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 variegate* 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 glucose 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, Ca^{2+} ATPase ,Mg $^{2+}$ ATPase and 5'nucleotidase along with proteolytic activity of brain extract were assessed. The experimentalfindings 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[1]. 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[2]. 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[3].

Earlier classified as rich man's disease, diabetes mellitus has now spread among masses[5]. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and eventually 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[6]. 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[7]. Activities of enzyme connected to glucose metabolism and neuronal activities have been studied as a function of diabetes[8].

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[9]. 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, there by affecting blood glucose[10]. *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[11].

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 powered 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 1ml/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 (45mg/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

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

Glucose Tolerance Test

The glucose tolerance test or study on glucose loaded rats[12] 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 using heamoglucostrips (Life scan, Johnson and Johnson Ltd) and reported as fasting blood glucose. The animals were then fed with glucose (4g/kg) and blood was withdrawn from the tail vein every 30min for the next 3hours and the glucose level was estimated. The results obtained using glucometer was confirmed by glucose oxidase kit method[13].

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 hypoglycaemic 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 and 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 [14] and total protein [15] 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) [16], superoxide dismutase (SOD) [17], catalase(CAT) [18], 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], Mg^{2+}-ATPase activity [24], Ca^{2+}-ATPase activity [25], and 5' 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'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 was the best among the five extracts with a high glycemic index of 81.9% (Fig 1 & 2) and a significant increase in body weight (Fig 3). Phytochemical screening of aqueous extract of *Erythrina 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 200,400,600mg/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 400mg/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 diocia* and *Ficus bengalensis* [28].
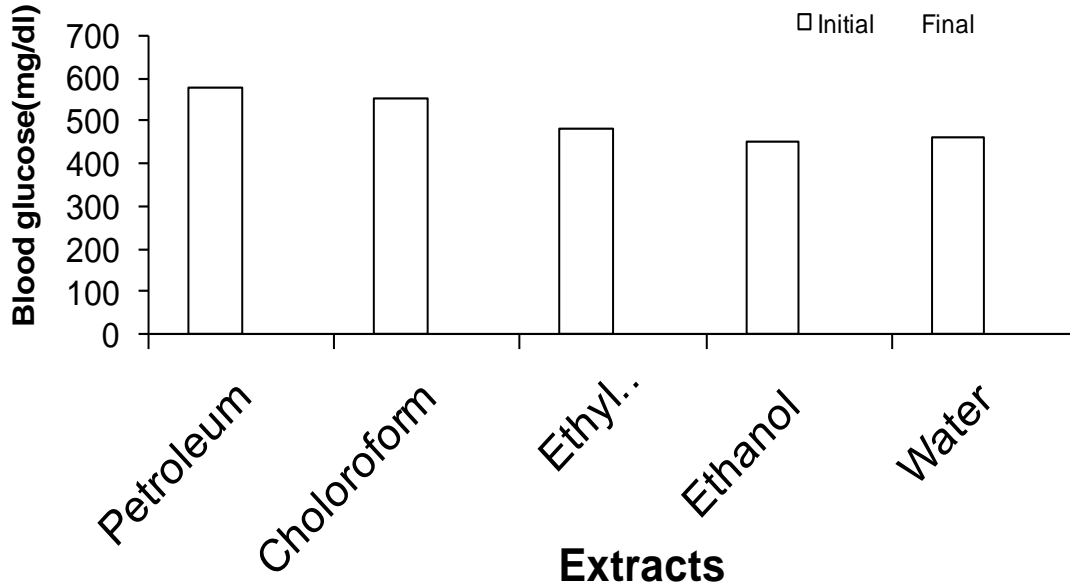

Fig. 1: Levels of blood glucose with various extracts of *E. variegata* in pilot study

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

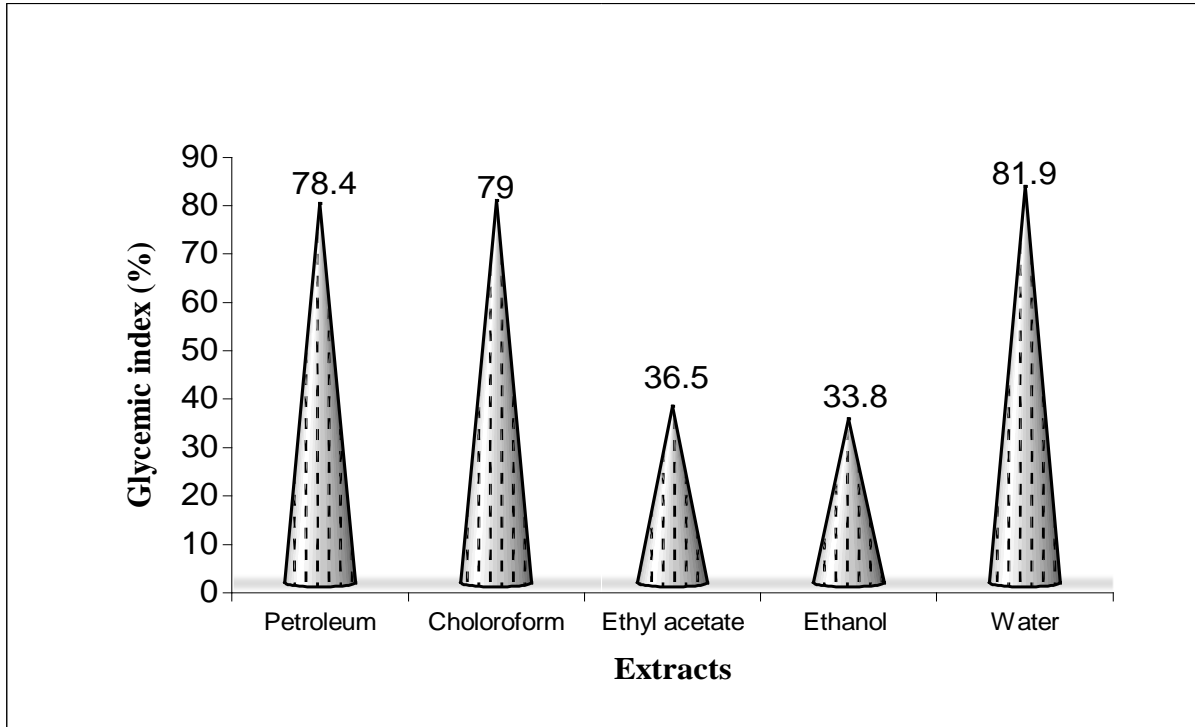* - Initial and final blood glucose level compared, significant at 5% level (t<0.05)

