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Research Article

Carbohydrate Metabolizing Enzymes Activity in Streptozotocin Induced Diabetic Rats Treated with Aqueous Extract of *Erythrina variegata* L. Bark

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ABSTRACT

Alteration in carbohydrate metabolizing enzymes in diabetes and the antidiabetic potential of aqueous extract of *Erythrina variegata* L. bark in streptozotocin (STZ) induced diabetic in Wistar albino rats was investigated. Diabetes was induced in rats by single intraperitoneal injection of streptozotocin (45mg/kg body weight). Three days after STZ induction, the diabetes rats were treated with aqueous extract of *Erythrina variegata* L. bark orally at the dose of 400 mg/kg body weight daily for 30 days period. Glibenclamide (2mg/kg, orally) was used as standard drug. In addition, changes in body weight, hepatic glycogen content and carbohydrate metabolic enzymes were estimated by standard protocols. The results showed that the aqueous extract of *E. variegata* L. bark significantly increases the body weight, glycogen content and normalize the carbohydrate metabolizing enzymes. From this study, it can be concluded that *E. variegata* L. possesses considerable anti-diabetic property in streptozotocin induced diabetic rats.

Keywords: *Erythrina variegata* L., hexokinase, glycogen, glycogen phosphorylase, glucose-6- phosphatase

INTRODUCTION

According to WHO, the word diabetes mellitus is described as a metabolic disorder of several etiology characterized by chronic hyperglycemia with turbulence of carbohydrate, fat and protein metabolism resulting from deficiency in insulin secretion, insulin action, or both[1]. Worldwide the numbers of cases of diabetes is rising gradually. There are numerous medicines presented in the market to treat diabetes mellitus but no drug is found to be fully efficient and secure. Plants and plant-derived products have proven to be effectual and safe in the cure of various kind of diabetes mellitus[2]. Medicinal plants consist of a number of active components, therefore they are used for the treatment of large number of infectious aliments. Today the enormous traditional facts of medicinal plants is playing significant role in the development of new drugs[3]. The genus Erythrina contain about 110 species of trees and shrubs. The name "coral tree" is employ as a collective word for these plants. Coral tree is indigenous to the Old World tropics, perhaps originated from India to Malaysia, but is native of ancient Westward to Zanzibar and Eastward to Eastern Polynesia[4]. Different parts of the plant have been utilized as a natural medicine in nervine sedation, opthalmia, asthma, epilepsy and skin disorders. The bark of the plant is astringent, febrifuge, anti-bilious and antihelmintics. The bark and leaves are used in several usual treatment, as well as paribhadra, an Indian preparation said to obliterate pathogenic parasites and reduce joint pain[5]. The aim of the present study has been designed to investigate the effect of aqueous extract of *Erythrina variegata* L. bark on alterations of carbohydrate metabolizing enzymes in streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Plant material

The plant materials 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 aqueous extract

The powdered plant material (bark), 50 g was weighed and extracted with 250 ml water for 72 h using an occasional shaker. The supernatant was collected and concentrated at40ºC. It was stored at 4ºC in an air-tight bottle for further studies.

Animals

Wistar albino rats of either sex weighing about 150–180 g were procured from the animal house of Karpagam University, Coimbatore, India. The animals were under standard conditions and were housed in polypropylene cages with a wire mesh top and a hygienic bed of husk in a specific pathogen free animal room under controlled conditions of 12 hr light and 12 hr dark cycle, with temperature of 24 ± 2ºC, relative humidity of 50 ± 10% and fed with rodent diet and water *ad libitum*. The study was accepted by Institutional Animal Ethical Committee constituted for the purpose of CPCSEA (Approved No:

