Aloe Vera Leaves (Aloe barbadensis Miller) Extract Attenuate Oxidative Stress of Hepatic Tissue in Streptozotocin-induced Diabetic Rats

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Abstract: Oxidative stress is suggested as a mechanism underlying diabetes mellitus complications. The main objective of this study was to investigate the effect of Aloe vera leaves (Aloe barbadensis Miller) extract on oxidative stress of hepatic tissue in streptozotocin (STZ)-induced diabetic rats. The lipid peroxidation product, malondialdehyde (MDA) and reduced glutathione (GSH) content was measured to assess free radical activity in the liver tissues. The enzymatic activities of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) were measured as indicators of antioxidation in liver tissue. Wistar rats were made diabetic with a single injection of STZ (75 mg/kg i.p.). Rats were randomly separated into four groups, of 10 animals each: Group 1, healthy control rats; Group 2 non-diabetic rats treated with 50 mg/kg b.w./day intraperitoneal injection of Aloe vera extract; Group 3, diabetic rats; Group 4, diabetic rats treated with Aloe vera extract (50 mg/kg b.w./day, i.p.) for 8 weeks. At the end of experiment, MDA contents of the liver tissue in Groups 3 was found to be significantly increased as compared with Group 1 (P<0.05) and liver MDA level in Group 4 were significantly decreased as compared with Group 3 (P<0.05). The GSH, SOD, CAT and GSH-Px contents of the liver in Group 3 were significantly decreased as compared to Groups 1 (P<0.05) and were increased in Group 4 as compared to Group 3 (P<0.05). The results obtained, demonstrated that Aloe vera extract alleviate oxidative stress of hepatic tissue in streptozotocin-induced diabetic rats.

Keywords: Aloe Vera Leaves (Aloe Barbadensis Miller) • Oxidative Stress • Diabetes Mellitus • Liver • Rat

INTRODUCTION

Diabetes mellitus is a serious metabolic disorder which is a major source of ill health all over the world and its incidence is expected to increase by 5.4% in 2025 [1]. Diabetes mellitus is characterized by hyperglycemia and is associated with disturbances in carbohydrate, protein and fat metabolism which occurs secondary to an absolute (type I) or relative (type II) lack of insulin [2]. Oxidative stress occurs when the balance between oxidant and antioxidant systems shifts in favour of the former leading to the generation of free oxygen radicals. Reactive oxygen species are involved in the pathogenesis of many diseases including hypoxia, hypercholesterolemia, atherosclerosis, hypertension, ischemia reperfusion injury and heart failure [3,4]. It has been shown that patients with diabetes mellitus have increased oxidative stress and impaired antioxidant defense systems, which appear to contribute to the initiation and progression of diabetes-associated complications [5]. There is convincing experimental and clinical evidence that the generation of reactive oxygen species is increased in both types of diabetes. Normally, the level of oxidative stress is modulated by antioxidant defense systems [6]. Diabetes-linked alterations in antioxidant defense system enzymes such as catalase, glutathione peroxidase, superoxide dismutase have been demonstrated [5]. The negative impact of diabetes on the retinal, renal, nervous and cardiovascular systems is well recognized, yet little is known about its effect on the liver [7, 8]. However, Lipid peroxidation and antioxidant status of hepatic tissue were studied by Feillet-Coudray and...
Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical disease. More attention has been paid to the protective effects of natural antioxidants against chemically induced toxicities [10]. Herbal formulations with a simultaneous antioxidant effect would thus be more useful in the management of diabetes mellitus. Their use alone or in combination with oral hypoglycemic agents or insulin may help in the better control of blood glucose level in diabetic subjects. Natural antioxidants strengthen the endogenous antioxidant defenses from reactive oxygen species (ROS) and restore and optimal balance by neutralizing the reactive species. They are gaining immense importance by virtue of their critical role in disease prevention. In this context, Aloe vera can rightly be mentioned as a plant of considerable interest.