Fig. 2: Glycemic index of various extracts of *E. variegate* in pilot study

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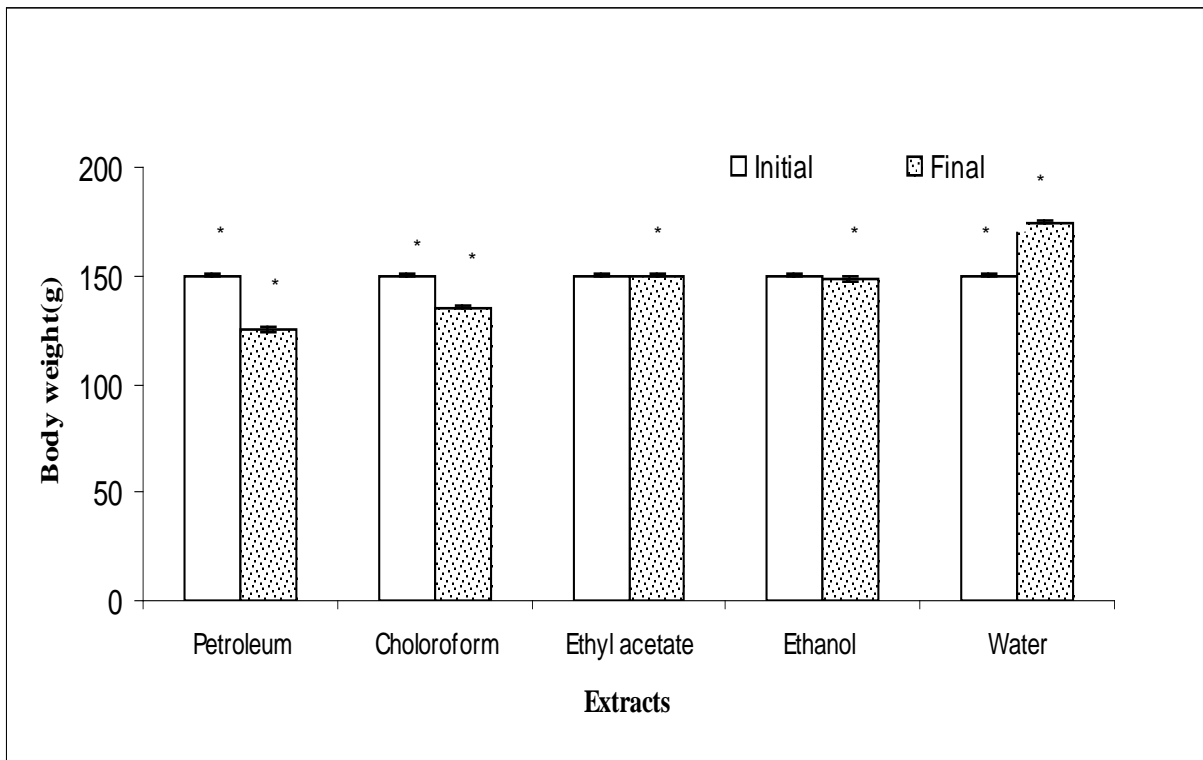

Fig. 3: Body weight of rats with different extracts of *E. variegata* in pilot study

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

* - Initial and final body weight compared, significant at 5% level (t<0.05)
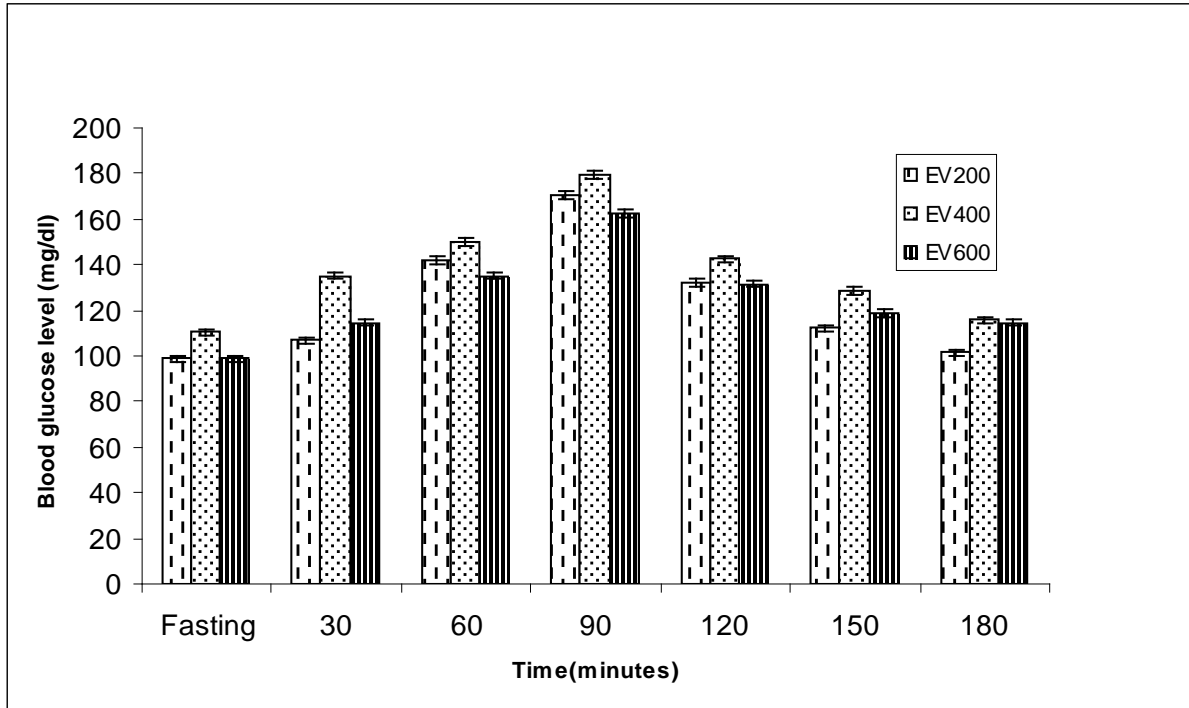
Fig. 4: Effective dose determination - Oral glucose tolerance test