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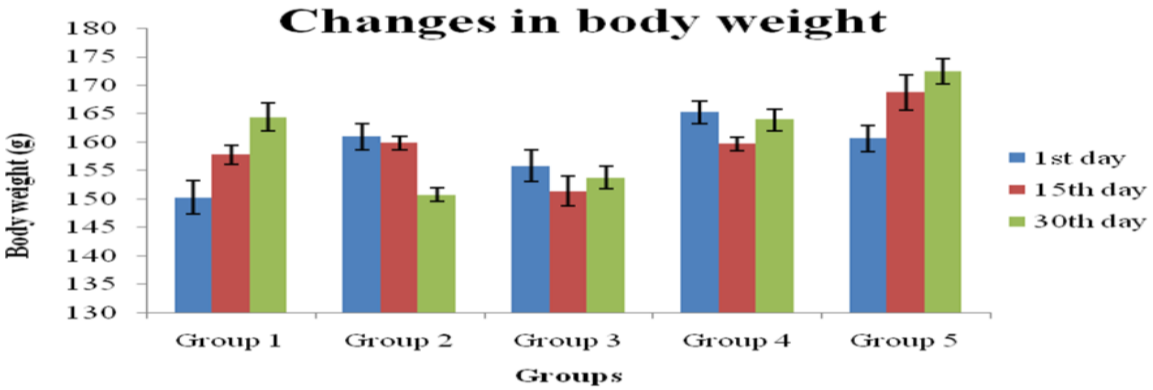

Figure 1: Effect of *Erythrina variegata* L. bark extract on body weight in STZ induced diabetic rats. Values are given as Mean ± S.D for 5 rats in each group

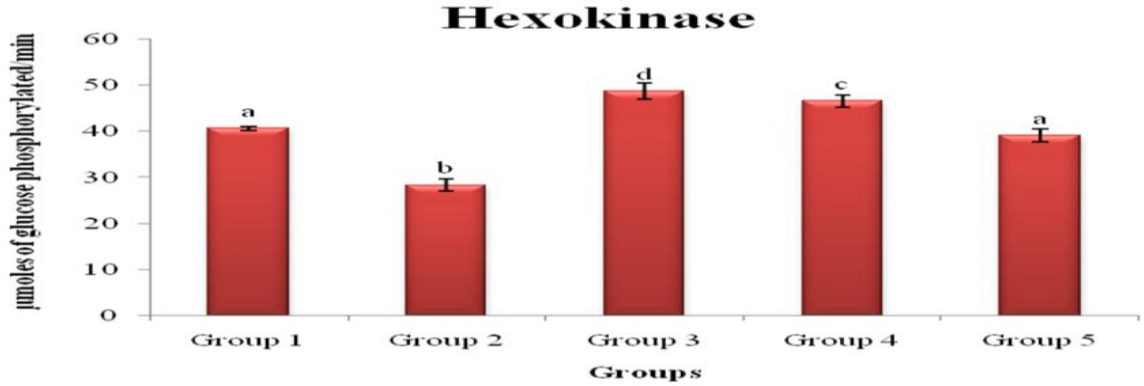

Figure 2: Effect of *Erythrina variegata* L. bark extract on Hexokinase in STZ induced diabetic rats. Values are given as Mean ± S.D for 5 rats in each group. Values not sharing a common superscript letter(a-d) differ significantly (DMRT).

