



Antioxidant effect of *Aloe vera* gel extract in streptozotocin-induced diabetes in rats

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Abstract:

In the present study, an attempt has been made to evaluate the presence of antioxidant property in the alcoholic extract of *Aloe vera* leaf gel. Oral administration of *Aloe vera* gel extract at a concentration of 300 mg/kg to diabetic rats significantly decreased the levels of blood glucose, glycosylated hemoglobin and increased hemoglobin. The increased levels of lipid peroxidation and hydroperoxides in tissues of diabetic rats were reverted back to near normal levels after the treatment with gel extract. The extract treatment also resulted in a significant increase in reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase in the liver and kidney of diabetic rats. These results clearly show the antioxidant property of *Aloe vera* gel extract. The extract was also more effective than glibenclamide in restoring the values of these parameters.

Key words:

Aloe vera, alcoholic extract, streptozotocin-induced diabetes, enzymatic antioxidants

Introduction

In diabetes mellitus, chronic hyperglycemia produces multiple biochemical sequelae, and diabetes-induced oxidative stress could play a role in the symptoms and progression of the disease [10]. Oxidative stress in cells and tissues results from the increased generation of reactive oxygen species and/or from decreases in antioxidant defense potential [12]. Several hypotheses have been put forth to explain the genesis of free radicals in diabetes. These include autoxidation processes of glucose, the non-enzymatic and progressive glycation of proteins with the consequently increased formation of glucose-derived advanced glycosylation end products (AGEs), and enhanced glucose flux through the polyol pathway [35, 49]. Elevated genera-

tion of free radicals resulting in the consumption of antioxidant defense components may lead to disruption of cellular functions and oxidative damage to membranes and may enhance susceptibility to lipid peroxidation [4]. Under physiological conditions, a widespread antioxidant defense system protects the body against the adverse effects of free radical production [15]. The antioxidant defense system represents a complex network with interactions, synergy and specific tasks for a given antioxidant [38]. The efficiency of this defense mechanism is altered in diabetes and, therefore, the ineffective scavenging of free radicals may play a crucial role in determining tissue damage [52].

Sulfonylureas such as glibenclamide stimulate insulin secretion from the existing pancreatic β -cells and are widely used to treat type 1 diabetes. Gliben-

clamide principally acts by inhibiting ATP-sensitive K^+ (K_{ATP}) channels in the plasma membrane [3]. The inhibition of ATP sensitive channels leads to membrane depolarization, activation of voltage-gated Ca^{2+} channels, increased Ca^{2+} influx, a rise in cytosolic (Ca^{2+}) and thereby insulin release. Glibenclamide is often used as a standard drug in streptozotocin (STZ)-induced moderate diabetic model to compare the antidiabetic properties of variety of compounds [18]. Though sulfonylureas are valuable in treatment of diabetes, their use is restricted by their limited action, pharmacokinetic properties, secondary failure rates and accompanying side effects [21]. Recent decades have shown a resurgent interest in traditional plant treatments for diabetes. Plants often contain substantial amounts of antioxidants including α -tocopherol (vitamin E), carotenoids, ascorbic acid (vitamin C), flavonoids and tannins [25], and it has been suggested that antioxidant action may be an important property of plant medicines used in diabetes. *Aloe vera* is a perennial plant belonging to the family of Liliaceae, which includes about 360 species [22]. Taxonomists now refer to *Aloe barbadensis* as *Aloe vera* [8]. Aloe is the one of the few medicinal plants that has maintained its popularity for a long period of time. The plant has stiff gray-green lance-shaped leaves containing clear gel in a central mucilaginous pulp. Clinical evaluations have revealed that the pharmacologically active ingredients are concentrated in both the gel and rind of *Aloe vera* leaves. Our previous experimental results were highly encouraging as they revealed that level of blood glucose was significantly lower after oral administration of ethanolic extract of *Aloe vera* gel in glucose load condition and in STZ-induced diabetes [39].

The present investigation was carried out to study the effect of *Aloe vera* leaf gel extract on tissue lipid peroxides and enzymatic antioxidants in rats with STZ-induced diabetes.

