



Research Article

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## Antidiabetic activity of aqueous extract of *Erythrina variegata* L. bark in streptozotocin induced diabetic rats

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### ABSTRACT

Traditional plant healing for diabetes has shown a surging significance in the last few decades. Therefore, the purpose of this study was to evaluate the hypoglycemic effect of the aqueous extract of *E. variegata* bark in diabetic rats. Diabetes was induced in rats by intraperitoneal administration of 45 mg/kg of streptozotocin (STZ). The aqueous extract of *E. variegata* was administered in the range of 400 mg/kg body weight during the study period of 30 days. The diabetic rats showed an increase in levels of blood glucose and glycosylated haemoglobin (HbA1c) and a decrease in the levels of insulin and haemoglobin (Hb). Treatment with the aqueous extract of *E. variegata* significantly decreased blood glucose and HbA1c ( $p < 0.05$ ) in diabetic rats and increased the levels of insulin and Hb. The histology of pancreatic islet cells was normal in non-diabetic animals, whereas in diabetic treated rats, *E. variegata* helped islet rejuvenation manifested by preservation of cell size. This study showed that the aqueous extract of *E. variegata* exerted a hypoglycemic effect. In addition, the extract positively functions on pancreas and provide evidence for its traditional usage in the control of diabetes.

**Keywords:** *E. variegata*, Diabetes, Insulin, Hb, HbA1c

### INTRODUCTION

Diabetes Mellitus (DM) is an assembly of metabolic diseases manifested as the presence of higher concentrations of glucose in the blood because of improper production of insulin from pancreas or inactivity of cells to the produced insulin. It is characterized by periodic or persistent hyperglycemia, which produces classical symptoms such as polyuria, polydipsia and polyphagia. In view of the formulations with the synthetic drugs caused side effects, the research was initiated with an upshot of bioactive antidiabetic principles from plant origin [1,2]. The substantial traditional knowledge of medicinal plants is playing an important role in the development of new drugs [3,4]. In recent years prominence is on the development of drugs from plants for the treatment of various diseases including diabetes mellitus, the incidence of which is very high all over the world especially in India [5-7].

Specific diabetic risk feature such as glycation of protein including haemoglobin and coagulation abnormalities, and subsequent endothelial dysfunction, may also throw in to cardiovascular complications. Glycosylated peptides are elevated several folds in diabetics and the use of the glycosylated hemoglobin (HbA1c) assay for long-term diabetic monitoring of diabetic control is gaining a great deal and acceptance [8].

*Erythrina variegata* L. (Fabaceae), commonly known as Tiger's Claw, is a thorny deciduous tree grown in tropical and subtropical regions of Eastern Africa, Southern Asia and Northern Australia. Different parts of *E. variegata*

have been used traditionally as sedative, antiasthmatic, anti epileptic for treatment of convulsion, fever, inflammation, bacterial infection, insomnia, helminthiasis, cough, cuts, and wounds [9-11]. The bark of the plant is also functional as an astringent, an antihelminthic and for ophthalmia and skin diseases [12]. The leaves are used in fever and inflammation. The juice of the leaves is used to relieve ear ache and toothache and if mixed with honey and ingested to kill tapeworm, roundworm and threadworm. The root of this plant is used in the treatment of cancer, convulsions and to treat pimples [13].

This study was therefore undertaken to evaluate the effect of *Erythrina variegata* L. bark antihyperglycemic activity in streptozotocin induced diabetic rats.

## EXPERIMENTAL SECTION

### Plant material

The plant specimens for the proposed study were collected from Kodaikannal, Dindigul district, Tamil Nadu, India. The plant was authenticated by Dr. G.V.S Moorthy, Botanical Survey of India, TNAU campus Coimbatore, with the voucher number BSI/SRC/5/23/2013-14/Tech/1500.

### Preparation of extract

About 50g of shade dried powdered material was added with 250 ml of water. The container is kept in a shaker for a period of 24 hours. The extract is then filtered, concentrated and dried. This dried viscous material obtained will be used for the analysis.