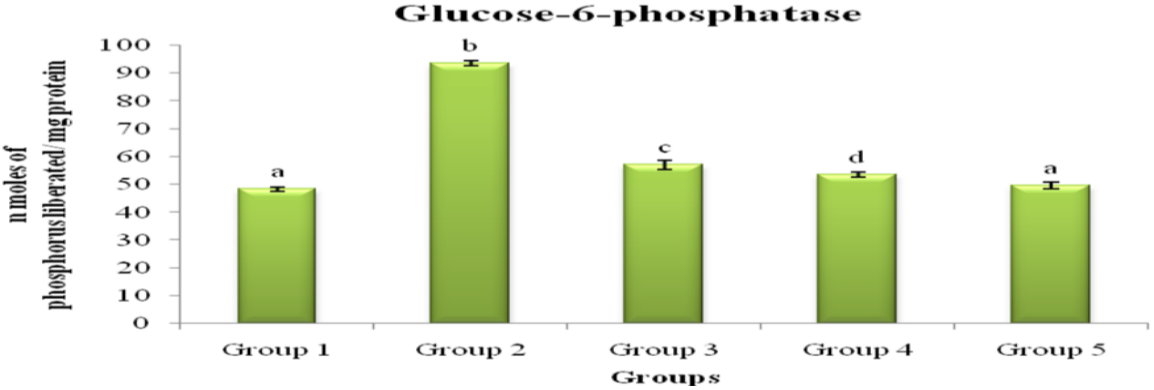

Figure 3: Effect of *Erythrina variegata* L. bark extract on Glucose -6 -phosphatase in STZ induced diabetic rats. Values are given as Mean ± S.D for 5 rats in each group. Values not sharing a common superscript letter(a-d) differ significantly (DMRT).

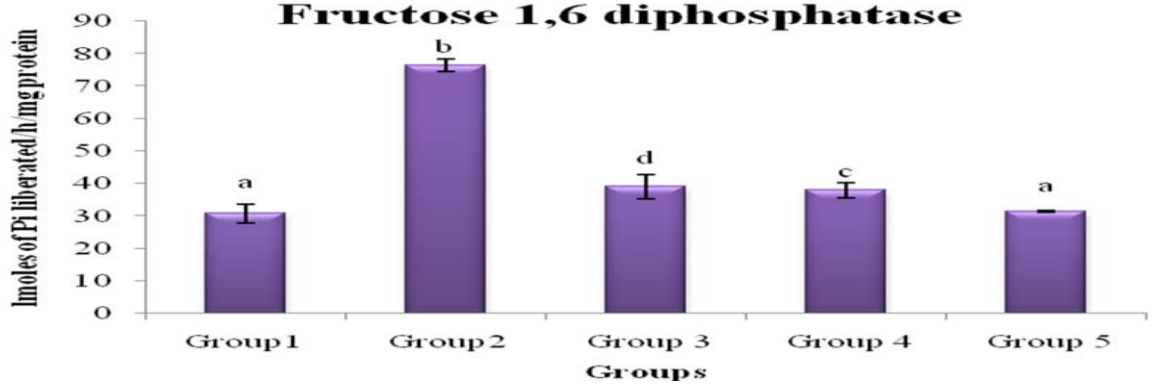

Figure 4: Effect of *Erythrina variegata* L. bark extract on Fructose 1,6 diphosphatase in STZ induced diabetic rats. Values are given as Mean ± S.D for 5 rats in each group. Values not sharing a common superscript letter (a-d) differ significantly (DMRT).

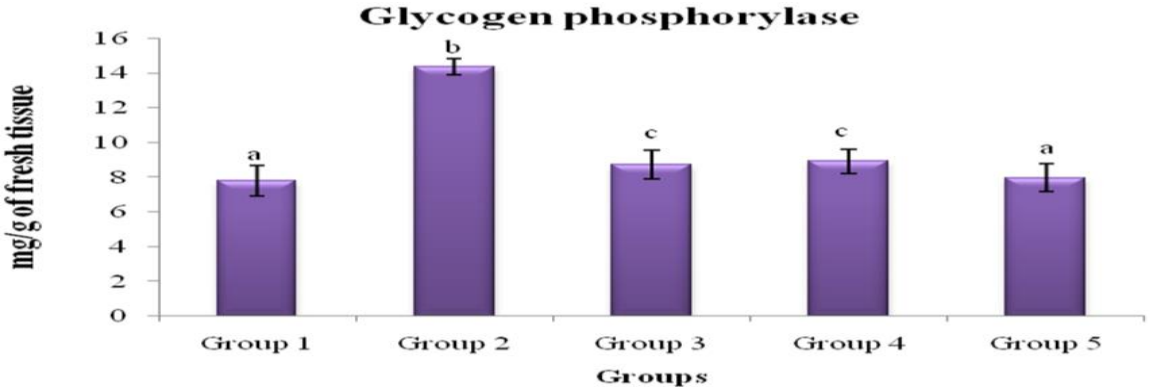

Figure 5: Effect of *Erythrina variegata* L. bark extract on Glycogen phosphorylase in STZ induced diabetic rats. Values are given as Mean ± S.D for 5 rats in each group. Values not sharing a common superscript letter (a-c) differ significantly (DMRT).