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

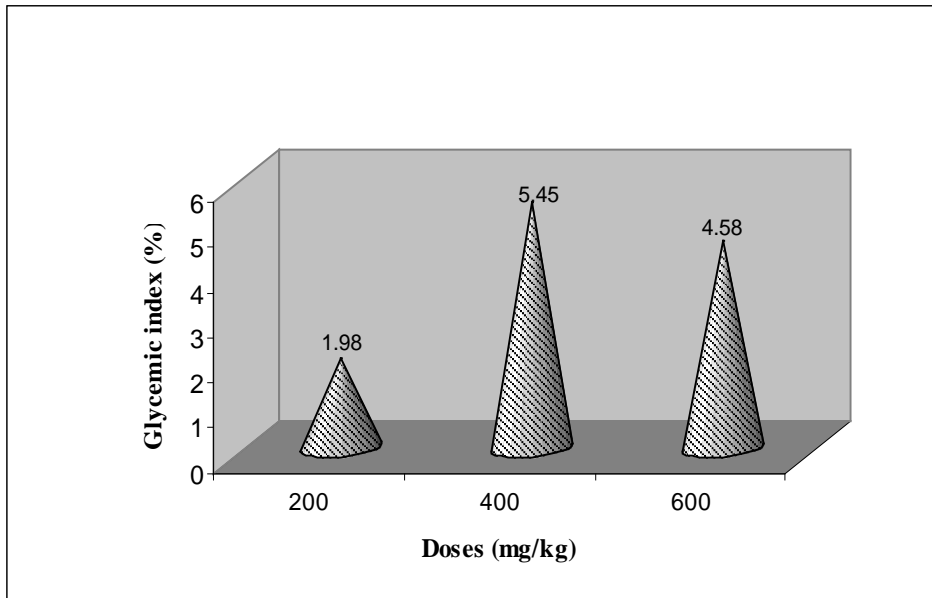EV – *Erythrina variegata*

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

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

Safety of the aqueous extract of *Erythrina variegata* bark was confirmed by the acute toxicity study where the extract did not show any mortality upto a dose of 2000mg/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[29] any compound or drug with the oral LD $_{50}$ 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.
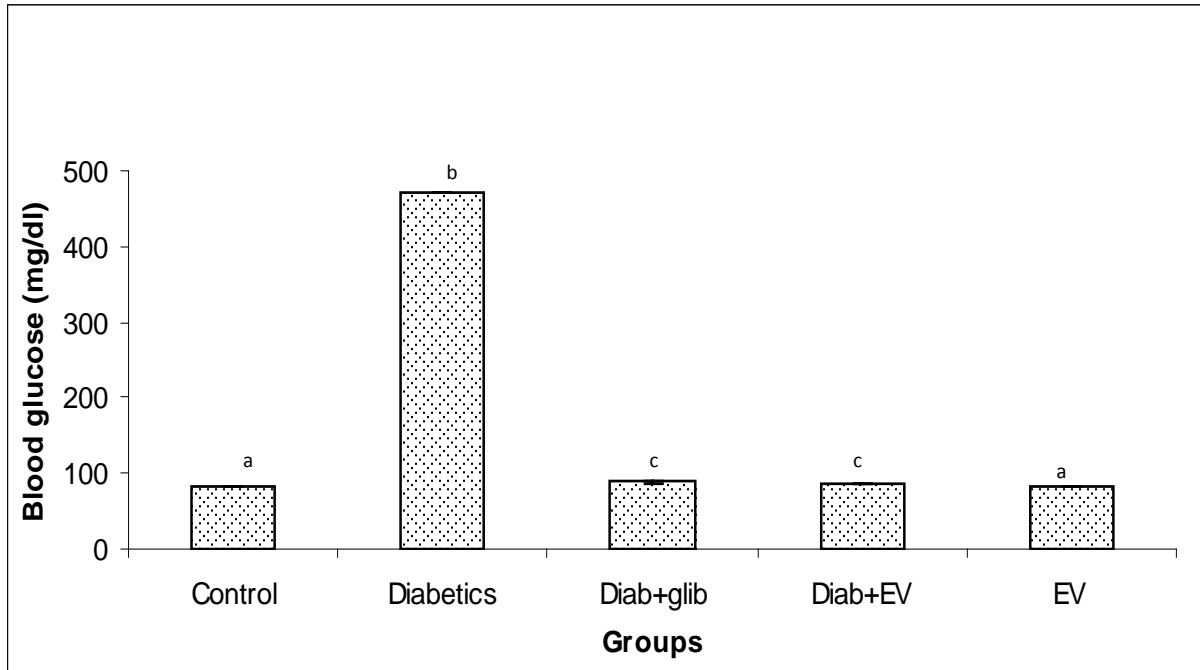
Fig. 6: Effect of *E. variegata* on blood glucose level of control and diabetic rats on 30th day of treatment