### Experimental animals

Adult albino rats weighing about 150-180 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 hrs 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, Bengaluru) with free access to tap water. The rats received humane care according to the criteria outlined in Principles of Laboratory Animal Care, 1985. The study was approved by Institutional Animal Ethics Committee (IAEC) and the experiments were conducted according to the ethical norms and IAEC guidelines. Approval number: KU/IAEC/DST/106.

### Induction of diabetes

Rats were rendered diabetic by a single intraperitoneal injection of freshly prepared STZ (45 mg/kg body weight) in 0.1 M citrate buffer (pH 4.5) in the volume of 1 ml/kg body weight using sterile 25G needle. Diabetes was identified in rats by moderate polydipsia and marked polyuria. After 48 hrs of STZ administration, blood glucose levels were estimated in rats following overnight fasting. Rats with a blood glucose level ranging between 200 and 300 mg/dl were considered diabetic and used for the experiments.

### Experimental design for anti-diabetic activity

The experiment will be conducted with a total of 25 rats (15 diabetic surviving rats, 10 normal rats) divided into five groups of five animals each. Based on the GTT results 400 mg/kg of *Erythrina variegata* was selected for this study.

Group 1: Untreated rats (Control)

Group 2: Diabetes induced rats (45mg/kg of streptozotocin)

Group 3: Rats treated with 2 mg/kg of Glibenclamide

Group 4: Rats treated with 400 mg/kg of *Erythrina variegata*

Group 5: Rats treated with 400 mg/kg of *Erythrina variegata* alone

The test drug and reference standard drug was fed orally for a period of 30 days. After the experimental period, rats were sacrificed by cervical dislocation after giving chloroform in mild dose.

### Determination of anti-diabetic activity

Blood was collected, and the serum was separated by centrifugation at 20,000 rpm for 30 minutes and used for biochemical analysis. Pancreas was immediately dissected, washed in ice cold saline, patted dry and weighed. The tissues were fixed in 10% formalin immediately after removal from the animal to avoid decomposition. Embedding in paraffin wax was carried out by removal of water using alcohol dehydration and infiltration of chloroform as a solvent for the wax. Whole blood was used for hemoglobin and HbA1c then, serum was used for biochemical

parameters – Glucose and Insulin (Serum insulin levels were measured by the micro plate ELISA method using a commercial kit) using various kits of analytical grade.

### Statistical Analysis

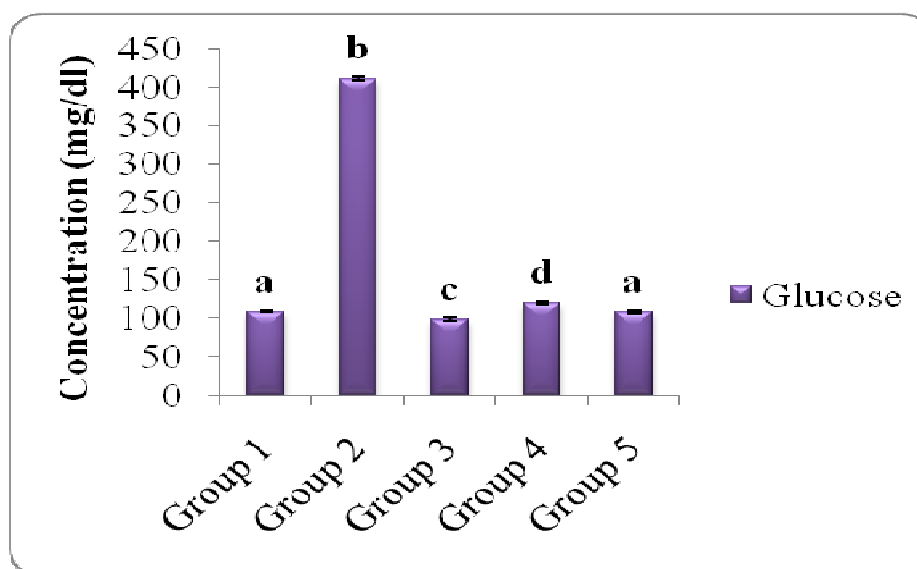
All samples were tested and analyzed in triplicates. Results were calculated as the Mean  $\pm$  SD (standard deviation) for each sample. Statistical analysis was done with one way analysis of variance (ANOVA) using SPSS version 16.0 and the individual comparisons were obtained by the Duncan Multiple Range Test (DMRT) [14]. The significant difference was judged to exist at a level of  $p < 0.05$ .