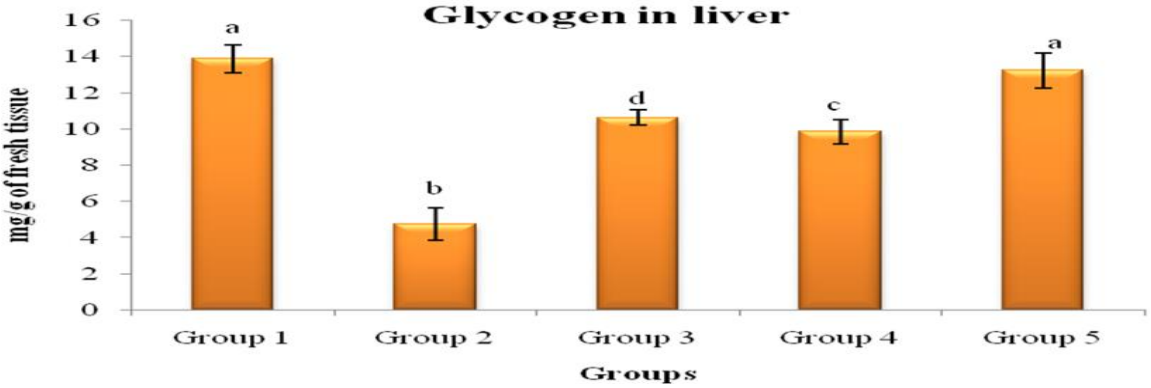

Figure 6: Effect of *Erythrina variegata* L. bark extract on glycogen in STZ-induced diabetic rats. Values are given as Mean ± S.D for 5 rats in each group. Values not sharing a common superscript letter (a-d) differ significantly (DMRT).