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
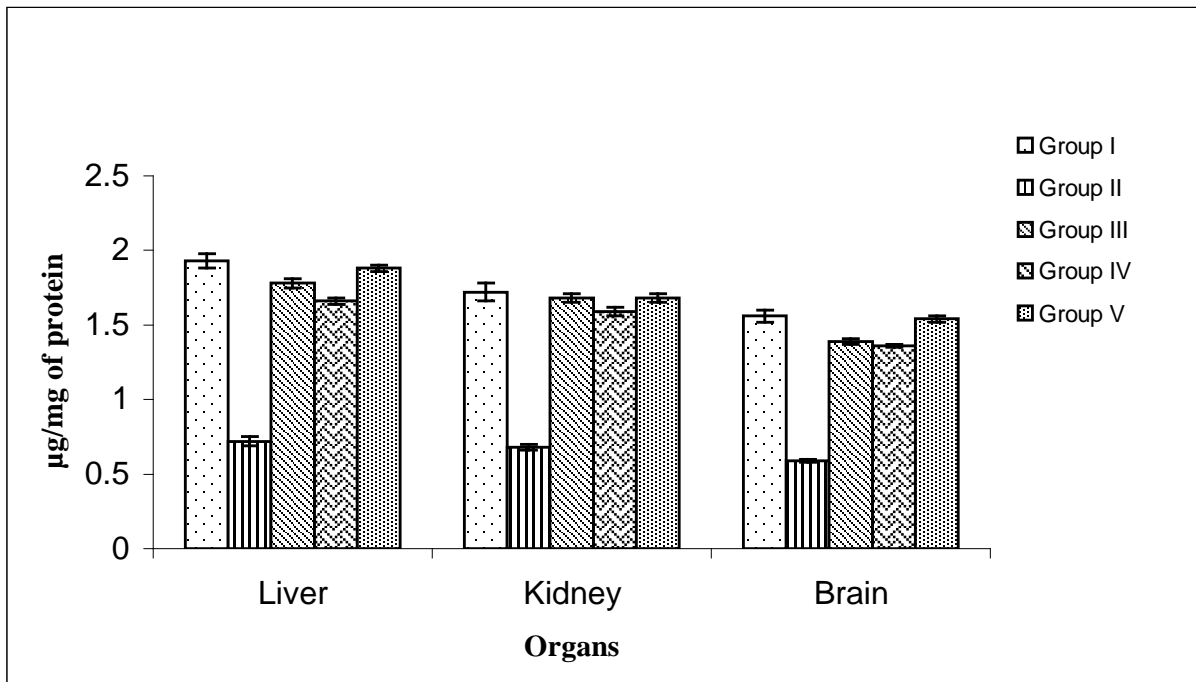

Fig. 7: Level of Vitamin C in liver, kidney and brain of control and experimental groups

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

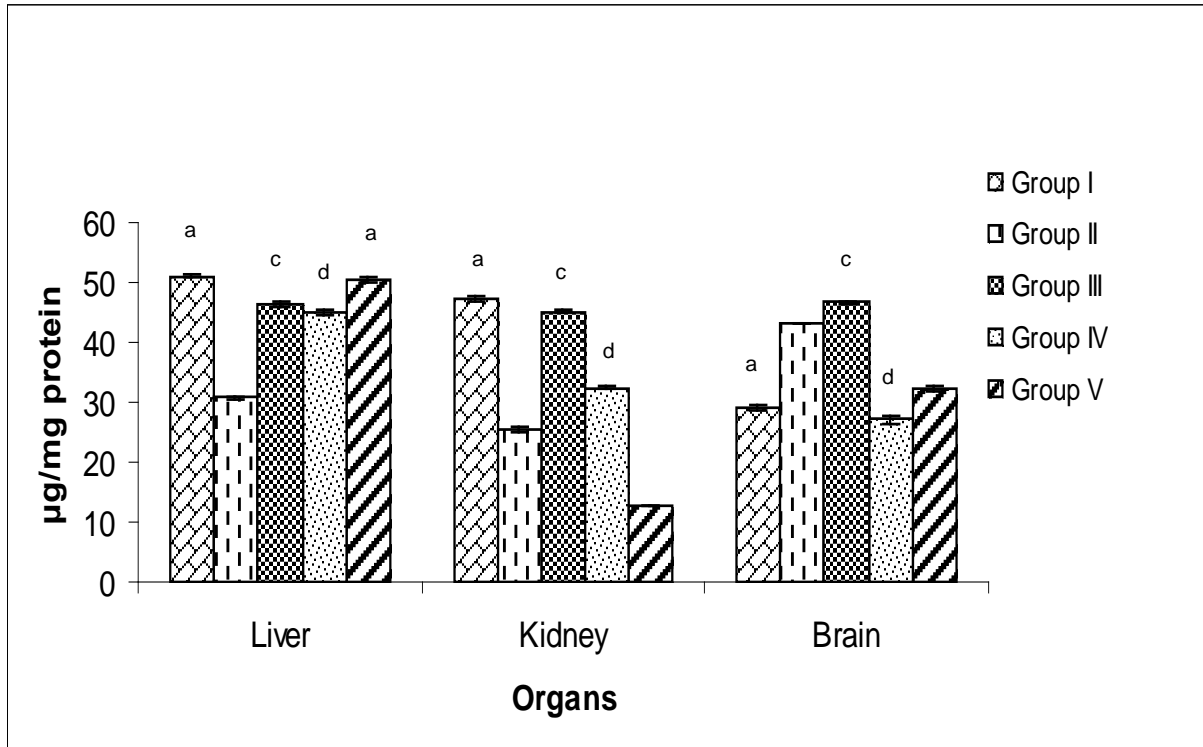Values not sharing a common superscript letter differ significantly (DMRT)