## RESULTS AND DISCUSSION

Herbal medications increase popularity due to an awareness that there is a lower incidence of adverse reaction to plant preparation than the synthetic pharmaceuticals [15,16]. Streptozotocin induced hyperglycemia in rodents is considered to be a good model for the preliminary screening of agents active against type II diabetes and it is widely used. Streptozotocin, N-{methylnitrocarbonyl}-D glucosamine, is a effective DNA methylating agent and acts as a nitric oxide donor in pancreatic cells.  $\beta$ -cells are particularly sensitive to damage by nitric oxide and free radicals because of their small levels of free radical scavenging enzymes [17].

In the present investigation, treatment with *Erythrina variegata* showed a significant antihyperglycemic activity. The maximum reduction in glucose level was observed in group receiving 400mg/kg of the extract. Findings of the present investigation revealed that STZ induced diabetes resulted in a significant increase in serum glucose level and significant decrease in serum insulin level. This diabetogenic effect could be due to the negative effect of streptozotocin on pancreatic islets. The mechanism of decreased insulin secretion could be attributed to the hyperglycemia that induced abnormalities in insulin action and secretion [18]. This momentous change to near normal glycemic concentration in streptozotocin induced diabetic rats, is an essential prompt for the liver to regress its normal homeostasis during experimental diabetes. The fundamental mechanism behind the hyperglycemia involves over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues [19].

Figure 1: Effect of *E. variegata* on glucose of control and treated rats

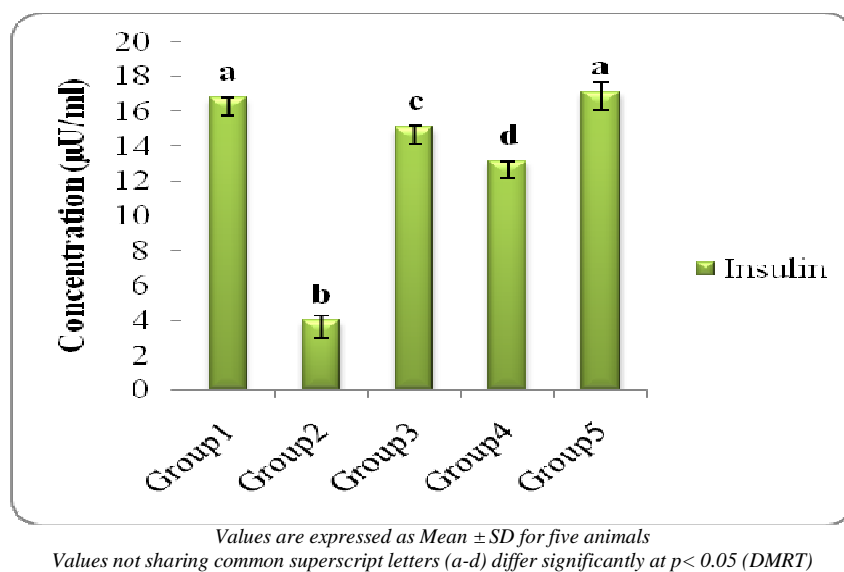


Values are expressed as Mean  $\pm$  SD for five animals  
Values not sharing common superscript letters (a-d) differ significantly at  $p < 0.05$  (DMRT)