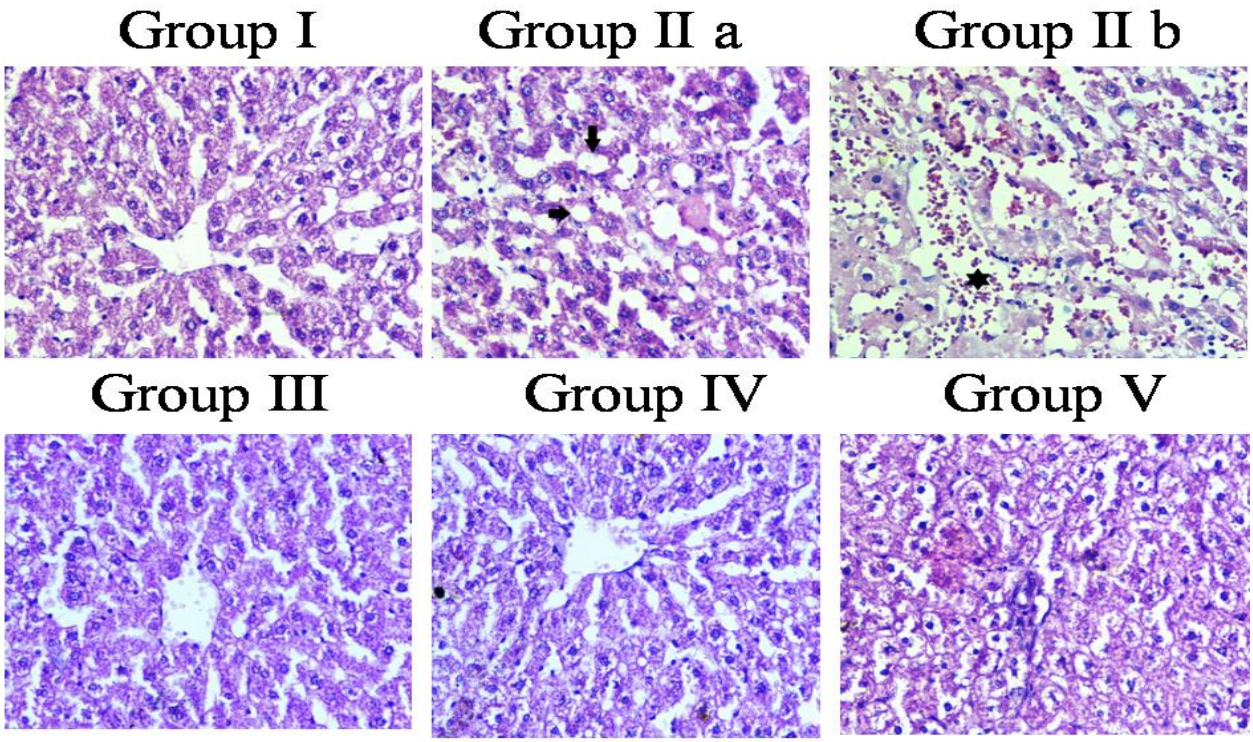

Figure: 7 Effect of *Erythrina variegata* L. bark extract on the histopathology of rat liver control and experimental rats.

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Induction of diabetes

Rats were rendered diabetic by a single intraperitoneal injection of freshly prepared streptozotocin (45 mg/kg body weight) in 0.1M citrate buffer (pH 4.5) in a volume

of 1 ml/kg body weight[6]. Normal rats received 1 ml citrate buffer as vehicle. Diabetes was identified in rats by moderate polydypsia and marked polyuria. After 48 hrs of streptozotocin administration, blood glucose levels were estimated in rats following overnight fasting. Rats with a blood glucose ranging between 200–400 mg/dl were considered diabetic and used for the experiments.

Experimental design

The animals were divided into five groups of five animals each. Based on the GTT results 400 mg/kg of *Erythrina variegata* L. bark was chosen for current investigation. Group I served as control animals given normal diet, Group II rats served as diabetic rats, Group III rats were induced with diabetes and treated with standard drug glibenclamide (2 mg/kg body weight) through oral intragastric tube, Group IV animals were induced with diabetes and treated with aqueous extract of *Erythrina variegata* L. bark (400 mg/kg body weight) and Group V control rats were treated with *Erythrina variegata* L. bark alone at a concentration of 400 mg/kg body weight. The extract was given every day through oral gastric tube for a period of 30 days. The animals will be weighed and dosed through oral intragastric tube every day. Blood glucose levels and body weight 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.

Sacrification of animals

After experimental period, the rats were kept for overnight fasted and sacrificed by cervical dislocation after giving chloroform in mild dose. Liver was immediately dissected out, washed and stored in 0.9% ice-cold saline for various biochemical evaluations and tissues (liver) were stored in 1% formalin and used for histopathological studies.

Preparation of tissue homogenate

A 10% homogenate of the washed tissues were prepared with 0.1 M Tris-HCl buffer pH 7.4 at 4ºC in a potter homogenizer, fitted with a Teflon plunger at 600 rpm for 3 min. The filtrate was used for further biochemical analysis.

Biochemical estimations

Liver carbohydrate metabolic enzymes like hexokinase[7], glucose-6- phosphatase[8], Fructose1,6-diphosphatase[9], glycogen phosphorylase[10] and Liver glycogen[11] were estimated by their respective protocols.

Histopathological studies

The anti-diabetic activity was confirmed through histopathological studies on liver kidney and pancreas of rats. They are removed from each animal after dissection and preserved in 10% formalin for histopathological observation by the method of Dunn[12].