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

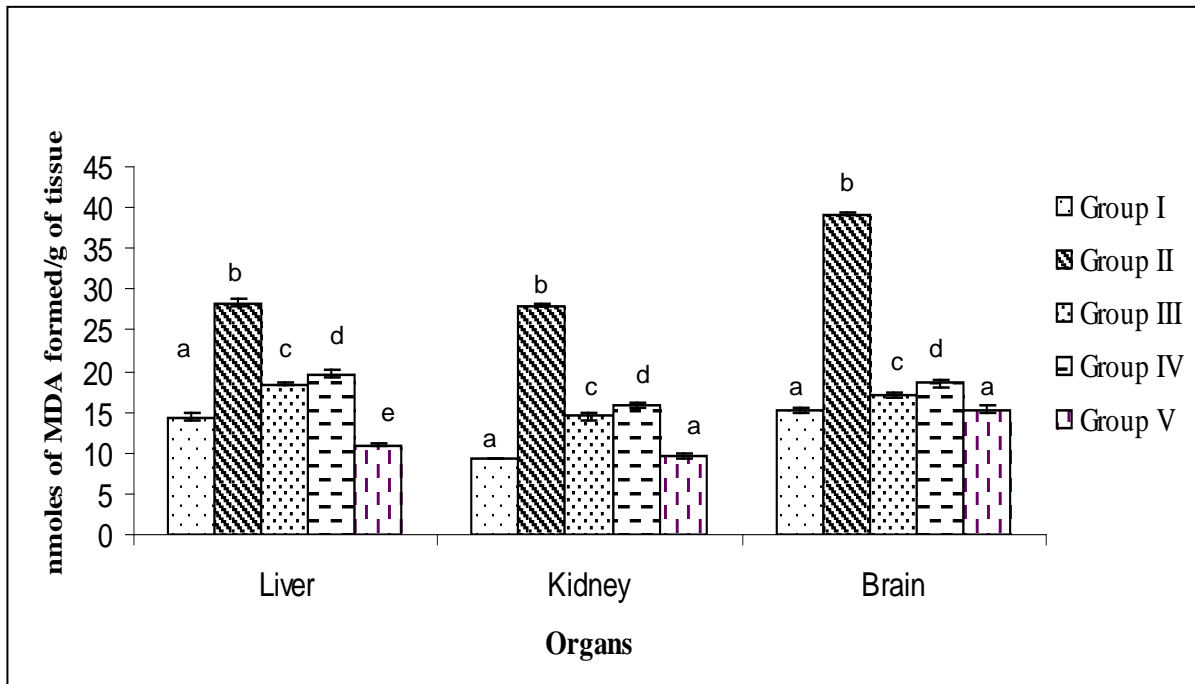Values not sharing a common superscript letter differ significantly (DMRT)

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

Particulars	Control (Group I)	Diabetic control (Group II)	Diabetic + Glibenclamide (Group III)	Diabetic + *E. variegate* (Group IV)	*E.variegata* alone treated (Group V)
0	89.2 ±0.23[a]NS	295.5±0.409[b]***	302.2±0.389[c]***	291.26±0.49[d]***	94.2±0.28[e]NS
15	93.13 ±0.29[a]NS	456.5±0.36[b]***	110±0.17[c]***	120.03±0.40[d]***	101.5±0.41[e]NS
30	82.1 ±0.32[a]NS	472.2±0.42[b]***	88.36 ±0.31[c]***	87.4±0.49[d]***	84.02±0.23[e]NS

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

a: 0th day compared with 15th day. *** Significance at p 0.01;

b: 15th day compared with 30th day. ** Significance at p 0.05

c: 0th day compared with 30th day.   NS - Not Significant

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 (400mg/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 the 30th day (Fig 6).

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 glycaemic 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[31]. Whereas after treatment both the values where comparable to those which received glibenclamide treatment. There was no significant difference in values obtained for group I and group V (Table 2)

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

Particulars	Control (Group I)	Diabetic control (Group II)	Diabetic +Glibenclamide (Group III)	Diabetic + *E. variegata* treated (Group IV)	*E.variegata* alone treated (Group V)
Urea (mg/dl)	22.37±0.17[ad]	39.30±0.21[b]	22.1±0.10[a]	25.14±0.13[c]	22.15±0.18[d]
Creatinine (mg/dl)	0.87±0.02[a]	2.39±0.45[b]	0.92±0.02[a]	1.09±0.01[c]	0.91±0.02[a]
SGOT (IU/L)	123.4±0.36[a]	272±0.29[b]	203.40±0.41[c]	204.3±0.37[d]	122.30±0.32[a]
SGPT (IU/L)	83.4±0.40[a]	185±0.13[b]	83.80±0.17[a]	98.5±0.45[c]	83.7±0.22[a]
ALP (IU/L)	259.7±0.57[a]	375±0.22[b]	290±0.58[c]	295±0.45[d]	255.23±0.19[e]
Protein (mg/dl)	7.76±0.44[a]	2.76±0.23[b]	6.79±0.02[c]	6.69±0.04[d]	7.12±0.23[e]

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 glucogenesis 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[33]. 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[34]. The restoration of ALT, ALP to their normal levels may be due to the presence of flavonoids in aqueous extract of *Erythrina variegata,* which are reported to be hepatoprotective agents. However administration of *Erythrina variegata* bark extract for thirty days was capable of lowering ALT and AST levels in diabetic groups. More over elevation of ALP was significantly reversed by treatment with aqueous extract of *Erythrina variegata.* No significant changes in hepatic and renal markers in serum indicate the safety of the extract on these organs.

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 rate. 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[35].

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[36]. The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia[37]. 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[38]. Under normal circumstances, insulin activates lipoprotein lipase and hydrolyses triglycerides[39].

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

Particulars	Control (Group I)	Diabetic control (Group II)	Diabetic + Glibenclamide (Group III)	Diabetic + *E. variegata* treated (Group IV)	*E.variegata* alone treated (Group V)
Cholesterol(mg/dl)	126.6±0.32[a]	239.5±0.39[b]	117.8±0.126[c]	136±0.32[d]	124.9±0.22[e]
Triglycerides (mg/dl)	75.5±0.44[a]	182.46±0.49[b]	91.26±0.58[c]	80.6±0.40[d]	74.6±0.37[e]
HDL (mg/dl)	24.77±0.34[a]	12.23±0.52[b]	23.8±0.18[c]	24.3±0.44[d]	25.6±0.47[a]

LDL (mg/dl)	78.7±0.23[a]	148.2±0.56[b]	76.7±0.39[c]	83.3±0.54[d]	78.37±0.37[e]
VLDL (mg/dl)	19.67±0.305[a]	43.39±0.47[b]	16.3±0.54[c]	18.06±0.27[d]	19.4±0.36[a]

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

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 diabetes, 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 lipolysis[40]. After supplementation with aqueous extract of *Erythrina variegata*, the alteration in lipid metabolism was partially attenuated as evidenced by decreased serum TG and TC levels and increased HDL concentration in diabetic rats. Treatment also produced a significant reduction in LDL and VLDL levels in diabetic rats. There were no significant changes in the lipid profile of rats which were given the *Erythrina variegata* extract alone indicate the inertness of the extract on these parameters.

