Research Article

Protective Effects of *Cistanche tinctoria* Aqueous Extract on Blood Glucose and Antioxidant Defense System of Pancreatic β-cells in Experimental Diabetes in Rats

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ABSTRACT

The aim of the present study is to evaluate the possible protective effects of '*Cistanche tinctoria* ' extract on the antioxidant defense systems of pancreas in streptozotocin (STZ) induced diabetes in rats. The levels of blood glucose and TBARS in pancreas were estimated in control and experimental groups of rats. The aqueous extract (ACE) was administered daily in doses of 200mg/kg body weight to streptozotocin induced diabetic rats for a period of 21 days. Evaluate the changes in the cellular antioxidant defense system such as the level of reduced glutathione and activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione-s-transferase were assayed in pancreatic tissue homogenate. The aqueous extract exerted a significant ($P<0.000$) antidiabetic effect in streptozotocin diabetic rats. Daily treatment with 200mg/kg body weight of ACE for 21 days not only brought a significant decrease on blood glucose level in STZ-induced diabetic rats, but also increased the antioxidant enzymes' activities. From this study it can be concluded that the aqueous extract of *C.tinctoria* causes antidiabetic and antioxidant activity in Streptozotocin induced in diabetic rats.

Keywords: Ci*stanche tinctoria*, Antidiabetic effect, diabetes, oxidative stress, streptozocin.

INTRODUCTION

Free radicals are continually produced in the body as a result of normal metabolic processes and interaction with environmental stimuli complications.[1] These unstable molecules are capable of causing cellular damage, which leads to cell death and tissue injury. The ROS can bind with most normal cellular components; they react with unsaturated bonds of membrane lipids, denature proteins, and attack nucleic acids.[2] The concentrations of ROS are modulated by antioxidant enzymes and non-enzymatic scavengers, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px).[3] A disturbance of the balance between formation of active oxygen metabolites and the rate at which they are scavenged by enzymic and nonenzymic antioxidants is referred to as oxidative stress.[4]

It has been established that oxidative stress lies at the root of a number of pathological processes and diseases such as cancers, atherosclerosis, rheumatic arthritis, haematological and neurodegenerative disorders are not exempt, with more making the list among which is diabetes mellitus.

Diabetes mellitus is a heterogeneous metabolic disorder characterized by high levels of blood glucose with disturbances of carbohydrate; lipid and protein metabolism resulting from defects in insulin secretion, insulin action or both.[5]

This serious, metabolic disorder affects approximately 4% of the population worldwide and is expected to increase by 5.4% in 2025.[6]

Ihara[7], examined oxidative stress markers in experimental diabetic rats and found increased reactive oxygen species (ROS) in pancreatic islets. The pancreatic beta-cells, have required intricate mechanisms to defend against ROS toxicity. However, the reduced antioxidant capacity potentially makes pancreatic β-cells sensitive to ROS-mediated signal transduction and cellular response.

Thus, maintenance of β-cell oxidant status and their protection against oxidative damage might delay the onset of diabetes as well as the evolution of its complications.[8]

Cistanche tinctoria is a parasitic plant (Orobanchaceae) that is attached underground to the roots of the main host plants (*Tamarix gallica*, *Calligonum comosum* and *Pulicaria* sp) and grows by absorbing nutrients from the host plant. The parasite is widely distributed in North Africa, Arabia, and Asian countries.

As a rare traditional medicinal plant, the dried whole plant is used for the treatment of abdominal pains, diarrhoea, Muscle contractions, bruises, gynaecological diseases; stimulant of lactation and Diabetes. No information was found on the pharmacological of this plant, while a search on its toxicity appears negative.

The present study is conducted to systematically evaluate the antihyperglucemic effect of *C.tinctoria* aqueous extract in STZ-induced diabetic rats.

In addition, this work determines whether the pancreas was subjected to oxidative damage during experimental diabetes as well as to examine the associated changes in antioxidant status.