Figure 1, Shows the effect of treatment with the extract on blood glucose levels. In *E. variegata* treated group shows a significant antihyperglycemic effect which was evident from the decrease in blood glucose at the end of 30 days of experimental period. The initial readings of blood glucose level in Group 4 were  $286.40 \pm 2.748$  and after treatment with *E. variegata* it was found to be  $118.04 \pm 1.89$  slightly higher than that of standard drug group. In standard

group 3 initial blood glucose level was  $273.38 \pm 2.739$  and the post test was  $97.68 \pm 2.12$  which showed that the standard drug produced maximum hypoglycemic effect and the statistical analysis was extremely significant. Untreated diabetic rat group showed increase in blood glucose level throughout the entire study period. Initially blood glucose level of untreated diabetic control group 2 was  $289.23 \pm 1.38$  and after 30 days of test period the blood glucose level was increased to  $410.57 \pm 0.92$ . The plasma glucose lowering activity was compared with that of glibenclamide, the reference oral hypoglycemic drug used for many years to treat diabetes mellitus, which stimulate pancreatic  $\beta$  cells [9]. There are countless reports available to support the multiple mechanisms of antidiabetic plants to exert their blood glucose lowering effect, such as inhibition of carbohydrate metabolizing enzymes, enhancement of insulin sensitivity, regeneration of damaged pancreatic islet  $\beta$ -cells and enhancement of insulin secretion and release [20].

Figure 2: Effect of *E. variegata* on insulin of control and treated rats

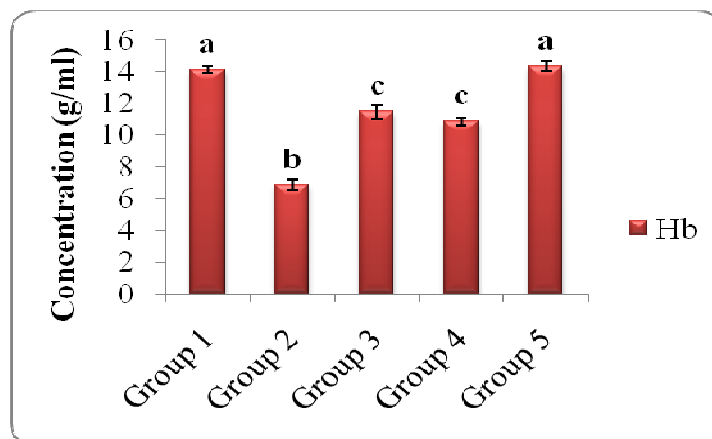


Insulin and some oral antihyperglycemic drugs is the keystone of the diabetes treatment, although they have important adverse effects and cannot always preserve glycemia and prevent diabetes complications significantly [21]. Insulin plays a key function in glucose homeostasis along the side of a counter regulatory hormone, glucagon, which raises serum glucose. Carrier proteins (GLUT 1- 5) are essential for glucose uptake into cell and its function is impaired in insulin deficiency. The mode of action of the active compound(s) of the plant material is probably mediated throughout enhanced secretion of insulin from the  $\beta$ -cells of langerhans or through extra pancreatic mechanism [22]. Effect of oral administration of *E. variegata* extract on serum insulin in diabetic rats is showed in Figure (2). Results indicated that serum insulin level of the diabetic positive control group significantly decreased after 30 days STZ administration compared with the normal control group ( $p < 0.05$ ). The oral administration of *E. variegata* and glibenclamide increased the levels of blood insulin in treated diabetic rats significantly ( $p < 0.05$ ) compared with the untreated control group. It may be suggested that the mechanism of hypoglycemic action of *E. variegata* is similar to glibenclamide. From the results of this study, it appears that still insulin producing cells are functioning and the stimulation of insulin release could be responsible for most of the metabolic effects.

In DM, the hemoglobin level is decreased, because the surplus glucose produced in the body reacts with the hemoglobin to form glycosylated hemoglobin [23]. Hematological complications consist mostly of abnormalities in the function, morphology and metabolism of erythrocytes, leukocytes and platelets. Literature has shown that intake of medicinal compounds or drugs can modify the normal range of hematological parameters [24,25]. Furthermore, it has been exposed by the present study that hemoglobin levels in diabetic control (untreated group) showed abnormalities. This might due to the destruction of matured Red Blood Cells (RBC) leading to low haemoglobin count (Hb), because of reaction of excess glucose with the haemoglobin to gives rise to glycosylated haemoglobin with diminish in RBC [26]. Figure 3 implies that group 2 showed decreased haemoglobin levels upon treatment with the plant extract and glibenclamide the stage was brought back to near normal. This might also be responsible in improving the immune system being weak due to the generation of reactive oxygen species as a result of

streptozotocin induction and shows that the aqueous extract may not have negative effect on the bone marrow, kidney and haemoglobin metabolism [27].