Changes in antioxidants status in tissues

Oxidative stress is a condition of reduction in antioxidative 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].

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

Particulars	Control (Group I)	Diabetic control (Group II)	Diabetic + Glibenclamide (Group III)	Diabetic + *E. variegate* treated (Group IV)	*E.variegata* alone treated (Group V)
GPx (µg of GSH/mg of protein)	32.4±0.42[a]	12.46±0.31[b]	26.5±0.32[c]	24.6±0.44[d]	31.8±0.53[a]
Superoxide dismutase(Units/mg of protein)	6.71±0.09[a]	6.93±0.22[a]	3.8±0.24[b]	6.73±0.22[a]	7.77±0.49[a]
Catalase (µ moles of H_2O_2 utilized /min/mg/protein)	68.07±0.14[a]	39.58±0.35[b]	64.56±0.36[c]	62.76±0.23[d]	68.76±0.45[a]
GST(µmoles of CDNB- Conjugate formed/mg of protein)	8.36±0.03[a]	4.4±0.14[b]	7.37±0.17[c]	6.95±0.27[d]	8.53±0.21[a]

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

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

Particulars	Control (Group I)	Diabetic control (Group II)	Diabetic + Glibenclamide (Group III)	Diabetic +*E. variegate* treated (Group IV)	*E.variegata* alone treated (Group V)
GPx (µg of GSH/mg of protein)	31.6±0.47[a]	17.3±0.32[b]	29.16±0.27[c]	27.53±0.45[d]	31.63±0.49[a]
Superoxide dismutase (Units/mg of protein)	2.43±0.45[a]	0.936±0.05[b]	2.06±0.42[ac]	1.67±0.01[c]	2.24±0.22[ac]
Catalase (µ moles of H_2O_2 utilized /min/mg/protein)	27.5±0.48[a]	18.07±0.08[b]	24.51±0.44[c]	22.54±0.51[d]	27.83±0.44[a]
GST(µmoles of CDNB- Conjugate formed/mg of protein)	7.1±0.03[a]	3.067±0.13[b]	6.43±0.41[c]	5.83±0.27[d]	7.74±0.04[ac]

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

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

Particulars	Control (Group I)	Diabetic control (Group II)	Diabetic + Glibenclamide (Group III)	Diabetic + *E. variegate* treated (Group IV)	*E.variegata* alone treated (Group V)
GPx (µg of GSH/mg of protein)	40.67±0.52[a]	18.13±0.21[b]	36.42±0.37[c]	38.4±0.49[d]	40.15±0.31[a]
Superoxide dismutase (Units/mg of protein)	1.68±0.02[a]	0.87±0.02[b]	1.27±0.03[c]	1.18±0.02[d]	1.64±0.06[a]
Catalase (µ moles of H_2O_2	14.23±0.27[a]	8.46±0.49[b]	14.29±0.37[a]	12.46±0.45[c]	14.14±0.14[a]

utilized /min/mg/protein)				
GST(μmoles of CDNB-Conjugate formed/mg of protein)	5.34±0.51 [a]	2.16±0.04 [b]	4.65±0.05 [c]	4.43±0.05 [c]	5.88±0.02 [a]

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

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

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 [45]. 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 co-substrate of conjugating enzyme system [46]. Hyperglycemia can increase oxidative stress and change the redox potential of glutathione[47]. 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[48]. 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[49]. It also acts as a co-antioxidant by generating vitamin A, E and GSH from radicals. A decrease in the level of vitamin C was observed in liver, kidney and brain of diabetic rats. Such a fall in level of vitamin C could be due to the increased utilization of vitamin C in the deactivation of increased level of ROS or due to decrease in GSH level, since GSH is required in recycling of vitamin C[50]. Another possibility is that hyperglycemia inhibits ascorbic acid and its cellular transport. Since the chemical structure of vitamin C (ascorbic acid) is similar to that of glucose, it shares the membrane transport system with glucose and hence competes with it for its transport. Thus elevation in glucose concentration may depress natural antioxidants in the body like vitamin C[51]. Streptozotocin induced diabetic rats were found to have decreased GSH and Vitamin C levels in liver, kidney and brain as compared to control rats. Treatment with aqueous extract of *Erythrina variegata* and the standard drug, glibenclamide produced significant increase in the levels of these non-enzymatic antioxidants in group IV and group III respectively. Treatment of non diabetic rats with the plant extracts did not produce any effect on GSH and vitamin C levels (Fig 7 & 8).

Lipid peroxidation is one of the characteristic features of chronic diabetes. In diabetes, hypoinsulineamia increases the activity of fatty acyl coenzyme A oxidase, which initiates β oxidation of fatty acids, resulting in lipid peroxidation. Increased lipid peroxidation impairs membrane function by decreasing membrane bound enzymes and receptors. Its products (lipid radicals and lipid peroxides) are harmful to the cells in the body and associated with atherosclerosis, brain and kidney damage[52]. The streptozotocin induced hyperglycemic condition was maintained in Wistar albino rats till they were sacrificed on the 30th day. In the antioxidant studies, the disease control group showed a marked increase in LPO in liver, kidney and brain. Treatment with standard drug, glibenclamide and plant extract, *Erythrina variegata* brought down these values to near normal and the activity was highly significant. No significant changes were observed in *Erythrina variegata* alone treated animals (group V) when compared with control animals. The results are presented in Figure 9.