MATERIALS AND METHODS

Collection of Plant Material

Ariel part of *C. Tinctoria* (Desf.) Beck. (Orobanchaceae) were used in this study. Lower parts of the stem were collected in Mars, 2012, in the region of Ouregla, Algeria. The plant was identified[9-10] by the botanists in the Department of Biology (Annaba, Algeria).

Chemical Reagents

All chemicals were purchased from Sigma (USA), Aldrich (Milwaukee, USA), Fluka (Buchs, Switzerland), Tokyo Chemical Industry (TCI) and Merck (Germany).

Preparation of Extract

C.tinctoria aqueous extract (ACE) was prepared by boiling 50 g of the powder of the aerial part of the plant in a flask containing 1 L of water for 5 min. The extract was agitated and covered until it reached room temperature. The residue was removed by filtration and the extract was then suitably concentrated in a rotary evaporator (final concentration: 50 mg/mL). A sample was separated in order to determine the solid concentration, and then the extract was divided into aliquots stored at −20° C until further use. (Yield of aqueous extract was about 13.5%).

Animals and Experimental Design

Animal

Wistar rats (body weight 220 ± 20 g) used for experiments were obtained from Pasteur Institute (Algiers, Algeria). The rats were acclimatized for three week before starting the experiment. Before and during the experiment the rats were housed under controlled environmental conditions of temperature (22 ± 2°C) in a 12 h light and dark cycle, and were maintained on (unless otherwise stated) standard food pellets and tap water *ad libitum*.

Induction of Diabetes in Animals

Diabetes was induced by a single intraperitoneally (i.p.) injection of streptozotocin (STZ) in fasted rats at dose of 60 mg/kg body weight.[11] STZ was freshly dissolved in 0.1M cold sodium citrate buffer, pH 4.5. Three days after STZ injection, the glucose level of blood from the tail vein was determined, and hyperglycemic rats (blood glucose level > 200 mg/dl) were used as the diabetic rats for further experiments. Treatment with plant extract was started on the third day after STZ injection and continued for 21 days.

Experimental Design

The STZ-induced diabetic rats (mentioned above) were randomly divided into two groups (8 rats per group), and normal rats were used as the control group. Group I (n = 8): normal control (NC), normal rats were received 1ml distilled water Group II (n = 8): diabetic control (DC), the diabetic rats were received 1ml distilled water; Group III (n = 8): diabetic rats were treated with 200mg/kg/d of ACE for 21days. Weekly body weights were also recorded.

On the last day of experimentation, the animals were deprived of food overnight and sacrificed by cervical decapitation.

Tissue preparation

The Pancreatic tissue was excised, rinsed in ice-cold physiological saline and homogenized in 0.1mM phosphate buffer (pH 7.4). The homogenate was then centrifuged at 9000 ×*g* for 30 min at 4°C, and aliquots of supernatant were kept at -20°C until used for assays.

Assay of Non-enzymatic Antioxidants

Measurement of Reduced Glutathione (GSH)

Pancreas GSH content was determined by eliman method of Ellman[12] modified by Jollow[13], based on the development of yellow colour when DTNB (5, 5' dithiobis-(2-nitrobenzoic acid) is added to compounds containing sulfhydryl groups.

In brief, 0.8 ml of homogenate supernatant was added to 0.2 ml of 10% trichloroacetic acid, and then tubes were centrifuged at 3000 ×g for 5 min. Supernatant (0.5 ml) was mixed with 0.025ml of 0.01 M DTNB and 1 ml phosphate buffer (0.1 M, pH = 7.4). The absorbance at 412 nm was recorded. Finally, total GSH content was expressed as U GSH/g protein.

Measurement of Ascorbic Acid

Quantification of ascorbic acid was performed according to Jagota and Dani[14]. In brief two hundred microlitres of tissue homogenate or standard preparation of ascorbic acid was precipitated on ice with 800 µl trichloroacetic acid for 5 min. The samples were then centrifuged at 3000 rpm/min for five minutes.

