coma and disturbances in liver and kidney metabolism. In addition, these preparations are not ideal for use during pregnancy. Activities of enzyme connected to glucose metabolism and neuronal activities have been studied as a function of diabetes.

The medicinal plants might provide a useful source of new oral hypoglycemic compounds for development of pharmaceutical entities or as a dietary adjunct to existing therapies. Many plants have been reported effective for treating diabetes, though their mechanism of action is not known. Plants may act on blood glucose through different mechanisms. Some of them may have insulin like substances, some may inhibit insulinase activity, and some others may increase β cells in pancreas by activating regeneration of these cells. The fiber of the plant may also interfere with carbohydrate absorption, thereby affecting blood glucose. Erythrina variegata is a medium sized deciduous small tree with prickly stems and branches, leaves with triangular leaflets and large coral red flowers. It is found throughout the tropics in cultivation. A wide spectrum of biological activities has been reported for different parts of the plant. Erythrina plants produce alkaloids, flavonoids and terpenes and are commonly used in folk medicine.

MATERIALS AND METHODS

Collection of plant material

The bark of Erythrina variegata was procured from the Trichur, Kerala and was identified by the Botanical Survey of India, Tamilnadu Agricultural University (TNAU), Coimbatore and authenticated by Dr.G.V.S.Moorthy. (Voucher no: BSI/SRC/5/23/09-10/Tech-990).

Extraction

The bark was air dried at 25°C for 5 days in the absence of sunlight and powdered well using a mixer and stored in an air tight container. The powdered medicinal plant material (50 g) was taken and subjected to successive solvent extraction (250ml) with increasing order of polarity like petroleum ether, chloroform, ethyl acetate, ethanol and water. The extraction was carried out for 16 hours with increasing order of polarity.

Experimental animals

Adult male albino rats weighing about 150-200 g were obtained from the animal house of Karpagam University, Coimbatore and...
were used for the study. Rats were housed in polycarbonate cages in a room with a 12-hour day-night cycle, at constant temperature of 22°C and humidity of 45-64%. During the experimental study rats were fed on pellets (Gulmohur rat feed, Lipton India, Bangalore) with free access to tap water. The rats received humane care according to the criteria outlined in Principles of laboratory animal care formulated by the National Society for Medical Research and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Induction of experimental diabetes

Rats were rendered diabetic by a single intraperitoneal injection of freshly prepared streptozotocin (STZ-45 mg/kg body weight) in 0.1M citrate buffer (pH 4.5) in a volume of 1 ml/kg body weight. Normal rats received 1 ml citrate buffer as vehicle. Diabetes was identified in rats by moderate polydypsia and marked polyuria. After 48 h of streptozotocin administration, blood glucose levels were estimated in rats following overnight fasting. Rats with a blood glucose ranging between 200–300 mg/dl were considered diabetic and used for the experiments.

Pilot Study

The various extracts of Erythrina variegata bark namely petroleum ether, chloroform, ethylacetate, ethanol and water extracts were tested for antidiabetic activity so as to determine which among them had the most potent antidiabetic activity. The animals were rendered diabetic after a single intraperitoneal administration of streptozotocin (45 mg/kg body weight). 200 mg/kg of various extracts were given orally to different group of rats (3 rats were used for each group, control rats received physiological saline) for a period of seven days. Initial and final body weight as well as blood glucose levels were noted and glycemic index was calculated by the formula

\[ \text{Glycemic index} = \frac{\text{Initial blood glucose - final blood glucose} \times 100}{\text{Final blood glucose}} \]

% Glycemic index = Initial blood glucose - final blood glucose \times 100

Final blood glucose

Glucose Tolerance Test

The glucose tolerance test or study on glucose loaded rats was performed on overnight fasted normal rats. The animals were divided into 4 groups each containing 4 rats. Group 1 was treated with normal saline. Group 2-4 receives 200, 400, 600 mg/kg of aqueous bark extract of Erythrina variegata.

The animals were given their respective doses of Erythrina variegata bark extract for seven days along with normal diet. At the end of the seventh day the rats were put on overnight fasting for 14hrs. On the next day, blood was drawn from tail vein and blood sugar was determined using one touch electronic glucometer by the formula

\[ \text{% Glycemic index} = \frac{\text{Initial blood glucose} \times 100}{\text{Final blood glucose}} \]

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\[ \text{% Glycemic index} = \frac{\text{Initial blood glucose} \times 100}{\text{Final blood glucose}} \]

Acute Toxicity Study

The aqueous extract was tested for its acute toxicity (if any) in Wistar strain of male albino rats weighing between 150-180g. To determine acute toxicity of single oral administration of E. variegata, different doses of the aqueous extract namely 500, 1000 and 2000 mg were administered orally to 3 groups of five animals each. Another group of five rats served as control and this received 1ml of physiological saline. The animals were observed continuously for initial 3hrs and intermittently for the next 6hrs following drug. Mortality and general behavior of animals were observed periodically for 24hrs.