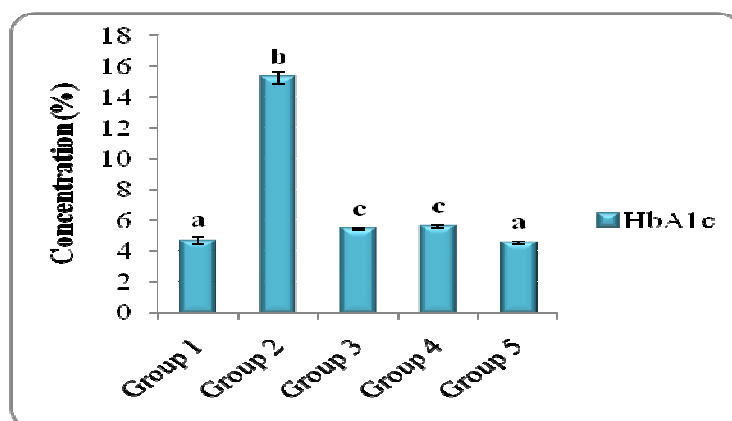
Figure 3: Effect of *E. variegata* on Hemoglobin of control and treated rats



Values are expressed as Mean  $\pm$  SD for five animals  
Values not sharing common superscript letters (a-c) differ significantly at  $p < 0.05$  (DMRT)

HbA1c is considered as an investigative marker and helps to know about degree of protein glycation, longterm blood sugar level and correlation of diabetes connected complications [28,29]. HbA1c has been found to be increased over a long period of time in diabetes because the excess of glucose present in blood reacts with haemoglobin to form glycosylated haemoglobin [30]. The rate of glycation is proportional to the concentration of blood glucose. In the present study, streptozotocin induced diabetic rats showed significant increase in HbA1c level compared with normal rats (Figure 4). The treatments with the aqueous extract of *E. variegata* and glibenclamide showed a significant decrease in the content of glycosylated haemoglobin that could be due to an improvement in glycemic status.