Thereafter, five hundred microliters of the supernatant was subsequently diluted with double distilled water to 2 mL and mixed with two hundred microliters of Folin-Ciocalteus reagent, diluted in double distilled water (1:10), the ascorbate reduced the Folin-Ciocalteau solution yielding a blue colour. After 10 min the absorbance of the samples was measured at 760 nm in a spectrophotometer.

Estimation of Lipid Peroxidation (malondialdehyde)

Lipid peroxidation in the pancreatic tissue was estimated colorimetrically by thiobarbituric acid reactive substances TBARS method of Ohkawa[15]. A principle component of TBARS being malondialdehyde (MDA), a product of lipid peroxidation. In brief, 2.5 ml of 20% trichloroacetic acid and 1.0 ml of 0.67% TBA are added to 0.5 ml of tissue homogenate (KCl 1.15%), then the mixture is heated in a boiling water bath for 30 min. The resulting chromogen is extracted with 4.0 ml of n-butyl alcohol and the absorbance of the organic phase is determined at the wavelength of 530 nm. The MDA contents were calculated using 1, 1, 3, 3-tetraethoxypropane as standard and the results are expressed as nmol of malondialdehyde/g of tissue.

Assay of Pancreas Enzymatic Antioxidants

Assay of Catalase (CAT) Activity

Catalase (E.C.1.11.1.6) activity was measured according to Aebi[16] method. The 0.1ml of the tissue homogenate was pipette into cuvette containing 1.9 mL of 50mM phosphate buffer, pH7.0. Reaction was started by the addition of 1.0mL of freshly prepared 30%(v/v) hydrogen peroxide (H_2O_2). The rate of decomposition of $H_2O_2$was measured spectrophotometrically from changes in absorbance at 240nm for 2min. The enzyme activity was calculated by using an extinction coefficient of 0.043 mM^{-1} cm^{-1}. Activity of enzyme was expressed as units mg^{-1} protein.

Assay of Glutathion Peroxidase (GSH-Px) Activity

Glutathion peroxidase (E.C.1.11.1.9) activity was measured by the method described by Floche and Gunzler.[17] The reaction mixture contained 0.3ml of 0.1M phosphate buffer, pH 7.4, 0.1mL of 10mM sodium azide, 0.3ml of enzyme, 0.2ml 2mM glutathione and 0.1 ml of 1mM H_2O_2. The contents were incubated at 37°C for 10min, followed by the termination of the reaction by the addition of 0.5 ml TCA 5%, centrifuged at 5000 rpm for 5 min. The supernatant was collected. 0.2 ml phosphate buffer (0.1 M, pH 7.4) and 0.7 ml DTNB (10 mM) were added to 0.1 ml supernatant. After mixing, the absorbance of the product was read at 420 nm and expressed as nmol mg^{-1} protein.

Assay of Superoxide Dismutase (SOD) Activity

Pancreatic SOD (E.C.1.15.11) activity was measured by inhibition of the formazan formation according to the method (xanthine/xanthine oxidase test) of Beauchamp and Fridovich.[18] The reaction mixture contained the following solutions: 2.25 ml of 0.05 M Tris/HCl buffer, pH 8.3, including 0.15 mM Na_2EDTA, 0.2 ml nitroblue tetrazolium chloride (3 mg per 10 ml buffer), 0.1 ml xanthine oxidase solution, and 0.1 ml xanthine solution (23 mg xanthine, 0.3 ml 1 N NaOH). The reaction was started by adding an aliquote of the xanthine oxidase solution. After incubation for 1-2 min at 25° C the activity was followed for 5 min at 560 nm. One unit of SOD is defined as the amount of enzyme which inhibits the formazan formation to 50 %.

Assay of Glutathione-S-transferase (GST) Activity

Glutathione-S-transferase (GST) (EC2.5.1.18) catalyzes the conjugation reaction with glutathione in the first step of mercapturic acid synthesis. The activity of GST was measured according to the method of Habig[19].

