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ORIGINAL ARTICLE

Evaluation of Antioxidants in the Kidney of Streptozotocin Induced Diabetic Rats

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Abstract Diabetes mellitus is one of the most common endocrine metabolic disorders. Dual endocrine deficits of impaired insulin action (insulin resistance) and inadequate insulin secretion create an environment of chronic hyperglycemia and general metabolic disarray. Oxidative stress plays an important role in diabetic pathogenesis. Oxidative stress induced by streptozotocin (STZ) has been shown to damage pancreatic beta cell and produce hyperglycemia in rats. The present study was made to evaluate the antioxidant activity of ethanolic extract of the *Evolvulus alsinoides* in STZ induced rats. The antioxidant activities were done by using standard protocols. For histopathological analysis, the pancreatic tissues of all experimental groups were fixed with 10 % formalin for 24 h then the samples were stained with hematoxylin–eosin for the microscopic observation. Our results showed the significant decrease in lipid peroxidation and increases in the antioxidant (both enzymatic and nonenzymatic) levels after treatment with standard as well as the *E. alsinoides*. There is no significant difference between control and plant alone group rats. The histopathology reports also revealed non-toxic effect and protective effect of *E. alsinoides* in the kidney of STZ induced diabetic rats. Our result indicated that the *E. alsinoides* extract effectively increased the antioxidant level thereby it prevents oxidative stress during diabetes mellitus and also it showed the protective effect on kidney of STZ induced rats. Hence it can be used to maintain the antioxidant level during diabetes mellitus.

Keywords Enzymatic antioxidants · Non-enzymatic antioxidant · LPO · Streptozotocin · *Evolvulus alsinoides*

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Introduction

Diabetes mellitus is considered as one of the five leading causes of death in the world. About 150 million people are suffering from diabetes worldwide, which is almost five times more than the estimates 10 years ago and this may double in the year 2030 [1]. It is characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative deficiency in insulin secretion or insulin action [2]. In diabetes mellitus, chronic hyperglycaemia produces multiple biochemical consequence and diabetes-induced oxidative stress could play a role in the symptoms and progression of the disease [3].

Oxidative stress may result in overproduction of oxygen free-radical precursors and/or decreased efficiency of the antioxidant system [4]. The oxygen free-radical generation is associated with auto-oxidation of glucose, impaired glutathione metabolism, alterations in the antioxidant enzymes and formation of lipid peroxides [5–7]. There are various endogenous defense mechanisms against free radicals, such as the enzymes GSH, SOD, GPx and CAT, whose activities eliminate superoxide, hydrogen peroxide and hydroxyl radicals [8]. Oxidative stress is increased in experimental models of streptozotocin (STZ)-induced diabetes mellitus [9, 10]. If the diabetic state is associated with a generalized increase in tissue oxidative stress, it might well be reflected in the changes in tissue antioxidant

system. So the present study was aimed to test the antioxidant enzymes and lipid peroxidation profile in the kidney of treated and untreated diabetic rats.

Materials and Methods

Plant Material

The whole plant of *Evolvulus alsinoides* (L.) L. used for the investigation was obtained from Coimbatore District, Tamilnadu, India. The plant was authenticated by Dr. P. Satyanarayana, Botanical Survey of India, TNAU Campus, Coimbatore. The voucher number is BSI/SRC/5/23/2011-12/Tech.-514. Fresh plant material was washed under running tap water, air dried and powdered.

Sample Extraction

100 g of dried plant powder was extracted in 500 ml of ethanol in an orbital shaker for 72 h. Repeated extraction was done with the same solvent till clear colorless solvent is obtained. Obtained extract was evaporated and stored at 0–4 °C in an air tight container.

Animals

Wistar albino rats weighing about 150–180 g were procured from Karpagam University Animal house, Coimbatore, India. The animals were kept under standard conditions, fed with rodent diet and water. The study was approved by Institutional Animal Ethical Committee constituted for the purpose of CPCSEA.

Induction of Experimental 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 a volume of 1 ml/kg body weight [11]. Diabetes was identified in rats by moderate polydypsia and marked polyuria. After 48 h of STZ administration, blood glucose levels were estimated and rats with a blood glucose ranging between 200 and 400 mg/dl were considered diabetic and used for the experiments.

Experimental Protocol

The animals were divided into five groups of six animals each. Group I served as a control; group II consisted of STZ-induced diabetic rats; group III consisted of STZ-induced diabetic rats treated with glibenclamide (1.25 mg/kg body weight/day/rat); groups IV consisted of STZ-induced diabetic rats treated ethanolic extract of *E. alsinoides* (150 mg/kg body weight/day/rat) and group V were normal rats treated with ethanolic extract of *E. alsinoides* (150 mg/kg body weight/day/rat).