Aloe vera (Aloe barbadensis Miller) is a perennial succulent belonging to the Liliaceal family. It is a cactus-like plant that grows in hot, dry climates [11]. It is used in Ayurveda for managing painful conditions and is also mentioned in folk medicine of Arabian Peninsula for management of diabetes [12]. Perhaps its survival in a harsh environment encourages people to believe that Aloe vera has wound-healing and antibiotic effects. It is, therefore, less than fortuitous that Aloe vera has been reported to possess immunomodulatory, antiinflammatory, UV protective, antiprotozoal and wound-and burn-healing promoting properties. The whole gel extract of Aloe vera has been reported to have various pharmacologic properties, specifically to promote wound, burn and frost-bite healing, in addition to having antiinflammatory, antifungal, hypoglycemic and gastroprotective properties [11]. Of those claims, Aloe vera’s antiinflammatory and wound healing has been the most extensively studied. The whole gel extract was found to have antiinflammatory activity on carrageenan-induced edema in rat paws [13]. Moreover, it was found to enhance wound tensile strength and antiinflammation [14]. Topically administered Aloe vera preparations inhibited inflammation in the croton oil-induced edema assay [15]. The immunomodulatory activity of Aloe vera gel has also been widely studied. The topical application of Aloe vera gel extract to the skin of UV-irradiated mice improved UV-induced immune suppression [16]. It is possible that Aloe vera activate anticancer immunity and produce therapeutic benefits in terms of stabilization of disease and survival in patients with advanced solid tumors [17].

Aloe vera gel also showed hypoglycemic activity on insulin-dependent diabetes mellitus and non-insulin-dependent diabetes mellitus rats, though it was found to be more effective in non-insulin dependent diabetes [18]. The acute and chronic effects of Aloe vera gel were studied on the plasma glucose levels of alloxan-diabetic mice and was found to reduce plasma glucose levels [19,20]. Aloe vera gel has also been found to have antifungal activity and is believed that Aloe vera likely contains antibiotic substances to help to prevent infection [21].

Considering the various beneficial effects of Aloe vera (Aloe barbadensis Miller), this study was designed to evaluate the antioxidant activity of Aloe vera leaves extract in hepatic tissue of streptozotocin-induced diabetes in rats. To our knowledge, this is the first investigation on the effect of Aloe vera leaves extract on the antioxidant status of liver tissue in experimental diabetic rats. We report on the effect of Aloe vera leaves extract on liver tissue oxidative parameters in rats with streptozotocin-induced diabetes.

MATERIALS AND METHODS

Chemicals: Streptozotocin was from Sigma (St. Louis, MO, USA). All other chemicals used were of analytical grade. All chemicals used in this study were of analytical grade and obtained from Nanjing Jiancheng Bioengineering Institute, Nanjing, China.

Plant: Aloe vera leaves were collected from a single garden plant, to obtain a fresh extract. Identification of the plant was done by Department of Cultivation and Development of Institute of Medicinal Plants, Islamic Azad University, Iran.

Preparation of the Extract: Some 300 g of the clean fresh plant leave material was ground using an electrical grinder. The extraction was carried out using 70% ethanol. The mixture was agitated over the mechanical shaker for 12 h. The resulting mixture was filtered and the filtrate concentrated into a residue over water bath [22]. The yield was 11.5% (w/w). Consequently the residue from the extract was dissolved in saline and used in the study.

Induction of Diabetes Mellitus: Diabetes was induced by intravenous injection of streptozotocin (Sigma, St. Louis, Mo, USA) into the tail vein at a dose of 65 mg/kg body
weight. STZ was extemporaneously dissolved in 0.1 M cold sodium citrate buffer, pH 4.5. After 18 h, animals with fasting blood glucose levels greater than 120-250 mg/dl were considered diabetic and then included in this study [23,24]. Fasting blood glucose was estimated by using one touch glucometer (Accu-chek sensor) of Roche Diagnostics, Germany.

**Animal Treatment:** Forty healthy male Wistar rats (about 180-200 g body weight) were purchased from Animal House, Islamic Azad University. All animals were conditioned at room temperature at a natural photoperiod for 1 week before experiment execution. A commercial balanced diet and tap water ad libitum were provided. The duration of experiment was 8 weeks. The rats were randomly divided into 4 groups (10 rats each) as the following: Group 1, healthy control rats received isotonic saline solution (ISS, 10 ml/kg) intraperitoneally; Group 2 non-diabetic rats were treated with 50 mg/kg b.w. /day intraperitoneal (i.p.) injection of Aloe vera extract; in Group 3, diabetic rats administered by ISS (10 ml/kg) was given through Intraperitoneal (i.p.) route; Group 4, diabetic rats were treated with Aloe vera leaves extract (50 mg/kg b.w. /day, i.p.) for a period of 8 weeks. The animals of different groups were sacrificed under light anesthesia (diethyl ether) 1 day after the end of the treatment.