The reaction mixture contained 0.05 ml of 1-chloro-2,4-dinitro benzene (20 mM), 0.84ml phosphate buffer (0.1M, pH 6.5), 0.01 pancreas supernatant and 0.1 ml of GSH (0.3 mg GSH/ml in 0.1 M phosphate buffer, pH 7.4) change in color was monitored by recording absorbance (340 nm) at 30 s intervals for 5 min. The enzyme activity was expressed in µmole conjugate/min/mg protein.

Statistical analysis

The data were analyzed by one way analysis of variance (ANOVA) followed by Tukey's test. All the results were expressed as mean± S.E.M. for eight rats in each group. A difference in the mean values of $p<0.05$ was considered to be statistically significant.

RESULTS

Effect of ACE on changes of body weight, pancreas weight, and blood glucose levels in diabetic rats.

Table 1 presents the effect of aqueous extract of *C.tinctoria* on changes on body weight, pancreas weight and blood glucose levels in diabetic rats. There was no significant intra-group variation in the basal body weight of the rats. However, the body weight of the animals in the NC group increased significantly from 211±4.058 g to 231±5.19 g, while the weight of the diabetic rats decreased remarkably from 215±4.34 g to 185±10.04 g. Oral administration of ACE at that dose of 200mg/kg bw significantly increased the body weight, compared to untreated diabetic rats, though there is no statistical significance. There were no significant changes in the panaceas weight of the test group compared to the diabetic control.

As illustrated in Table 1, the changes in fasting blood glucose levels of different experimental groups during the experimental period. There was a significant elevation in blood glucose level in STZ-diabetic rats compared to normal rats. The administration of ACE extract produced marked antihyperglycemic effect in diabetic rats. The fasting blood glucose decreased by 59.97% after treatment. The difference between the experimental and diabetic control rats in lowering the fasting blood glucose levels was statistically significant.

Table 1: Effect of oral administration of ACE on body weight, Pancreas weight and blood glucose in streptozotocine-induced diabetic rats on 21st day.

Parameters studied	NC	DC	DC + ACE (200mg/kg bw)
Initial body weight (g/day)	211.61 ± 4.05	215.15 ± 4.76	226.94 ± 4.34
Final body weight (g/day)	231.71 ± 5.19*	185.8 ± 10.4	231.53 ± 6.67*
Pancreas weight (g/100g bw)	0.40 ± 0.01	0.36 ± 0,06	0.48 ± 0,07
Blood glucose levels (mg/dl)	101.32 ± 4.04	442.0 ± 1,8[a]	176.9 ± 33,6[b]

Each value is mean ± SEM of eight rats in each group.

*$P < 0.01$. Initial *vs.* Final body weight

[a]$P < 0.01$.by comparison with normal rats.

[b]$P < 0.01$.by comparison with streptozotocin diabetic rats.

Evaluation of Redox Status in Pancreatic Tissue

To measure oxidative stress markers in pancreatic tissue, we evaluated oxidative damage to lipids, specifically, TBARS. A significant increase in the concentration of

TBARS was observed in the pancreas of diabetic animals compared to controls (Figure 1). Diabetic animals treated with 200 mg/kg of ACE showed a reduction in the concentration of TBARS compared to untreated diabetic animals.

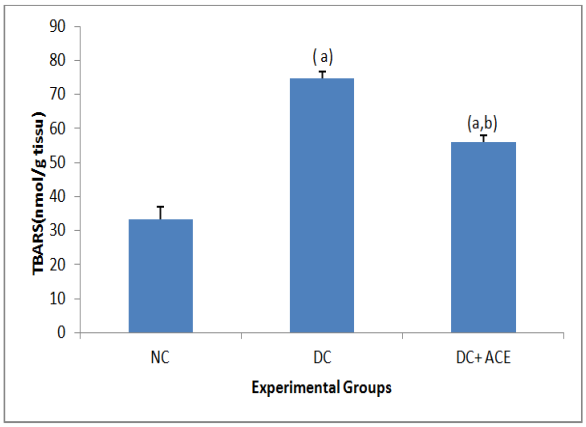

Figure 1: Effect of ACE on the level of TBARS in pancreas of experimental groups of rats. Data are presented as the mean ± SEM (n = 8). N C, control (untreated); DC, diabetic; DC+ACE, diabetic treated with 200mg/kg body mass. Statistically significant differences (p ≤0.01) are between the following groups: (a) NC and DC; (b) DC and DC+EAC.