Increased lipid peroxidation under diabetic conditions may be due to increased oxidative stress in the cells as a result of depletion of antioxidant scavenging enzymes like SOD, CAT, GPx and GSH. The extract showed a significant reduction in basal LPO in liver, kidney and brain tissues compared to diabetic control. Thus, it can be concluded that *Erythrina variegata* aqueous extract has ability of providing promising antioxidant potential.

Enzymatic activities in the rat brain

The neurological consequences of diabetes mellitus in the Central Nervous System (CNS) are now receiving greater attention. Cognitive deficits, along with morphological and neurochemical alterations illustrate that the neurological complications of diabetes are not limited to peripheral neuropathies[53]. Glucose utilization is decreased in the brain during diabetes[54], providing a potential mechanism for increased vulnerability to acute pathological events. Oxidative stress, leading to an increased production of reactive oxygen species (ROS), as well as lipid peroxidation, is increased in diabetes[55] and also by stress in euglycemic animals[56]. Similarly, oxidative damage in rat brain is increased by experimentally induced hyperglycemia[57]. Lipid peroxidation products are also increased in the brains of type 1 diabetic rats[58] and type 2 diabetic mice[59]. Diabetes and stress mediated increases in oxidative stress, as well as decreases in antioxidant activity, may make the brain more vulnerable to subsequent pathological events.

The present study shows that diabetes induces 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[60, 61]. 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[62]. This pump is essential for the regulation of cell volume, uptake of nutrients, and regulation of neurotransmitter release and excitability properties of nerve tissue[63]. It has been proposed that alteration in this enzyme activity may represent an important neurotoxic mechanism for neurons[64]. The inhibition of such enzyme provokes an increased uptake of Na [+] and cytosolic free Ca 2[+], releasing of acetylcholine and decreasing the membrane potential of synaptosomes from cerebral cortex [65]. Decreased Na [+]- K [+] ATPase activity leads to neuron-selective lesion in the brain [66] 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 Ca 2[+]-ATPase activity was noticed. A number of mechanisms regulate plasma Ca 2[+]-

ATPase Viz conformational transition by calmodulin, acidic phospholipids and fatty acids, PKC-mediated phosphorylation and calcium itself[67]. Diabetes-related ATPase activity changes in cerebral microvessels may depend on altered blood-brain barrier functions[68]. Moreover the decrease in Ca^{2+}-ATPase activity was related to protein glycosylation and lipid peroxidation. Ca^{2+}-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[69]. The reversal of Ca^{2+}-ATPase activity in *Erythrina variegata* treated and glibenclamide treated diabetic rats towards normal level shows the normal functioning of Ca^{2+}-ATPase .No significant change was observed in plant extract alone treated rats due to the normal activity of Ca^{2+}-ATPase.

STZ induced diabetes resulted in a decrease in Mg^{2+} ATPase activity. Mg^{2+}ATPase is to control the intracellular Mg^{2+} concentration, changes of which can modulate the activity of Mg^{2+} dependent enzymes and regulate rates of protein synthesis and cell growth[70]. Mg^{2+}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[71]. In the present study, Mg^{2+}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 [72, 73]. Administration of *Erythrina variegata* plant extract and glibenclamide to diabetic rats reverted the diminished Mg^{2+}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, Ca^{2+}-ATPase and Mg^{2+}-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

Particulars	Control (Group I)	Diabetic control (Group II)	Diabetic + Glibenclamide (Group III)	Diabetic +*E. variegate* treated (Group IV)	*E.variegata* alone treated (Group V)
Proteolytic activity(µmoles of TE /hr /mg of protein)	4.73±0.19[a]	8.75±0.04[b]	5.83±0.05[c]	6.03±0.14[c]	4.84±0.04[a]
Na^+-K^+ATPase(µmoles ofPL /hr/mg of protein)	2.25±0.04[a]	1.76±0.01[b]	1.62±0.04[c]	1.55±0.04[c]	2.24±0.01[a]
Ca^{2+}ATPase(µmoles of PL/hr/mg of protein)	1.06±0.03[a]	0.646±0.03[b]	0.96±0.03[c]	1.05±0.03[a]	1.15±0.04[d]
Mg^{2+}ATPase(µmoles ofPL/hr/mg of protein)	1.45±0.01[a]	0.475±0.019[b]	1.18±0.018[c]	1.24±0.01[d]	1.48±0.02[a]
5'Nucleotidase(µmoles of PL/hr /mg of protein)	1.22±0.05[a]	2.16±0.05[b]	1.24±0.03[a]	1.42±0.22[c]	1.16±0.05[a]