Experimental Setup

The Wistar strain of male albino rats weighing between 150-180g was used for the study. A total of 25 rats (15 diabetic surviving rats, 10 normal rats) were used and they were divided in to five groups of five animals each. Group I served as untreated control. Group II was diabetic induced. Group III and Group IV were diabetic rats received standard oral hypoglycemic agent, glibenclamide (2mg/kg b.wt) and aqueous bark extract E. variegata (400mg/kg,b.wt) respectively. Group V was normal rats treated with E variegata (400mg/kg,b.wt) alone.

The animals were weighed and dosed through oral intragastric tube every day. Blood glucose levels were measured in normal and experimental rats in initial, 15th and 30th days of treatment using electronic glucometer. The test drug and reference standard drugs were fed orally for 30days. The study was terminated in overnight fasted rats at the end of 30days. The rats were sacrificed by cervical dislocation after giving mild anesthesia using chloroform. Blood and serum were separated. Liver, whole brain and kidneys were immediately dissected out, washed and stored in 0.9% ice cold saline for various biochemical evaluations of liver and pancreas were put in 1% formalin for histopathological studies.

Collection of blood, kidney, brain and liver from the rat

After the experimental regimen, the animals were sacrificed by cervical dislocation under mild chloroform anesthesia. Blood was collected on decapitation and serum was separated by centrifugation (for 20 min at 2000 rpm). Liver, whole brain and kidneys were immediately dissected out, washed and stored in 0.9% ice cold saline for various biochemical evaluations of liver and pancreas were put in 1% formalin for histopathological studies.

Estimation of biochemical parameters in serum and tissues

Serum glucose and total protein were estimated. Serum Cholesterol, triglycerides, HDL, urea, creatinine, SGOT, SGPT alkaline phosphatase, VLDL and LDL were assayed using diagnostic reagent kit manufactured by Span diagnostics Ltd. Certain biochemical indices like protein, glutathione (GSH) 15, superoxide dismutase (SOD) 17, catalase(CAT) 15, glutathione peroxidase (GPx) 19, ascorbic acid (Vitamin C) 20, basal lipid peroxidation (LPO) 21 and Glutathione - S -Transferase (GST) 22 were estimated in brain, liver and kidney.

Biochemical assays done on the rat brain

To appraise the effect of diabetes on rat brain, the following parameters in brain were monitored namely protease activity, Na+/K+-ATPase activity 23, Mg2+-ATPase activity 24, Ca2+-ATPase activity 25, and S' nucleotidase activity 26.

Statistical Analysis

Results are expressed as Mean ± SD of five individual experiment and the statistical significance was evaluated by one way analysis of variance (ANOVA) using SPSS version (10.0) and the individual comparisons were obtained by the Duncan multiple range test (DMRT). A value of p<0.05 was considered to indicate a significant difference between groups. Comparison with in the group were achieved by student’s t test and compared at p<0.05 and p<0.01.

RESULTS AND DISCUSSION

Changes in serum parameters

Assessment of hypoglycemic activity of different extracts was carried out by pilot study. Pilot study confirms that water extract of E. variegata showed the presence of various chemical constituents, mainly flavonoids which may be responsible for its antidiabetic properties. 27.
Effective dose determination was carried out by performing GTT in normal healthy rats. A constant decrease in blood glucose level during GTT in healthy rats (Fig 4) was seen after single oral administration of variable doses 200, 400, 600 mg/kg body weight of aqueous extract for seven days. Since the highest glycemic index of 5.45 (Fig 5) was associated with the dose of 400 mg/kg, this dose was identified as the most effective dose. Such a phenomenon of less hypoglycemic response at a higher dose is not uncommon with indigenous plants and has already been observed in Annona squamosa, Trichosanthes dioica, and Ficus bengalensis.

Fig. 1: Levels of blood glucose with various extracts of *E. variegata* in pilot study

Values are expressed as Mean ± S.D of three individual experiments

* - Initial and final blood glucose level compared, significant at 5% level (t<0.05)
Fig. 2: Glycemic index of various extracts of *E. variegata* in pilot study

Values are expressed as Mean ± S.D of three individual experiments

Fig. 3: Body weight of rats with different extracts of *E. variegata* in pilot study

Values are expressed as Mean ± S.D of three individual experiments

* - Initial and final body weight compared, significant at 5% level (t<0.05)
Values are expressed as Mean ± S.D of four individual experiments.