Statistical analysis

All the data were expressed as Mean±Standard deviation of a number of experiments. Statistical significance was evaluated using one-way analysis of variance (ANOVA) using SPSS version 16.0 (SPSS, Cary, USA) and the individual comparisons were obtained by the Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Medicinal plants are the backbone of traditional medicines and have been the subjects of very intense pharmacological studies[13]. Diabetes is one of the most common metabolic disorders in worldwide. It is a disorder of carbohydrate metabolism in which sugars in the body are not oxidized to produce energy due to lack of the pancreatic hormone, insulin. Streptozotocin (STZ) treatment results in diabetes due to the destruction of beta-cells of the pancreas that secrete insulin[14]. Liver is the candidate organ involved in glucose homeostasis. It is the main location for glycolysis, a method where glucose is degraded and gluconeogenesis, where glucose is produced from lactate, amino acids and glycerol. The action of enzymes like hexokinase, glucose-6-phosphatase, glycogen phosphorylase and fructose-1,6-diphosphatase was noticeably altered, consequential in hyperglycemia, which directs to the pathogenesis of diabetic complications[15].

Change in body weight control and experimental rats

In the present work initial, middle and final body weight of experimental animals were calculated, whereas food and water consumption were evaluated on a every day basis. Though the food and water intake of diabetic rats were greater than before during the experimental period, the weight gain was significantly reduced when compared to control animals. Induction of diabetes with STZ is related with a characteristic loss of body weight, which is due to augmented muscle wasting and loss of tissue[16]. Figure 1 exhibit the changes in body weight of normal and experimental rats in each group at initial and final day of treatment. Significant decrease in body weight of diabetic control rats were observed when compared with normal control rats. On the other hand, significant increase in body weight was observed after treatment with plant extract treated groups and glibenclamide treated group indicating the possible role of the extract in restoration of protein responsible for body weight. There were no alteration found in body weight of plant alone group as that of control.

Change in carbohydrate metabolizing enzymes in control and experimental rats

Defects in carbohydrate metabolizing mechanism and dependable efforts of the physiological systems to correct the imbalance in carbohydrate metabolism place an overexertion on the endocrine system, which directs to the deterioration of endocrine control.

Continuing deterioration of endocrine control exacerbates the metabolic disturbances by altering carbohydrate-metabolizing enzymes and leads to diabetes[17]. Recently there has been a growing interest in hypoglycemic agents from natural products, especially those derived from plants. Plant sources are usually considered to be non-toxic, with fewer side effects than synthetic sources. Many medicinal plants have been found to be useful for the successful management of diabetes[18]. Figure 2 demonstrate the effect of *Erythrina variegata* L. bark on hepatic carbohydrate metabolising enzymes. The action of hexokinase (HK) is significantly