**Measurement of Antioxidant Activity:** The rat's liver were removed immediately and washed in normal saline and homogenate 10% prepared in 1.15% w/v of potassium chloride. The homogenate was centrifuged in 7000 ×g for 10 minutes at 4°C and supernatant were used for measurement of oxidative stress by estimation of reduced glutathione (GSH) and determination of malondialdehyde (MDA) as well as antioxidant enzymes (AOE) such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). GSH, MDA, SOD, CAT and GSH-Px were measured by using commercially available kits according to the manufacturer's protocol (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Reduced glutathione (GSH) content was determined according to Sedlak and Lindsay [25]. GSH reacts with 5,5'-dithiobis-2-nitrobenzoic acid and the absorbance spectra of the product have a maximum absorbance at 410 nm. The results were expressed as imol/gwt.Liver homogenate MDA levels were expressed as nmol MDA per mg protein and antioxidant activity was expressed as arbitrary units per mg protein. Degree of lipid peroxidation in liver tissue homogenates was determined in terms of thiobarbituric acid reactive substances (TBARSs) formation by following the protocol of Esterbauer and Cheesman [26]. SOD activity was measured by Nishikimi method [27] and was modified by Kakkar method [28]. Each unit of SOD activity was determined as required enzyme concentration for prohibition of creation color at 1 minute, under study conditions. CAT activity was measured by Claiborne method [29] and was based on hydrogen peroxide breakdown. GSH-Px activity was measured by Rotruck method [30] and was expressed as micromole of GSSG /minute/milligram of protein, based on blow reaction:

\[2H_2O+GSSG→H_2O_2+2GSH\]

**Statistical Analysis:** The Statistical Package for Social Sciences (SPSS Inc. Chicago, IL, USA), version 13.0, was used for statistical analysis. All data are presented as mean ± SEM. Before statistical analysis, all variables were checked for normality and homogeneity of variance by using the Kolmogorov-Smirnoff and Levene tests, respectively. The data obtained were tested by ANOVA followed by Tukey's post-hoc multiple comparison test. \(P<0.05\) was considered statistically significant.

**RESULTS**

Results of the effect, of daily treatment of Aloe vera leaves extract at the dose of 50 mg/kg for 8 weeks on blood glucose levels of experimental rats werepresented in Figure 1. The Aloe vera leaves extract produced significant hypoglycemic effect in normal (\(P<0.05\)) and diabetic (\(P<0.01\)) rats after 8 weeks of administration.

Figures 2-6 showed the effects of Aloe vera leaves extract on antioxidative activity of liver tissue in diabetic rats. MDA contents of the liver tissue in Groups 3 was found to be significantly increased as compared to Group 1 (\(P<0.05\)) and liver MDA level in Group 4 were significantly decreased as compared to Group 3 (\(P<0.05\)). The GSH, SOD, CAT and GSH-Px contents of the liver in Group 3 were significantly decreased as compared to Groups 1 (\(P<0.05\)) and GSH, SOD, CAT and GSH-Px activity were increased in Group 4 as compared to Group 3 (\(P<0.05\)).
**Fig. 1:** Comparison of the effect of *Aloe vera* leaves extract on blood glucose levels among the experimental groups (mean±SEM). *P<0.05, compared to Group 1, compared to Group 3.*

**Fig. 2:** Comparison of the effect of *Aloe vera* leaves extract on liver GSH content among the experimental groups (mean±SEM). *P<0.05, compared to Group 1, compared to Group 3.*

**Fig. 3:** Comparison of the effect of *Aloe vera* leaves extract on liver MDA content among the experimental groups (mean±SEM). *P<0.05, compared to Group 1, compared to Group 3.*

**Fig. 4:** Comparison of the effect of *Aloe vera* leaves extract on liver SOD activity among the experimental groups (mean±SEM). *P<0.05, compared to Group 1, compared to Group 3.*

**Fig. 5:** Comparison of the effect of *Aloe vera* leaves extract on liver CAT activity among the experimental groups (mean±SEM). *P<0.05, compared to Group 1, compared to Group 3.*

**Fig. 6:** Comparison of the effect of *Aloe vera* leaves extract on liver GSH-Px activity among the experimental groups (mean±SEM). *P<0.05, compared to Group 1, compared to Group 3.*

**DISCUSSION**

Intraperitoneal injection of *Aloe vera* leaves extract significantly produced reductions of blood glucose levels in healthy normal rats. In addition, *Aloe vera* leaves extract caused significant hypoglycemic effect in diabetic rats. Such a phenomenon of hypoglycemic response with *Aloe vera* leaves extract has already reported [12].