**EV – Erythrina variegata**

Fig. 4: Effective dose determination - Oral glucose tolerance test

Fig. 5: Glycemic index of various doses of *E. variegata* in GTT of albino rats

Safety of the aqueous extract of *Erythrina variegata* bark was confirmed by the acute toxicity study where the extract did not show any mortality up to a dose of 2000 mg/kg body weight in male albino rats. There were no signs of lethality, hyperactivity, sedation, respiratory rate or toxic symptoms. Even at this high dose there were no gross behavioral changes. It reported any compound or drug with the oral LD₅₀ estimate greater than 1000 mg/kg could be considered of low toxicity and safe. This supports that the aqueous extract of *Erythrina variegata* bark was found to be safe up to the dose of level 2000 mg/kg body weight.
Fig. 6: Effect of *E. variegata* on blood glucose level of control and diabetic rats on 30th day of treatment

Values are expressed as Mean ± S.D of five individual experiments  
Values not sharing a common superscript letter differ significantly (DMRT)  
Diab+Glib - Diabetes + Glibenclamide treated group  
Diab+EV - Diabetes + *E. variegata* treated group  
EV - *E. variegata* alone treated group

Fig. 7: Level of Vitamin C in liver, kidney and brain of control and experimental groups

Values are expressed as Mean ± S.D of five individual experiments  
Values not sharing a common superscript letter differ significantly (DMRT)
Fig. 8: Level of GSH in liver, kidney and brain of control and experimental groups

Values are expressed as Mean ± S.D of five individual experiments

Values not sharing a common superscript letter differ significantly (DMRT)

Fig. 9: Lipid peroxidation in liver, kidney and brain of control and experimental groups

Values are expressed as Mean ± S.D of five individual experiments

Values not sharing a common superscript letter differ significantly (DMRT)

Table 1: Effect of Erythrina variegata on level of blood glucose (mg/dl) at various stages of study
The 30th day (Fig 6). The permeability of liver cell membrane 33. In the present study, the permeability of liver cell membrane 33. In the present study, STZ induces hepatocellular damage, which results in leakage of AST, ALT compared to control group as shown in Table 2. Furthermore STZ induces a hepatocellular damage, which is indicated by AST, ALT reversed by treatment with aqueous extract of *Erythrina variegata* for thirty days was capable of lowering ALT and AST levels in diabetic rats. However administration of *Erythrina variegata*, which are reported to be hepatoprotective agents. The increased levels of transaminases, which are active in the liver, are accelerated, resulting in increased blood glucose levels. The capacity of the extract to decrease the elevated blood glucose level to normal glycemic level may be an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes 30.

STZ diabetic rats were found to have significantly elevated serum creatinine and urea levels as compared to non diabetic control rats. This is because STZ diabetic rats have diminished ability to filter urea and creatinine from blood and excrete them in urine. This is another characteristic change in diabetes. Whereas after treatment both groups became comparable to those which received glibenclamide treatment. There was no significant difference in values obtained for group I and group V (Table 2).

Thus the aqueous extract of *Erythrina variegata* was almost equal to glibenclamide in its efficiency in lowering blood glucose level in STZ induced diabetic model. When the plant extract alone treated rats were compared with control rats there was no significant change was observed in blood glucose level. The capacity of the extract to decrease the elevated blood glucose level to normal glycemic level may be an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes.

Table 1 shows the effect of aqueous extract of *Erythrina variegata* on blood glucose in normal and experimental animals at the end of 0, 15 and 30 days of study. The level of blood glucose was significantly increased in diabetic rats when compared to normal rats. Oral administration of plant extract (400 mg/kg body weight) and glibenclamide (2 mg/kg body weight) to diabetic rats significantly decreased the blood glucose. In *Erythrina variegata* treated groups, although a significant antihyperglycemic effect was evident from day 15 onwards, decrease in blood glucose was maximum at the end of 30th day (Fig 6).

Table 2: Effect of *Erythrina variegata* on blood glucose in normal and experimental animals at the end of 0, 15 and 30 days of study. Values are expressed as Mean ± S.D of five individual experiments.

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Control (Group I)</th>
<th>Diabetic control (Group II)</th>
<th>Diabetic + Glibenclamide (Group III)</th>
<th>Diabetic + <em>E. variegata</em> treated (Group IV)</th>
<th><em>E. variegata</em> alone treated (Group V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>89.2 ±0.23</td>
<td>295.5±0.40</td>
<td>302.2±0.39</td>
<td>291.2±0.40</td>
<td>94.2±0.28</td>
</tr>
<tr>
<td>15</td>
<td>93.1±0.29</td>
<td>456.5±0.36</td>
<td>11.0±0.17</td>
<td>120.3±0.40</td>
<td>101.5±0.41</td>
</tr>
<tr>
<td>30</td>
<td>82.1±0.32</td>
<td>472.2±0.42</td>
<td>88.36±0.31</td>
<td>87.4±0.49</td>
<td>84.02±0.23</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.D of five individual experiments.