Effect of EAC on the Levels of Non enzymatic Antioxydants

The change in the levels of nonenzymatic antioxidants such as vitamin C and GSH in pancreatic tissue of experimental and control groups of rats are represented in Table 2. Diabetic rats showed a significant (P<0.01) decrease in the levels of vitamin C and GSH compared to control rats. Treatment with *C.tinctoria* aqueous extract reversed the level of GSH to near control levels when compared to diabetic rats. However a marked a small unsignification increase in the concentration of vitamin C is observed.

Table 2: Effect of ACE on non enzymatic antioxidants (GSH, Vit C) in experimental groups of rats.

Parameters studied	NC	DC	DC+ ACE (200mg/kg bw)
GSH (U /g protein)	8.20 ± 0.86	3.72 ± 0.42[a]	7.31 ± 1.42[b]
Ascorbic acid (mg/g wet tissue)	51.59 ± 2.95	28.06 ± 4.10[a]	37.11 ± 2.70[a]

Each value is Mean ± SEM of eight rats in each group.

[a]*P* < 0.01.by comparison with normal rats.

[b]*P* < 0.05.by comparison with streptozotocin diabetic rats.

Effects of *Cistanche tinctoria* extract on STZ-induced changes in the antioxidant enzyme activities

Table 3 shows the changes in the activities of enzymatic antioxidants such as superoxide dismutase (SOD), Catalase (CAT), glutathione perioxidase (GSH-Px), and glutathione-s-transferase (GST) in pancreatic tissues of control and experimental groups of rats. Significantly decreased activities of these enzymes were observed in STZ-induced diabetic rats. The oral administration of ACE to diabetic rats showed a significant increase in the activities of SOD, CAT, GsT and GSH-Px and restored these activities to near control levels.

Table3: Antioxidant enzymes activities (SOD, CAT, GSH-Px and GsT) in the pancreas tissue of adult rats (controls and experimental groups).

Parameters studied	NC	DC	DC+ ACE (200mg/kg bw)
SOD	3.51 ± 0.15	1.95 ± 0.26[a]	3.345 ± 0.49[b]
CAT	20,24 ± 1.02	8,15 ± 0.45[a]	16,31 ±0.89[b]
GSH-Px	8.08 ± 0.88	3.60 ± 0.43[a]	6.01 ± 1.64[b]
GST	6.31 ± 0.22	3.39 ± 0.24[a]	6.14 ± 0.56[b]

Activity is expressed as: 50 % of inhibition of formazan formation /min/mg of protein for SOD; µM of hydrogen peroxide decomposed/min/ mg of protein for catalase; nM of glutathione oxidized/min/mg of protein for GSH-Px, U/min/mg of protein for GST. Values are given as mean ± SEM for groups of eight rats in each. Values are statistically significant at P<0.01. Statistical significance was compared within the groups as follows: a) comparison with normal rats. b) comparison with streptozotocin diabetic rats.

DISCUSSION

Diabetes is a metabolic defect characterized with developed hyperglycemia after the insufficiency of insulin release from the pancreas, increased oxidative stress, non-enzymatic glycolization, lipid peroxidation and changed antioxidative defence system after being exposed to free radicals.[20] The use of phytochemicals compounds on tissues which regulates glucose metabolism, is an interesting area to explore.