In the present study significant decline in GSH level and antioxidant enzymes activity as well as increased lipid...
peroxidation in the liver tissue of rats reflects oxidative stress of the liver in experimental diabetes. These results are in line with the findings reported by Feillet-Coudray et al., who observed that STZ-induced diabetes in rat accompanied with an increase in the susceptibility to lipid peroxidation [9]. The data of our study also revealed that daily treatment of Aloe vera extract markedly improves antioxidant status of liver tissue of rats with streptozotocin-induced diabetes.

The role of oxidative lipid and protein damage in the pathogenesis of the diabetic state has been investigated extensively [31, 32]. Oxidative stress, leading to an increased production of ROS, as well as lipid peroxidation is increased in diabetes [33-35]. Researches on the role of lipid peroxidation in diabetic complications are hampered by the complexity of products formed [36] and limitations in the various assays for measuring the status and products of lipid peroxidation. However, TBA (thiobarbituric acid) test measuring the malonaldehyde formation is still the reliable method for assay [37].

GSH (an important part of the non-enzymatic antioxidant system) is a major non-protein thiol in living organisms, which plays a central role in co-ordinating the body’s antioxidant defense processes. Perturbation of GSH status of a biological system can lead to serious consequences. Elevation in MDA level and reduction in GSH stores of liver tissue of diabetic rats suggest that oxidative stress due to free-radical damage is one of the possible mechanisms in the pathophysiology of diabetic hepatopathy. On administration of Aloe vera extract, the MDA levels have decreased and the GSH levels have increased. This indicates that in the presence of Aloe vera extract there is an improvement in the oxidative stress. Increased oxidative stress in the tissues of streptozotocin diabetic rats was similarly reported. This was said to be a contributory factor in the development of the complications of diabetes [38,39]. Free radicals are the chemically most reactive substances in the human or animal organism [40,41]. The unbalance between formation and detoxification of free radical species results in the progression of oxidative stress [42]. Oxidative stress causes serious damage in biomolecules such as proteins [42,44-47], lipids [48,49] and nucleic acids [50,51] and leads to the development of a wide spectrum of serious diseases [52], e.g. some types of neoplasia [53, 54], inflammation processes [55], ischaemic and reperfusion states [56], acute pancreatitis [57], atherosclerosis [58], or diabetes mellitus [59] and diabetic complications [60]. The findings of Kakkar et al. study suggest that oxidative stress starts at early onset of diabetes mellitus and increases progressively [61]. Therefore, the structural damage to hepatic tissue or other complications of diabetes mellitus may be due to oxidative stress.

SOD, CAT and GPx constitute a mutually supportive team of defense against ROS. SOD is a metalloprotein and is the first enzyme involved in the antioxidant defense by lowering the steady-state level of O2. In hyperglycaemia, glucose undergoes autooxidation and produces superoxide and it produces free radicals that in turn lead to lipid peroxidation in lipoproteins. CAT is localized in the peroxisomes or the microperoxisomes, which catalyses the decomposition of H2O2 to water and oxygen and thus protects the cell from oxidative damage produced by H2O2. GPx catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide. In our study, decline in the activities of these enzymes in liver tissue of streptozotocin -induced animals and attainment of near normalcy in Aloe vera extract treated rats indicate oxidative stress elicited in hepatic tissue of diabetic rats had been nullified due to the effect of the extract. This observation perfectly agrees with those of Parihar et al. [62,63] who demonstrated hypoglycaemic and antioxidant activity of Aloe vera extract in streptozotocin induced diabetic mice.

The results of the present study demonstrated that daily treatment of diabetic rats by Aloe vera extract markedly improves antioxidant status in liver tissue. It is therefore likely that Aloe vera extract is prophylactic against diabetic complications and ameliorates diabetic hepatopathy through its antioxidant potential. Hyperglycemia is the primary symptom of diabetes and is blamed for the complications of diabetes because elevated glucose concentration directly injures cells and induces lipid peroxidation [64]. Whether this reflects oxidative stress induced liver injury or direct glycemic injury of liver remains to be determined. However, the obtained results suggested that Aloe vera extract consumption can be use as an option for the prevention of advanced liver disease known as diabetic hepatopathy.

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REFERENCES