Table 2: Effect of *Erythrina variegata* on urea, creatinine, ALP, SGOT, SGPT and protein in serum of control and experimental groups

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Control (Group I)</th>
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<th><em>E. variegata</em> alone treated (Group V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mg/dl)</td>
<td>22.37±0.17</td>
<td>39.30±0.21</td>
<td>22.1±0.10</td>
<td>25.14±0.13</td>
<td>22.15±0.18</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.87±0.02</td>
<td>2.39±0.45</td>
<td>0.92±0.02</td>
<td>1.09±0.01</td>
<td>0.91±0.02</td>
</tr>
<tr>
<td>SGOT (IU/L)</td>
<td>123.4±0.36</td>
<td>272.0±0.29</td>
<td>203.4±0.41</td>
<td>204.3±0.37</td>
<td>122.3±0.32</td>
</tr>
<tr>
<td>SGPT (IU/L)</td>
<td>83.4±0.40</td>
<td>185.0±13.03</td>
<td>83.80±0.17</td>
<td>98.5±0.45</td>
<td>83.7±0.22</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>259.7±0.57</td>
<td>375.0±0.22</td>
<td>290.0±0.58</td>
<td>295.0±0.45</td>
<td>255.2±0.19</td>
</tr>
<tr>
<td>Protein (mg/dl)</td>
<td>7.76±0.44</td>
<td>2.76±0.23</td>
<td>6.79±0.02</td>
<td>6.69±0.04</td>
<td>7.12±0.23</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.D of five individual experiments.

The increased levels of transaminases, which are active in the liver, are accelerated, resulting in increased blood glucose levels. The capacity of the extract to decrease the elevated blood glucose level to normal glycemic level may be an essential trigger for the liver to revert to its normal homeostasis during experimental diabetes 30.

STZ diabetic rats were found to have significantly elevated serum creatinine and urea levels as compared to non diabetic control rats. This is because STZ diabetic rats have diminished ability to filter urea and creatinine from blood and excrete them in urine. This is another characteristic change in diabetes. Whereas after treatment both groups became comparable to those which received glibenclamide treatment. There was no significant difference in values obtained for group I and group V (Table 2).

Table 3: Effect of *Erythrina variegata* on lipid profile of control and experimental groups

<table>
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<tr>
<th>Particulars</th>
<th>Control (Group I)</th>
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<th><em>E. variegata</em> alone treated (Group V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>126.6±0.32</td>
<td>239.5±0.39</td>
<td>117.8±0.12</td>
<td>136±0.32</td>
<td>124.9±0.22</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>75.5±0.44</td>
<td>162.4±0.49</td>
<td>91.6±0.58</td>
<td>80.6±0.40</td>
<td>74.6±0.37</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>24.77±0.34</td>
<td>122.5±0.52</td>
<td>23.8±0.18</td>
<td>24.3±0.44</td>
<td>25.6±0.47</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.D of five individual experiments.

Values not sharing a common superscript letter differ significantly (DMRT)

The increased levels of transaminases, which are active in the absence of insulin because of increased availability of amino acids in diabetes, are responsible for increased glucogenes is and ketogenesis observed in diabetes 32. The present results show that injection of STZ induces a hepatocellular damage, which is indicated by significant increase in AST, ALT and ALP in diabetic group as compared to control group as shown in Table 2. Furthermore STZ induces hepatocellular damage, which results in leakage of AST, ALT and ALP from liver cytosol to the blood stream and/or may change the permeability of liver cell membrane 32.

In the present study, aqueous extract of *Erythrina variegata* significantly decreased AST, ALT and ALP enzyme activities in diabetic rats. The improvements in the levels of the enzymes studied are a consequence of an improvement in the carbohydrate, fat and protein metabolism. The restoration of AST and ALT after treatment also indicates a revival of insulin secretion 32.

Streptozotocin induced diabetes significantly lowered the serum protein level (Table 2). The serum protein level of untreated diabetic rats was 2.76±0.23 mg/dl as compared to 7.76±0.44 mg/dl in normal rats. Subsequent treatment with glibenclamide and plant extract for thirty days reversed the serum protein level to 6.79±0.02 and 6.69±0.04 respectively. With insulin deficiency, the oxidation of branched chain amino acids in muscle and uptake of alanine (the principle glycogenic amino acid) by the liver is accelerated, resulting in increased gluconeogenesis and augmented protein catabolism. The accompanying rise in glucose levels is most likely due to an increased conversion of ingested protein into glucose and to a decreased glucose removal rate 32.