Materials and Methods

Preparation of *Aloe vera* gel extract

Aloe vera powder was prepared from *Aloe vera* leaf gel according to the published procedure [11] with slight modifications. Mature, healthy and fresh leaves

of *Aloe vera* having a length of approximately 75 to 90 cm were washed with fresh water. The leaves were cut transversely into pieces. The thick epidermis was selectively removed. The solid gel in the center of the leaf was homogenized. The resulting mucilaginous, thick and straw colored homogenate was lyophilized. Then the lyophilized sample was extracted using 95% ethanol. The filtrate was collected and evaporated to dryness under reduced pressure in a rotary evaporator. The residue was stored in dry sterilized small containers at 4°C until further use. An aqueous suspension which is the form customarily used in folk medicine was prepared by dissolving suitable amount of ethanol free extract of *Aloe vera* leaf gel to get the desired concentration. The drug solutions were prepared freshly each time and administered intragastrically. The dosing schedule used was once per day.

Animals used

Male albino rats of Wistar strain weighing about 160–200 g were obtained from Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai and were selected as the animal model. The animals were maintained on standard rat feed supplied by Hindustan Lever Ltd., Bangalore, India and water *ad libitum*. The experiments were conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (Approval no. 360/01/A/CPCSEA). Animals described as fasted had been deprived of food for 16 h but had been allowed free access to water.

Induction of experimental diabetes

After fasting, diabetes was induced by intraperitoneal (*ip*) injection of STZ (Sigma, St. Louis, Mo., USA) dissolved in 0.1 M cold sodium citrate buffer, pH 4.5, at a dose of 55 mg/kg [44]. The control rats received the vehicle alone. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. After a week time for the development of diabetes, the rats with moderate diabetes having glycosuria and hyperglycemia (blood glucose range of above 250 mg/dl) were considered as diabetic rats and used for the experiment.

Experimental procedure

The rats were divided into four groups of ten animals in each group as follows.

- Group I Normal control rats
- Group II STZ-induced diabetic control rats
- Group III Diabetic rats given *Aloe vera* leaf gel extract (300 mg/kg) in aqueous solution daily using an intragastric tube for 21 days
- Group IV Diabetic rats given glibenclamide (600 µg/kg) in aqueous solution daily using an intragastric tube for 21 days

After 21 days of the treatment, the fasted rats were sacrificed by cervical decapitation. Blood was collected in heparinized tubes and used for the estimation of hemoglobin and glycosylated hemoglobin. The liver and kidney were dissected out, washed in ice-cold saline, patted dry and weighed.

Determination of blood glucose, hemoglobin and glycosylated hemoglobin

Fasting blood glucose was estimated by o-toluidine method [42]. Hemoglobin was estimated by the method of Drabkin and Austin [9]. Glycosylated hemoglobin was assayed according to the method of Nayak and Pattabiraman [33].

Determination of thiobarbituric acid reactive substances (TBARS), hydroperoxides and reduced glutathione (GSH)

TBARS were estimated by the method of Ohkawa et al. [36]. Hydroperoxides were determined by the method of Jiang et al. [20], glutathione was estimated by the method of Sedlak and Lindsay [43].

Assay of antioxidant enzymes and protein

The activity of superoxide dismutase (SOD) was assayed by the method of Misra and Fridovich [32]. Catalase (CAT) was assayed by the method of Takahara et al. [48]. The activities of glutathione peroxidase (GPx) and glutathione-S-transferase (GST) were assayed according to the method of Rotruck et al. [41], and Habig et al. [14], respectively. Protein content in tissue homogenate was measured by the method of Lowry et al. [27].

Statistical analysis

All the grouped data were statistically evaluated with SPSS/7.5 software. Hypothesis testing methods included one-way analysis of variance (ANOVA) followed by least significant difference (LSD) test; p values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as the mean \pm SD for six animals in each group.