Biochemical Studies

After 45 days of treatment the animals were sacrificed under chloroform anesthesia. Liver and kidneys was quickly excised off, a portion of liver washed with saline and liver homogenate was prepared using 0.1 M phosphate buffer, pH 7.4. The liver homogenate was centrifuged and the supernatant was used for the determination of lipid peroxidation [12], enzymatic antioxidant like superoxide dismutase [13], catalase [14], glutathione peroxidase [15], glutathione reductase [16], glucose-6-phosphate dehydrogenase [17] and non-enzymatic antioxidants like total reduced glutathione [18], vitamin C [19] and vitamin E [20].

Histological Observation

One portion of the kidney in all the experimental groups was fixed in 10 % formalin for histological observation. It was done by Dunn, 1974 [21] method.

Statistical Analysis

The values were expressed as mean ± SD. The statistical analysis was carried out by one way analysis of variance using SPSS (version 10) statistical analysis program. Statistical significance was considered at $p < 0.05$.

Result and Discussion

Antioxidants are substances or nutrients in our foods which can prevent or slow the oxidative damage to our body. When our body cells use oxygen, they naturally produce free radicals (by-products) which can cause damage. Antioxidants act as "free radical scavengers" and hence prevent and repair damage done by these free radicals. Health problems such as heart disease, muscular degeneration, diabetes mellitus, cancer etc. are all contributed by oxidative damage [22]. Hence the enzymatic and nonenzymatic antioxidant levels were measured in diabetic rats before and after treatment with standard as well as plant extract.

The diabetic state is associated with a generalized increase in tissue oxidative stress, which might be reflected in the changes in the tissue antioxidant system. Results of the changes in antioxidant enzymes are presented in Table 1. All enzymatic antioxidants like SOD, catalase, glutathione reductase, GPx and glucose-6-phosphate dehydrogenase levels were decreased in diabetic condition due to the glycation of proteins. After the treatment with

Table 1 Enzymatic antioxidant levels in kidney of diabetic condition before and after treatment with *Evolvulus alsinoides* of control and experimental groups

Groups	SOD	Catalase	Glutathione reductase	GPx	Glucose-6-phosphate dehydrogenase
Control	3.31 ± 0.33^{b}	0.72 ± 0.057^{b}	3.18 ± 0.23^{b}	1.96 ± 0.08^{d}	3.22 ± 0.05^{b}
Diabetic control	1.10 ± 0.53^{a}	0.305 ± 0.30^{a}	1.96 ± 0.92^{a}	0.76 ± 0.21^{a}	2.45 ± 0.16^{a}
Diabetic + glibenclamide	2.98 ± 0.20^{b}	0.66 ± 0.030^{b}	3.02 ± 0.62^{b}	1.51 ± 0.10^{b}	3.19 ± 0.05^{b}
Diabetic + *Evolvulus alsinoides*	3.27 ± 0.46^{b}	0.64 ± 0.09^{b}	3.06 ± 0.28^{b}	1.59 ± 0.08^{b}	3.04 ± 0.05^{b}
Evolvulus alsinoides alone	3.35 ± 0.52^{b}	0.74 ± 0.09^{b}	3.65 ± 0.38^{b}	1.86 ± 0.29^{d}	3.48 ± 0.21^{d}

Values are expressed as mean ± SD for six animals in each group. Values not sharing common superscript letters $^{(a–d)}$ differ significantly at $p < 0.05$ (DMRT)

Table 2 Non-enzymatic antioxidant levels in kidney of diabetic condition before and after treatment with *Evolvulus alsinoides* of control and experimental groups

Groups	Total reduced glutathione	Vitamin C	Vitamin E
Control	25.54 ± 1.17^{c}	2.97 ± 0.10^{d}	3.02 ± 0.27^{b}
Diabetic control	13.82 ± 2.05^{a}	1.11 ± 0.05^{a}	1.63 ± 0.68^{a}
Diabetic + glibenclamide	21.83 ± 5.43^{b}	2.35 ± 0.69^{b}	2.95 ± 0.17^{b}
Diabetic + *Evolvulus alsinoides*	$22.98 \pm 0.65b^{c}$	2.18 ± 0.23^{b}	2.83 ± 0.74^{b}
Evolvulus alsinoides alone	29.03 ± 1.18^{d}	2.99 ± 0.11^{d}	3.02 ± 0.24^{b}

Values are expressed as mean ± SD for six animals in each group. Values not sharing common superscript letters $^{(a–d)}$ differ significantly at $p < 0.05$ (DMRT)