Lipids play a vital role in pathogenesis of diabetes mellitus. Abnormalities in lipid profile are one of the most common complications in diabetes mellitus found in 40% of diabetic cases 32. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia 32. In the present study, elevated levels of serum lipids such as cholesterol, LDL, VLDL and triglyceride and diminished levels of HDL were noticed in diabetic rats. Diabetes induced hyperlipidemia is attributable to excess mobilization of fat from adipose tissue due to underutilization of glucose 32. Under normal circumstances, insulin activates lipoprotein lipase and hydrolyses triglycerides 32.
were no significant changes in the lipid profile of rats which were given the extract on these parameters.

Values not sharing a common superscript letter differ significantly (DMRT)

Various parameters of blood lipid profiles were tested in the normal and diabetic rats. The results are summarized in Table 3. The levels of TC, LDL, VLDL and TG were significantly elevated and levels of serum HDL was decreased in diabetic control group as compared to normal control rats. In case of insulin deficiency, there is increased lipolysis leading to hyperlipidemia. In insulin deficient diabetics, the concentration of free fatty acids is elevated as a result of free fatty acid outflow from fat depots, where the balance of free fatty acid esterification- triglyceride lipolysis cycle is displaced in favor of acid outflow from fat depots, where the balance of free fatty acid esterification- triglyceride lipolysis cycle is displaced in favor of lipolysis.\(^40\) After supplementation with aqueous extract of *Erythrina variegata*, the alteration in lipid metabolism was partially attenuated like SOD, CAT, GPX and GST.\(^41\) Antioxidants thus play an important role to protect the human body against damage by reactive oxygen species. The cellular radical scavenging systems include enzymes such as superoxide dismutase (SOD), which scavenges the superoxide ions by catalyzing its dismutation and catalase (CAT), a heme enzyme which removes hydrogen peroxide.\(^42\) Catalase destroys hydrogen peroxide in high concentration by catalyzing its two-electron dismutation into oxygen and water.\(^43\) SOD catalyzes the one electron dismutation of superoxide into hydrogen peroxide and water. The decreased activities of CAT and SOD thereby result in the increased production of hydrogen peroxide and oxygen by auto oxidation of glucose and non-enzymatic glycation.\(^44\)

Table 4: Effect of *Erythrina variegata* on the activities of antioxidant enzymes GPX, SOD, GST and catalase in liver of control and experimental groups

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</tr>
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<tbody>
<tr>
<td>GPX (µg of GSH/mg of protein)</td>
<td>32.4±0.4(^a)</td>
<td>12.4±0.3(^b)</td>
<td>26.5±0.32(^c)</td>
<td>24.6±0.44(^d)</td>
<td>31.8±0.53(^e)</td>
</tr>
<tr>
<td>Superoxide dismutase (Units/mg of protein)</td>
<td>6.71±0.09(^a)</td>
<td>6.93±0.22(^b)</td>
<td>3.8±0.24(^c)</td>
<td>6.73±0.22(^d)</td>
<td>7.77±0.49(^e)</td>
</tr>
<tr>
<td>Catalase (µmoles of H(_2)O(_2) utilized /min/mg of protein)</td>
<td>68.07±0.14(^a)</td>
<td>39.58±0.35(^b)</td>
<td>64.56±0.36(^c)</td>
<td>62.76±0.23(^d)</td>
<td>68.76±0.45(^e)</td>
</tr>
<tr>
<td>GST (µmoles of CDNB-Conjugate formed/mg of protein)</td>
<td>8.36±0.03(^a)</td>
<td>4.4±0.14(^b)</td>
<td>7.37±0.17(^c)</td>
<td>6.95±0.27(^d)</td>
<td>8.53±0.21(^e)</td>
</tr>
</tbody>
</table>

Values not sharing a common superscript letter differ significantly (DMRT)

Table 5: Effect of *Erythrina variegata* on the activities of antioxidant enzymes GPX, SOD, GST and catalase in kidney of control and experimental groups

<table>
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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>GPX (µg of GSH/mg of protein)</td>
<td>31.6±0.47(^a)</td>
<td>17.3±0.32(^b)</td>
<td>29.16±0.27(^c)</td>
<td>27.53±0.45(^d)</td>
<td>31.63±0.49(^e)</td>
</tr>
<tr>
<td>Superoxide dismutase (Units/mg of protein)</td>
<td>2.43±0.45(^a)</td>
<td>0.936±0.05(^b)</td>
<td>2.06±0.42(^c)</td>
<td>1.67±0.01(^d)</td>
<td>2.24±0.22(^e)</td>
</tr>
<tr>
<td>Catalase (µmoles of H(_2)O(_2) utilized /min/mg of protein)</td>
<td>27.5±0.48(^a)</td>
<td>18.07±0.08(^b)</td>
<td>24.51±0.44(^c)</td>
<td>22.54±0.51(^d)</td>
<td>27.83±0.44(^e)</td>
</tr>
<tr>
<td>GST (µmoles of CDNB-Conjugate formed/mg of protein)</td>
<td>7.1±0.03(^a)</td>
<td>3.067±0.13(^b)</td>
<td>6.43±0.41(^c)</td>
<td>5.83±0.27(^d)</td>
<td>7.74±0.04(^e)</td>
</tr>
</tbody>
</table>