TE- Tyrosine equivalents; PE- Phosphorous librated

Values are expressed as Mean ± S.D 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[74]. 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[75]. Thus, stimulation of brain ectonucleotidases may be used as another useful marker for behavior and cognitive disturbances found in diabetes [76, 77].
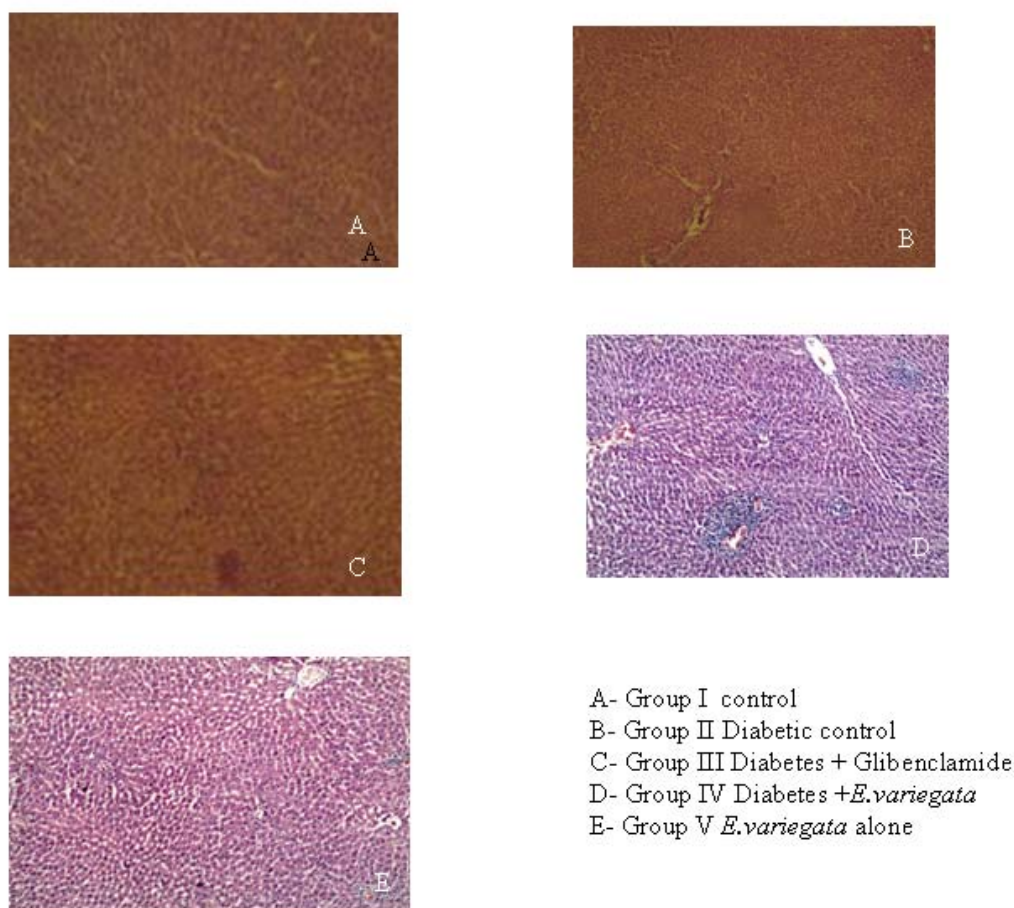
Table 7 revealed the diabetes induced stimulation of 5' nucleotidase activity as well as proteolytic activity when compared to the normal healthy rats. Administration of *Erythrina variegata* aqueous extract and glibenclamide down regulated the level of 5' nucleotidase as well as proteolytic activity 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[78]. 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.

HISTOPATHOLOGY OF LIVER

A- Group I control
B- Group II Diabetic control
C- Group III Diabetes + Glibenclamide
D- Group IV Diabetes +*E.variegata*
E- Group V *E.variegata* alone

Fig. 10: Histopathology of Liver

It is also known that streptozotocin destroys insulin secreting β-cells in the islets of Langerhans and their effect is irreversible[79]. The ultra structure 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 *invivo* 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.
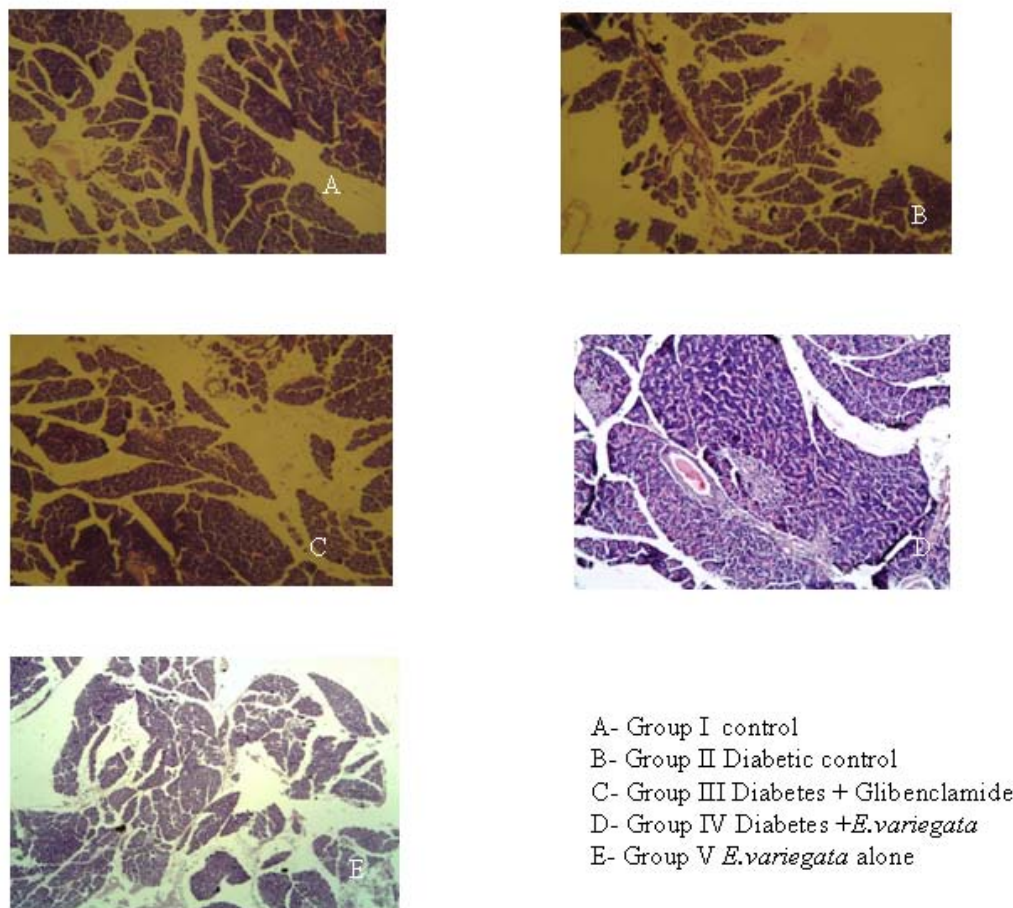
HISTOPATHOLOGY OF PANCREAS

A- Group I control
B- Group II Diabetic control
C- Group III Diabetes + Glibenclamide
D- Group IV Diabetes +*E.variegata*
E- Group V *E.variegata* alone

Fig. 11: Histopathology of Pancreas

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