The units were expressed as μg/mg protein

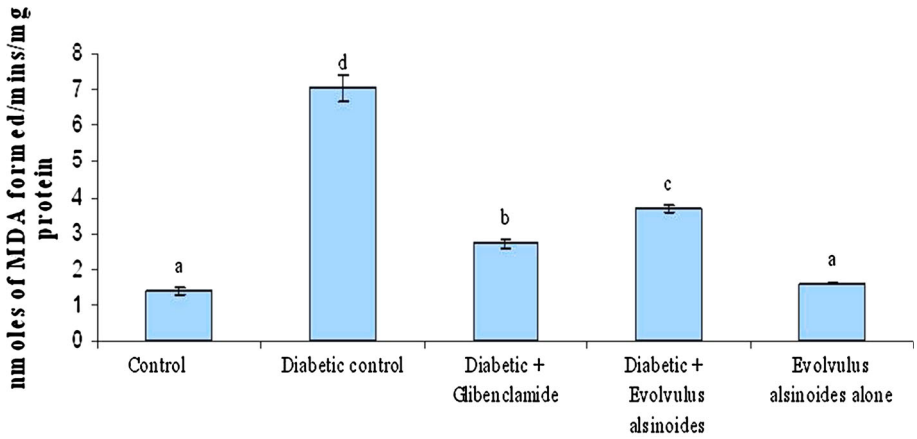

Fig. 1 Effect of *Evolvulus alsinoides* on lipid peroxidation in kidney of control and experimental rats

plant extract the antioxidant enzymes level reaches near to the normal level as that of standard. There is no significant difference found between control and plant alone group.

Units

Superoxide dismutase	Enzyme required for 50 % inhibition of NBT reduction/min/mg protein
Catalase	μmoles of H_2O_2 utilized/min/mg/protein
Glutathione peroxidase	μmoles of GSH utilized/min/mg/protein
Glutathione reductase	*n* moles of NADPH oxidized/min/mg protein
Glucose-6-phosphate dehydrogenase	min/mg protein

Oxidative stress is the imbalance between production and removal of reactive oxygen species (ROS). Increased oxidative stress contributes substantially to the pathogenesis of diabetic complications which is the consequences of