Values not sharing a common superscript letter differ significantly (DMRT)

Table 6: Effect of *Erythrina variegata* on the activities of antioxidant enzymes GPX, SOD, GST and catalase in brain of control and experimental groups

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Control (Group I)</th>
<th>Diabetic control (Group II)</th>
<th>Diabetic + Glibenclamide (Group III)</th>
<th>Diabetic + <em>E. variegata</em> treated (Group IV)</th>
<th><em>E. variegata</em> alone treated (Group V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPX (µg of GSH/mg of protein)</td>
<td>40.67±0.52(^a)</td>
<td>18.13±0.21(^b)</td>
<td>36.42±0.37(^c)</td>
<td>38.4±0.49(^d)</td>
<td>40.15±0.31(^e)</td>
</tr>
<tr>
<td>Superoxide dismutase (Units/mg of protein)</td>
<td>1.68±0.02(^a)</td>
<td>0.87±0.02(^b)</td>
<td>1.27±0.03(^c)</td>
<td>1.1±0.02(^d)</td>
<td>1.6±0.06(^e)</td>
</tr>
<tr>
<td>Catalase (µmoles of H(_2)O(_2))</td>
<td>14.23±0.27(^a)</td>
<td>8.46±0.49(^b)</td>
<td>14.29±0.37(^c)</td>
<td>12.46±0.45(^d)</td>
<td>14.14±0.14(^e)</td>
</tr>
</tbody>
</table>

Values not sharing a common superscript letter differ significantly (DMRT)

Changes in antioxidants status in tissues

Oxidative stress is a condition of reduction in antioxidant enzymes like SOD, CAT, GPX and GST.\(^41\) Antioxidants thus play an important role to protect the human body against damage by reactive oxygen species. The cellular radical scavenging systems include enzymes such as superoxide dismutase (SOD), which scavenges the superoxide ions by catalyzing its dismutation and catalase (CAT), a heme enzyme which removes hydrogen peroxide.\(^42\) Catalase destroys hydrogen peroxide in high concentration by catalyzing its two-electron dismutation into oxygen and water.\(^43\) SOD catalyzes the one electron dismutation of superoxide into hydrogen peroxide and water. The decreased activities of CAT and SOD thereby result in the increased production of hydrogen peroxide and oxygen by auto oxidation of glucose and non-enzymatic glycation.\(^44\)
The effect of aqueous extract of *Erythrina variegata* on enzymatic antioxidant variables in liver, kidney and brain are shown in Table 4, 5 and 6 respectively. Results showed that hepatic, renal and brain activity of catalase, superoxide dismutase, glutathione peroxidase and Glutathione-S-Transferase decreased significantly in STZ induced diabetic group (Group II). The normal control group maintained optimal value for activity of antioxidants. *Erythrina variegata* aqueous extract treatment in diabetic rats significantly increased the antioxidant enzyme activities and reversed them to their near normal values. The same phenomenon was seen in the results of glibenclamide treated groups (Group III).

An array of nonenzymatic antioxidants like vitamin C and GSH are involved in scavenging free radicals in vivo. Reduced glutathione (GSH), a tripeptide present in all cells, is an important antioxidant. It is essential to maintain structural and functional integrity of cells. It is involved in the synthesis of important macromolecules and in protection against ROS. Apart from its direct scavenging properties and ability to conjugate with several electrophilic intermediates that are capable of initiating lipid peroxidation, GSH also acts as a co-substrate of conjugating enzyme system. Hyperglycemia can increase oxidative stress and change the redox potential of glutathione. Decreased levels of GSH in liver, kidney and brain of diabetic rats may increase their susceptibility to oxidative injury. Reduction of oxidized form of glutathione requires NADPH, as a cofactor and enzyme glutathione reductase. The reduced availability of NADPH, which could be either due to reduced synthesis or increased mobilization of NADPH through some other pathway, could be also responsible for low levels of reduced glutathione in diabetic rats as compared to control rats. The distinct diminution of GSH content in tissues of diabetic animals and subsequent improvement towards their near normal values after *Erythrina variegata* treatment reveals that the extract could have either increased the biosynthesis of GSH and/or reduced oxidative stress, which ultimately reduced the degradation of GSH.