Results

Table 1 shows the level of blood glucose, total hemoglobin and glycosylated hemoglobin in normal and experimental rats. There was a significant elevation in blood glucose and glycosylated hemoglobin, while the level of total hemoglobin decreased during diabetes when compared with corresponding control group. Administration of *Aloe vera* and glibenclamide tended to bring the values to near normal and the effect was more pronounced in the group of rats treated with *Aloe vera*.

Tab. 1. Levels of blood glucose, hemoglobin and glycosylated hemoglobin in control and experimental groups of rats

Groups	Blood glucose (mg/dl)	Hemoglobin (g/dl)	Glycosylated hemoglobin (% Hb)
Normal control	85.10 \pm 2.92	13.8 \pm 0.85	5.8 \pm 0.7
Diabetic control	274.17 \pm 12.42*	10.34 \pm 0.67*	12.1 \pm 1.2*
Diabetic + <i>Aloe vera</i>	94.18 \pm 4.76*	12.58 \pm 1.01*	6.5 \pm 0.9*
Diabetic + glibenclamide	115.47 \pm 11.36*	12.25 \pm 1.07*	6.9 \pm 0.8*

Values are given as the mean \pm SD for groups of six animals each. Values are statistically significant at * p < 0.05. Diabetic control was compared with normal control rats. Diabetic + *Aloe vera* and diabetic + glibenclamide were compared with diabetic control

Table 2 shows the concentration of TBARS and hydroperoxides in the liver and kidney of normal and experimental rats. There was a significant elevation in tissue TBARS and hydroperoxides during diabetes compared to corresponding control group. Administration of *Aloe vera* gel extract and glibenclamide

significantly decreased the level of TBARS and hydroperoxides in the liver and kidney of diabetic group of rats and the effect was more pronounced in the group of rats treated with *Aloe vera*.

Tab. 2. Levels of lipid peroxides and hydroperoxides in liver and kidney of control and experimental groups of rats

Groups	TBARS (mM/100 g of tissue)		Hydroperoxides (mM/100 g of tissue)	
	Liver	Kidney	Liver	Kidney
Normal control	0.85 ± 0.03	1.29 ± 0.05	68.03 ± 0.93	55.37 ± 0.89
Diabetic control	1.77 ± 0.08*	2.21 ± 0.07*	94.55 ± 1.11*	74.67 ± 1.06*
<i>Diabetic + Aloe vera</i>	0.96 ± 0.07*	1.40 ± 0.02*	77.15 ± 2.00*	61.77 ± 0.90*
Diabetic + glibenclamide	1.47 ± 0.31*	1.82 ± 0.08*	84.40 ± 0.82*	68.88 ± 0.94*

Values are given as the mean ± SD for groups of six animals each. Values are statistically significant at * p < 0.05. Diabetic control was compared with normal control rats. Diabetic + *Aloe vera* and diabetic + glibenclamide were compared with diabetic control

Table 3 shows the content of GSH in the liver and kidney of normal and experimental rats. There was a significant decrease in the concentration of GSH in tissues during diabetes compared to the corresponding control group. Treatment with *Aloe vera* gel extract and glibenclamide increased the content of GSH in the liver and kidney of diabetic group of rats and the effect was more pronounced in the group of rats treated with *Aloe vera*.

Tab. 3. Level of reduced glutathione in liver and kidney of control and experimental groups of rats

Groups	GSH (mg/100 g of tissue)	
	Liver	Kidney
Normal control	49.57 ± 1.44	34.79 ± 1.99
Diabetic control	25.57 ± 1.49*	21.67 ± 1.27*
<i>Diabetic + Aloe vera</i>	43.77 ± 3.85*	31.40 ± 1.57*
Diabetic + glibenclamide	39.65 ± 1.84*	27.57 ± 1.20*

Values are given as the mean ± SD for groups of six animals each. Values are statistically significant at * p < 0.05. Diabetic control was compared with normal control rats. Diabetic + *Aloe vera* and diabetic + glibenclamide were compared with diabetic control

Tab. 4. Activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase in liver of control and experimental groups of rats