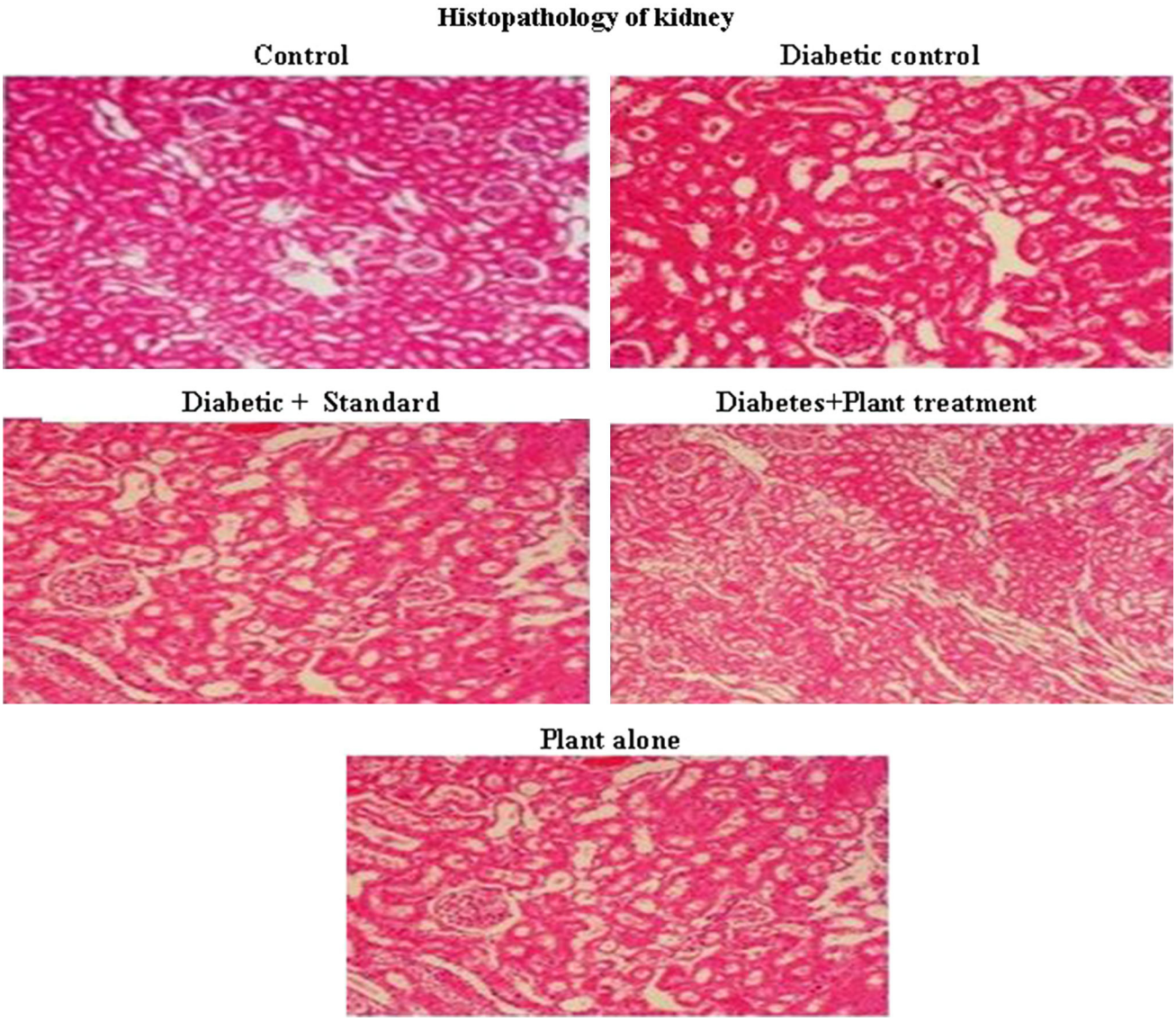

Fig. 2 Histopathology of kidney

either enhanced ROS production or attenuated ROS scavenging capacity. Several reports have shown the alterations in the antioxidant enzymes during diabetic condition [23, 24]. The antioxidative defense system like SOD and catalase showed lower activities in brain during diabetes and the results agree well with the earlier published data [25]. The decreased activities of SOD and catalase may be a response to increased production of H_2O_2 and O_2^- by the auto oxidation of excess glucose and non-enzymatic glycation of proteins e.g. glycation of SOD, catalase [26–28]. Hodgson and Fridovich [29] and Pigleot et al. 1990 [30] have reported the partial inactivation of these enzyme activities by hydroxyl radicals and hydrogen peroxide. GPx catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide and the reduction product of hydroperoxide [31]. In the present study, decline in the activities of these enzymes in STZ induced animals due to the oxidative stress elicited by STZ and attainment of normalcy in plant treated rats.

The non-enzymatic antioxidants activity in kidney of diabetes induced and treated groups were depicted in Table 2. In that all the non-enzymatic antioxidants like reduced glutathione, vitamin C and E showed a reduced level in diabetic control group and the increased level was found after the treatment with plant extract and standard drug. There is no significant difference was originated between control and plant alone group rats. Glutathione plays an important role in the endogenous non-enzymatic antioxidant system. It primarily acts as a reducing agent and detoxifies hydrogen peroxide in the presence of the enzyme glutathione peroxidase [32].Vitamin C, a potent water soluble non-enzymatic antioxidant effectively intercept oxidants in the

aqueous phase before they attack and cause detectable oxidative damage [33]. The observed decrease in the levels of vitamin C in the diabetic condition is consistent with previous reports [34]. Vitamin E is the main endogenous antioxidant which reacts with oxygen radicals and prevents free radical chain reaction to protect the membranes. Based on our findings our plant extract protects the organs from oxidative damage.

Lipid peroxidation is a characteristic of diabetes mellitus. Lipid peroxide-mediated tissue damage resulted in the development of both type I and II diabetes. In our study the increased levels of lipid peroxidation was found in diabetic control group. After treatment with plant extract the LPO level restored near to control in kidney (Fig. 1). Low levels of lipid peroxides stimulate the secretion of insulin, but when the concentration of endogenous peroxides increases, it may initiate uncontrolled lipid peroxidation, thus leading to cellular infiltration and islet cell damage in type I diabetes [35]. The most commonly used indicators of lipid peroxidation are TBARS products [36]. The increased lipid peroxidation in the tissues of diabetic animals may be due to the observed increase in the concentration of TBARS in the liver and kidney of diabetic rats [10, 37]. Our results showed that the levels of TBARS were high in kidney tissues of diabetic animals and which were restored to normal levels after treatment with *E. alsinoides* extract.

Figure 2 shows the histopathology of rat kidney. The Group II diabetic control rat shows more necrosis, when compared to Group I control (Fig. 2). But this is reversed by the administration of *E. alsinoides* in Group IV and III. Group V shows that the *E. alsinoides* did not show any toxicity to the rat kidney.

In conclusion, the results of the present study show that *E. alsinoides* brings back the antioxidant levels to normal in diabetes-induced rats and protection against tissue lipid peroxidation. In histology also the plant extract showed protective effect of the organ tissues. Future research to refine the extraction procedure of *E. alsinoides* could lead to improved pharmaceutical products.

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