Vitamin C is an excellent hydrophilic, dietary antioxidant and it readily scavenges ROS and peroxyl radicals. It also acts as a co-antioxidant by generating vitamin A, E and GSH from radicals. An important neuro toxic mechanism for neurons is the inhibition of the cell membrane, thereby generating the transmembranous Na⁺ -K⁺ gradient necessary for neuronal excitability. It catalyzes the hydrolysis of ATP and couples it to transport of Na⁺ and K⁺ across the cell membrane, thereby generating the transmembranous Na⁺ - K⁺ gradient. This pump is essential for the regulation of cell volume, uptake of nutrients, and regulation of neurotransmitter release and excitability properties of nerve tissue. It has been proposed that alteration in this enzyme activity may represent an important neurotoxic mechanism for neurons. The inhibition of such enzyme provokes an increased uptake of Na⁺ and cytosolic free Ca²⁺, releasing of acetylcholine and decreasing the membrane potential of synaptosomes from cerebral cortex. Decreased Na⁺ - K⁺ ATPase activity leads to neuron-selective lesion in the brain suggesting that inhibition of this enzyme may be used as useful indicator of brain neurodegenerative pathophysiology related to memory and cognitive disorders of diabetic state.

In the present study a decrease in Na⁺ - K⁺ ATPase activity in brain of diabetic rats compared with normal animals, a fact that may be an important factor in the pathogenesis of the central nervous system in the diabetic state. Na⁺ - K⁺ ATPase are a crucial enzyme responsible for maintaining the ionic gradient necessary for neuronal excitability. It catalyzes the hydrolysis of ATP and couples it to transport of Na⁺ and K⁺ across the cell membrane, thereby generating the transmembranous Na⁺ - K⁺ gradient. This pump is essential for the regulation of cell volume, uptake of nutrients, and regulation of neurotransmitter release and excitability properties of nerve tissue. It has been proposed that alteration in this enzyme activity may represent an important neurotoxic mechanism for neurons. The inhibition of such enzyme provokes an increased uptake of Na⁺ and cytosolic free Ca²⁺, releasing of acetylcholine and decreasing the membrane potential of synaptosomes from cerebral cortex. Decreased Na⁺ - K⁺ ATPase activity leads to neuron-selective lesion in the brain suggesting that inhibition of this enzyme may be used as useful indicator of brain neurodegenerative pathophysiology related to memory and cognitive disorders of diabetic state.

In the present study a decrease in the brain Ca²⁺-ATPase activity was noticed. A number of mechanisms regulate plasma Ca²⁺-

**Table 4**

<table>
<thead>
<tr>
<th>Values are expressed as Mean ± SD of five individual experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GST (µmoles of CDNB-Conjugate formed/mg of protein)</strong></td>
</tr>
<tr>
<td>5.34±0.51 a</td>
</tr>
<tr>
<td>2.1±0.04 b</td>
</tr>
<tr>
<td>5.8±0.02 a</td>
</tr>
</tbody>
</table>

Values not sharing a common superscript letter differ significantly (DMRT)
ATPase Viz conformational transition by calmodulin, acidic phospholipids and fatty acids, PKC-mediated phosphorylation and calcium itself. Diabetes-related ATPase activity changes in cerebral microvessels may depend on altered blood-brain barrier functions. Moreover the decrease in Ca2+-ATPase activity was related to protein glycosylation and lipid peroxidation. Ca2+-ATPase is sensitive to its phospholipids milieu and to polyunsaturated fatty acids. The content of these lipids may change in diabetes and may cause alterations in enzyme activity. The reversal of Ca2+-ATPase sensitive to its phospholipids milieu and to polyunsaturated fatty acid protein glycosylation and lipid peroxidation. Ca2+-ATPase is STZ induced diabetes resulted in a decrease in Mg2+ ATPase activity. Mg2+ ATPase is to control the intracellular Mg2+ concentration, changes of which can modulate the activity of Mg2+-dependent enzymes and regulate rates of protein synthesis and cell growth. Mg2+ ATPase play a role in endergonic process other than ion transport. It utilizes a pool of ATP that is not directly related to the change in free energy for sodium transport. In the present study, Mg2+-ATPase activity was significantly decreased in brain of STZ induced diabetic rats. This may be due to free radical induced lipid peroxidation and protein oxidation in cell membrane followed by the alteration of the membrane fluidity, enzyme properties and ion transport. Administration of Erythrina variegata plant extract and glibenclamide to diabetic rats reverted the diminished Mg2+ ATPase activity to near normal. No significant change in normal functioning of above mentioned enzyme was noticed in plant extract alone treated rats as compared to normal rats.

Administrations of either Erythrina variegata plant extract or glibenclamide prevents the inhibition of Na+-K+ ATPase activity of diabetic rat brains and consequently would attenuate the resultant neurotoxicity. This result suggest that the inhibition of both drugs on reactive species and lipid peroxidation may restore membrane fluidity and hence the functional ability of associated enzymes.