In the current study, STZ-induced diabetes was characterized by a severe loss in body weight, which has also been reported by other investigators.[21] The decrease in body weights compared to normal rats could be due to poor glycemic control. The excessive catabolism of protein to provide amino acids for gluconeogenesis during insulin deficiency results in muscle wasting and weight loss in diabetic untreated rats.[22] Oral administration of ACE partially improved the body weight in diabetic rats. An increase in the body weight of diabetic rats might be due to an improvement in insulin secretion and glycemic control.[23] Likewise, *C.tinctria* aqueous extract decreased polyphagia and polydipsia in treated-diabetic rats compared with diabetic group.

Hyperglycemia, as the most predominant characteristics of diabetes, is very dangerous for diabetic patients and animals.[24] In this study, it is observed a significant increase in the concentration of blood glucose in streptozotocin-induced diabetic rats. Oral administration of ACE might reverse the blood glucose in diabetic rats. Anti-hyperglycemic effect of medicinal plant extracts is

generally dependent upon the degree of β-cell destruction.[25] We suggested also that ACE extract might reverse the catabolic features of insulin deficiency by (i) stimulating peripheral glucose utilization, (ii) increasing glucose removal from blood or (iii) reducing glucose absorption from the gastrointestinal tract.

Oxidative stress in diabetes coexisted with a reduction in the antioxidant capacity, which could increase the deleterious effects of free radicals. The accumulation of free radical observed in diabetic rats is attributed to chronic hyperglycemia that alters antioxidant defense system as demonstrated by previous studies.[7-3] Lipid peroxidation of unsaturated fatty acids is frequently used as an indicator of increased oxidative stress and subsequent oxidative damage.[21] Lipid peroxidation impairs cell membrane function by decreasing membrane fluidity and causes free radical induced membrane lipid peroxidation including increased membrane rigidity, decreased cellular deformability and alters activity of membrane-bound enzymes and receptors, leading to disease.[26] The high level of the lipid peroxidation marker TBARS in diabetic rats is a reflection of insufficient antioxidant defenses in combating ROS-mediated damage. The results show that the pancreas of diabetic animals has increased oxidative damage, exemplified by the increased concentration of TBARS. Several studies also showed an increase in the concentration of TBARS in the pancreatic tissue of diabetic rats.[27-21] In the present study, the increased formation of lipid peroxidation in pancreas tissue of diabetic rats supported these findings. Oral administration of *C.tinctoria* aqueous extract to SZT diabetic rats abrogated the increased MDA levels suggesting that ACE might have a high antioxidant capacity to scavenge free radicals generated by reactive oxygen species and prevent radical damage.

Glutathione (GSH) is an important intracellular peptide with multiple functions ranging from antioxidant defense to modulation of cell proliferation.[28] It is well known that GSH is involved in the protection of normal cell structure and function by maintaining the redox homeostasis, quenching of free radicals and participating in detoxification reactions. It is a direct scavenger of free radicals as well as a co-substrate for peroxide detoxification by glutathione peroxidises.[29] Decreased levels of reduced glutathione are reported in the pancreas of the STZ-induced diabetic rats.[30] This reduction could be explained, according to previous studies,[31] by a decrease of GSH synthesis or an increase of its degradation induced by STZ oxidative stress.[30] In the present study, the elevation of GSH levels in pancreas was observed in the ACE treated diabetic rats. This indicates that the ACE can either increase the biosynthesis of GSH or reduce the oxidative stress leading to less degradation of GSH, or could have both effects.

Vitamin C or ascorbic acid is an excellent hydrophilic antioxidant in plasma and disappears faster than other antioxidants on exposure to reactive oxygen species.[32] Hypoinsulinemia and/or hyperglycemia inhibit ascorbic acid and cellular transport. As the chemical structure of ascorbic acid is similar to that of glucose, it shares the membrane transport system with glucose and hence competes with it for its transport.[30] The decreased level of ascorbic acid in diabetic rats may be due to either increased utilization as an antioxidant defense against increased reactive oxygen species or to a decrease in glutathione level, since glutathione is required for the recycling of ascorbic acid.[25]