Figure 4: Effect of *E. variegata* on HbA1c of control and treated rats

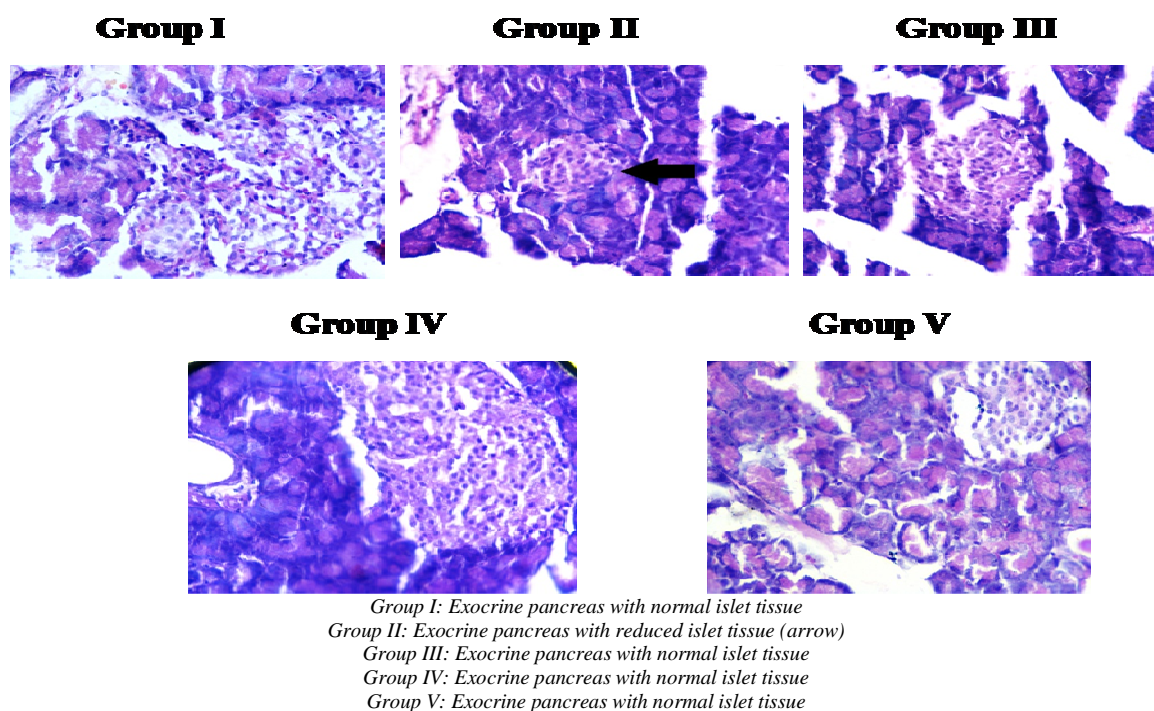


Values are expressed as Mean  $\pm$  SD for five animals  
Values not sharing common superscript letters (a-c) differ significantly at  $p < 0.05$  (DMRT)

HbA1c is used as a sign for estimating the degree of protein glycation in diabetes mellitus [31]. Evidence showed that glycation itself may persuade the formation of oxygen-derived free radicals in diabetic condition, and the level of HbA1c is considered as one of the markers of degree of oxidative stress in diabetes mellitus [32]. Administration of *E. variegata* to diabetic rats reduced the glycosylation of haemoglobin by virtue of its normoglycaemic activity and thus decreases the levels of glycosylated haemoglobin in diabetic rats. This normalisation of glycosylated haemoglobin indicates decreased glycation of proteins.

Changes in the islet cells of pancreas in all groups are given in Figure 5. The diameter of islets did not differ significantly in groups I and V of the experimental animals. Since the  $\beta$  cells were damaged due to the induction of diabetes, the islet size decreased significantly in group II of the experimental animals. Streptozotocin enters the beta cells via the glucose transporter (GLUT2) and causes alkylation of DNA. DNA damage induces establishment of poly ADP-ribosylation, a progression that is more important for the diabetogenicity. Improved ATP dephosphorylation after streptozotocin induction supplies a substrate for xanthine oxidase resulting in the formation of super oxide radicals, hydrogen peroxide and hydroxyl radicals. In addition streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage finally it undergoes destruction by necrosis [33]. The administration of the standard drug glibenclamide and *E. variegata* extract to group III and IV of the experimental animals resulted in the recovery of damaged  $\beta$  cells and the restoration of the diameter of the islets to that of the control group (I). It implies that treatment with *E. variegata* cause regeneration of ( $\beta$ -cell) and restored standard cellular size of islets without hemorrhage. It is thus apparent that the hypoglycemic effect is likely due to pancreatic mechanism and confirmed that the given extract is effective and can act in the treatment of diabetes.

Figure 5: Effect of *Erythrina variegata* L. bark extract on the histopathology of control and experimental rat pancreas



### CONCLUSION

It can be concluded from the data that *E. variegata* bark extract supplementation is advantageous in controlling the blood glucose level, HbA1c and also improves the Hb and insulin levels in experimental diabetic rats. This could be useful for prevention of diabetic disorders. Thus, the folk use of the plant for the control of diabetes may be supported by this study. The histopathologic studies undertaken on the islets demonstrated the recovery of damaged islets and an improvement in the number of  $\beta$  cells after treatment with the plant extract. It can thus be assumed that *E. variegata* bark extract has a therapeutic effect that alleviates diabetes mellitus. The actual chemical compound that is responsible for this antidiabetic effect requires further investigation.

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### CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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