The activity of Na+-K+-ATPase, Ca2+-ATPase and Mg2+-ATPases were significantly diminished in STZ induced diabetic rats when compared with control rats (Table 7). The activities of these enzymes were reverted to near normal in Erythrina variegata aqueous extract administered rats. The same effect was produced on administration of hypoglycemic drug, glibenclamide. No significant change was observed in Erythrina variegata aqueous extract treated drug control rats as compared to the normal control group.

Table 7: Effect of Erythrina variegata on the activities of various enzymes in brain of control and experimental groups

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Control (Group I)</th>
<th>Diabetic control (Group II)</th>
<th>Diabetic + Glibenclamide (Group III)</th>
<th>Diabetic + E. variegata treated (Group IV)</th>
<th>E. variegata alone treated (Group V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteolytic activity (µmoles of TE /hr/mg of protein)</td>
<td>4.7±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.75±0.04&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.83±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.03±0.14&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.84±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Na+-K+ ATPase (µmoles of PL /hr/mg of protein)</td>
<td>2.25±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.76±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.62±0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.55±0.04&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.24±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ca2+ ATPase (µmoles of PL/hr/mg of protein)</td>
<td>1.06±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.64±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.96±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.05±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.15±0.04&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mg2+ ATPase (µmoles of PL/hr/mg of protein)</td>
<td>1.45±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.75±0.019&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.18±0.018&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.24±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.48±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5'Nucleotidase (µmoles of PL/hr/mg of protein)</td>
<td>1.22±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.16±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.24±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.42±0.22&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.16±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

TE- Tyrosine equivalents; PE- Phosphorous liberated
Values are expressed as Mean ± SD of five individual experiments
Values not sharing a common superscript letter differ significantly (DMRT)

5' nucleotidase catalyzes the hydrolysis of AMP thus playing an important role in adenosine production. The present data revealed a marked increase in 5' nucleotidase activities in brain of diabetic rats when compared to the normal healthy groups, thus implying alteration of nucleotide hydrolysis. Stimulation of 5' nucleotidase, leads to enhancement of ATP, ADP and AMP hydrolysis and consequently an increment of adenosine production. The potential increase of brain adenosine level and a lower availability of ATP particularly affect the hippocampus synaptosomal fraction, since this region of brain plays a key role in memory and learning. Thus, stimulation of brain ectonucleotidases may be used as another useful marker for behavior and cognitive disturbances found in diabetes.

Table 7 revealed the diabetes induced stimulation of 5' nucleotidase activity as well as proteolytic activity when compared to the normal healthy rats. Administration of Erythrina variegata aqueous extract and glibenclamide down regulated the level of 5' nucleotidase as well as proteolytic activity and more or less regulated the deviation of enzyme activities to near normal levels. No significant changes were seen in the studied parameters on treating the normal rats with plant extract alone when compared to normal untreated group. The enzymatic control of nucleotide levels is important in the process of brain homeostasis which was exerted by the administration aqueous extract of Erythrina variegata and glibenclamide treated rats.

Histopathological Study
Histologically, liver section of STZ-induced diabetic rats showed marked structural alterations in the liver as a result of absence of insulin. The major alteration was periportal fatty infiltration and necrosis of hepatocytes. This damage is partially reversed by the Erythrina variegata bark extract treatment and is similar to that observed by glibenclamide treated groups. The normal animal treated with plant extract Erythrina variegata alone showed normal hepatic structure thus indicating that it did not produce any liver toxicity.
It is also known that streptozotocin destroys insulin secreting β-cells in the islets of Langerhans and their effect is irreversible. The ultrastructure of STZ diabetic pancreas showed considerable reduction in the islet of Langerhans and depleted islets. Aqueous extract of *Erythrina variegata* reduced blood glucose level in streptozotocin-induced diabetic rats. At testing period of 30 days similar results also have been found with glibenclamide. The pancreatic sections of the diabetic rats treated with *Erythrina variegata* as well as glibenclamide showed almost normal pancreatic cells.

The histopathological investigation along with the biochemical evaluation suggests the possibility of the islets regeneration and recovery of normal carbohydrate metabolism in treated group of aqueous extract of *Erythrina variegata*. The regenerative effect of the pancreatic cells by *Erythrina variegata* via exocrine cells of pancreas may enlighten the positive effects of these agents on the production of insulin. Reports on histopathological analysis of pancreas of the *Erythrina variegata* alone treated animals showed results that were very similar as that of the control group.

The present results constitute the *in vivo* evidence for antidiabetic activity of *Erythrina variegata* bark in STZ induced diabetic rats. Since the study of antioxidant enzymes is considered to be a reliable marker for evaluating the antioxidant efficacy of medicinal plant, these findings are suggestions of possible antioxidant role played by *Erythrina variegata* plant extract in addition to its antidiabetic effect.
ACKNOWLEDGEMENT

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REFERENCES