decreased in the liver of diabetic rats compared to those in normal control rats. Hexokinase (HK) is an isoenzyme that catalyzes the phosphorylation of glucose to glucose-6-phosphate thus playing a crucial function in tissue intermediary metabolism. There are four isoforms of mammalian hexokinases involved in the oxidation of glucose[18]. Decreased level of hexokinase in STZ induced diabetic rats can be accountable for diminished glycolysis which results in decreased utilization of glucose for energy production[19]. Administration of *Erythrina variegata* L. bark restore the enzyme activation implies its normalizing effect in diabetes. In the present study, hepatic HK activities were decreased in diabetic rats compared to those in normal rats. Decreased activity of hepatic HK in diabetic animals were previously shown by several investigators[20]. Figure 3 and 4 depicts the activities of gluconeogenic enzymes, such as glucose-6-phosphatase and fructose-1,6-bisphosphatase in liver of control and experimental rats. The activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase were significantly increased in liver of diabetic rats when compared to those in normal controls. The increased activities of these gluconeogenic enzymes in diabetic rats were decreased to near-normal levels after the administration of *Erythrina variegata* L. bark. The promising mechanism by which *Erythrina variegata* L. bark bring about the normalization of enzyme actions may be by potentiation of insulin liberation from β-cells of the islets of Langerhan's which might improve glucose utilization. Glucose-6-phosphatase, a key enzyme in the homeostatic regulation of blood glucose concentration, is expressed mainly in the liver and kidney and is critical in providing glucose to other organs during diabetes, prolonged fasting or starvation[21,22]. It catalyzes the dephosphorylation of glucose-6-phosphate to free glucose as the terminal step in gluconeogenesis and glycogenolysis[23]. Fructose-1, 6-bisphosphatase is one of the important regulatory enzymes of gluconeogenic pathway and it catalyze the rate limiting step of fructose 1-6-bisphosphate to fructose-6-phosphate[24]. Hepatic glucose production is raised in diabetic state and is associated with the impaired suppression of the gluconeogenic enzyme fructose 1, 6-bisphosphatase[25].Disturbed performance of carbohydrate metabolising enzymes in diabetic rats denoted that the carbohydrate metabolic pathways (glycolysis, glycogenolysis, glycogenesis and gluconeogenesis) were harshly disturbed, which was probably due to insulin deficit in body[26,27]. Figure 5 represents the effect of *Erythrina variegata* L. bark treatment on the activities of glycogen phosphorylase in liver of control and experimental groups of rats. A significant ($p < 0.05$) turn up in the activity of glycogen phosphorylase were noted in the liver of diabetic group of rats. Oral therapy with *Erythrina variegata* L. bark as well as glibenclamide to diabetic groups of rats restore the level of glycogen phosphorylase to close to normalcy when compared to control group of rats. Glycogen phosphorylase, a rate-limiting enzyme of glycogenolysis, cleaves (1→4) linkages to eliminate glucose molecules from the glycogen. This enzyme exists as a dimer with each subunit correlated to the essential cofactor pyridoxal phosphate, which donates the phosphate as an electron donor for release of glucose-1-phosphate[28]. From the figure 6, it is evident that the hepatic glycogen levels in diabetic control group animals without any treatment, showed significant decrease in hepatic glycogen levels (4.76 ± 2.89 mg equivalents/g wet tissue) when compared to the normal rats (13.88 ± 1.78 mg equivalents/g wet tissue). But the treatment of diabetic rats with *Erythrina variegata* L. bark significantly increased the liver glycogen (9.87 ± 1.67 mg equivalents/g wet tissue). The reduced glycogen store in diabetic rats has been attributed to the loss of glycogen synthase-activating system or the increased activity of glycogen phosphorylase[29]. The decrease in hepatic glycogen observed may be due to insufficient insulin and inactivation of glycogen synthetase in diabetic state[30]. In our previous study, the *Erythrina variegata* L. bark had good antioxidant effect and enzymatic alterations were reported against streptozotocin diabetic rats. So,these property may be due to the existence of alkaloids, flavonoids and glycosides in the bark extract of *Erythrina variegata* L.[31,32].

Histological studies in liver tissue of STZ-induced diabetic rats showed hepatocellular damage in the form of sinusoidal dilation, fatty changes, and extensive vacuolization with the disappearance of nuclei. This damage is partially reversed by the administration of *Erythrina variegata* L. significantly improved the histological architecture of liver in diabetic rats. Which is similar to that observed by glibenclamide treated groups. The normal animal treated with plant extract of *Erythrina variegata* alone showed normal hepatic structure thus indicating that it did not produce any liver toxicity.

Findings of the present study suggests that *Erythrina variegata* L. enhances the glycolytic enzymes and control glucose metabolism in streptozotocin-diabetic rats. Thus, *Erythrina variegata* L. possesses antihyperglycemic activity by stimulating the insulin production from the existing β-cells of pancreas. This study also shows that *Erythrina variegata* L. bark manage the increase in the levels of glucose by increasing glycolysis and by decreasing gluconeogenesis. This is possible as it controls the activities of the key enzymes of glycolysis.

Group I: Normal hepatocytes and central vein
Group IIa: Hepatocytes showing fatty change (arrows)
Group IIb: Congestion of hepatic sinusoids (star)
Group III: Normal hepatocytes and central vein
Group IV: Normal hepatocytes and central vein
Group V: Normal hepatocytes and portal tract

CONCLUSION

The present research clearly indicated that the aqueous extract of *Erythrina variegata* L. bark have promising activity against streptozotocin induced diabetic rats. Thus, the dietary supplementation of *E. variegata* L. bark may be helpful for the management of diabetes mellitus and prevention of diabetic complications. Future research to

refine the extraction procedure of *E. variegata* L. bark could lead to improved pharmaceutical products.

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CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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