The diabetogenic action of streptozotocin is associated with the generation of reactive oxygen species, which causes oxidative damage. This damage might play an important role in the progression and development of diabetes and its complications.[33-34] This can be prevented by the scavenging activity of enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase. Moreover, the deleterious reactive oxygen electrophiles are neutralized by the action of nonenzymatic antioxidant, reduced glutathione, which is formed by the activities of glutathione reductase and glutathione-S-transferase, respectively.[35]

Superoxide dismutase, an important intracellular antioxidative enzyme, plays a pivotal role in oxygen defense metabolism by intercepting and reducing superoxide to hydrogen peroxide.[20-26] Several studies have reported a reduced activity of SOD in experimental diabetes.[3-35-38] This diminished activity of SOD is the result of the over accumulation of superoxide anion in the cellular organelles,[39] inactivation by hydrogen peroxide[40] or by glycation of the enzyme.[41] Oral treatment of ACE caused a significant increase in SOD activities of the diabetic rats. This means that the ACE extract can reduce reactive oxygen free radicals and improve the activities of the tissue antioxidant enzymes.

Catalase, Another important free radical scavenger enzyme that breaks down hydrogen peroxide, which was produced by SOD, into water and reactive oxygen species.[20] The decrease in Catalase activity in diabetes could result from uncontrolled production of hydrogen peroxide due to the auto-oxidation of glucose, protein glycation and lipid oxidation.[35] The deficiency of this enzyme in beta cells causes increased oxidative stress and damaged beta cells.[42] In the present study, the reduction in CAT level in diabetic condition was observed. Our result is in agreement with the other results,[3-35-37-38] who reported a decrease in CAT level in diabetes mellitus. In our study, it was observed that the oral administration of ACE caused a significant increase in the activity of CAT of the diabetic rats and it may be due to the antioxidant activity of extract.

In experimental diabetes, it is observed reduced activities of the GSH-metabolizing enzymes, GSH-Px and GST. Glutathione peroxidise was a selenium-containing tetrameric glycoprotein involved in the detoxification of hydrogen peroxide into water and molecular oxygen,[3] in the presence of reduced glutathione (GSH) forming

oxidized glutathione (GSSG), and thus, it protects cell proteins and cell membranes against oxidative stress.[37] Glutathione peroxidases represent the major enzymatic defense against oxidative stress caused by hydroperoxides. They reduce hydrogen peroxide and organic hydroperoxides, such as fatty acid hydroperoxides, to the corresponding alcohols.[43] GST shows broad substrate specificity and detoxify a variety of electrophiles by conjugation to reduced GSH. GST also plays an important although indirect role in antioxidant defense, by eliminating toxic substances and preventing their subsequent deleterious effect.[44] The decrease of GSH-Px and GST may also be due to the decreased availability of its substrate, GSH, which has been shown to be depleted during diabetes.[37] In the present study, treatment of diabetic rats with aqueous extract of *C.tinctoria* induced an increase in GSH-Px and GST activity in pancreatic tissues to levels higher than those in control animals by 66.9% and 81% respectively. Moreover, the results of the present investigation are consistent with the results of sefi[3], Sivakumar and Subramanian,[35] and El-Missiry and El Gindy,[45] reporting the correlation between the increased lipid peroxides and decreased enzymatic antioxidants in experimental diabetes.

The enhanced activity of antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase and glutathione-s-transferase, can be very effective in scavenging the various types of oxygen free radicals and their products. Pancreatic β-cells may be protected from oxidative damage (induced by STZ) according to this reciprocal mechanism. Suggesting that the ACE has a direct or indirect preventive and protective effect in diabetes by decreasing oxidative stress and by preserving the integrity of pancreatic β-cell. These mechanisms may help explain why ACE has a protective effect in STZ-induced diabetic rats.

CONCLUSION

In conclusion, the present investigation shows that aqueous extract of *C. tinctoria* possess significant antidiabetic action in streptozotocin-induced diabetic rats. The present, investigation shows the favorable effect of ACE on pancreas antioxidant defense system in, addition to its antidiabetic effect.

Further detailed studies are in progress to elucidate the exact way by which they elicit their modulatory effects.

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