Groups	SOD	CAT	GPx	GST
Normal control	9.20 ± 0.42	83.33 ± 2.93	9.43 ± 0.43	7.12 ± 0.43
Diabetic control	3.70 ± 0.19*	42.20 ± 2.75*	5.44 ± 0.28*	3.25 ± 0.19*
<i>Diabetic + Aloe vera</i>	6.43 ± 0.42*	71.30 ± 3.60*	8.52 ± 0.43*	6.05 ± 0.19*
Diabetic + glibenclamide	5.08 ± 0.29*	66.20 ± 2.13*	7.08 ± 0.29*	5.52 ± 0.29*

Values are given as the mean ± SD for groups of six animals each. Values are statistically significant at * p < 0.05. Diabetic control was compared with normal control rats. Diabetic + *Aloe vera* and diabetic + glibenclamide were compared with diabetic control. Activity is expressed as 50% of inhibition of epinephrine autoxidation per min for SOD; μmoles of hydrogen peroxide decomposed per min per mg of protein for CAT; μmoles of glutathione oxidized per min per mg of protein for GPx; units per min per mg of protein for GST

Tab. 5. Activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase in kidney of control and experimental groups of rats

Groups	SOD	CAT	GPx	GST
Normal control	14.32 ± 0.43	43.28 ± 2.66	7.37 ± 0.09	6.03 ± 0.43
Diabetic control	8.23 ± 0.29*	26.20 ± 1.89*	4.50 ± 0.28*	2.42 ± 0.15*
<i>Diabetic + Aloe vera</i>	12.17 ± 0.57*	33.63 ± 2.92*	6.83 ± 0.43*	5.30 ± 0.28*
Diabetic + glibenclamide	10.70 ± 0.54*	31.58 ± 2.13*	6.23 ± 0.15*	4.75 ± 0.46*

Values are given as the mean ± SD for groups of six animals each. Values are statistically significant at * p < 0.05. Diabetic control rats were compared with normal control rats. Diabetic + *Aloe vera* and diabetic + glibenclamide were compared with diabetic control. Activity is expressed as 50% of inhibition of epinephrine autoxidation per min for SOD; μmoles of hydrogen peroxide decomposed per min per mg of protein for CAT; μmoles of glutathione oxidized per min per mg of protein for GPx; units per min per mg of protein for GST

Tables 4 and 5 show the activities of SOD, CAT, GPx and GST in the liver and kidney of normal and experimental rats. During diabetes there was a significant reduction in the activities of SOD, CAT, GPx and GST in tissues, such as liver and kidney. Treatment with *Aloe vera* gel extract and glibenclamide increased the activity of SOD, CAT, GPx and GST in diabetic group of rats and the effect was more pronounced in the group of rats treated with *Aloe vera*.

Discussion

STZ-induced experimental diabetes is a valuable model for induction of type I diabetes. Further, the STZ diabetic animals may exhibit most of the diabetic complications, namely, myocardial cardiovascular, gastrointestinal, nervous, vas deferens, kidney, and urinary bladder dysfunctions through oxidative stress [37]. During diabetes, the excess glucose present in the blood reacts with hemoglobin to form glycosylated hemoglobin. It has been reported that various proteins, including hemoglobin, albumin, collagen, LDL, or crystalline proteins undergo non-enzymatic glycation in diabetes [23]. The rate of glycation is proportional to the concentration of blood glucose [45]. Glycosylated hemoglobin has been found to be increased over a long period of time in diabetes [6]. There is an evidence that glycation itself may induce the formation of oxygen-derived free radicals in diabetic condition [13]. Therefore, the measurement of glycosylated hemoglobin is supposed to be a very sensitive index for glycemic control [19]. In the present study, the diabetic rats had shown higher levels of glycosylated hemoglobin compared to those in normal rats indicating their poor glycemic control. Treatment with *Aloe vera* showed a significant decrease in the glycosylated hemoglobin levels in the diabetic rats that could be due to an improvement in glycemic status. This suggests amelioration of oxidative stress due to hyperglycemia by the treatment with extract.

Lipid peroxidation is one of the characteristic features of chronic diabetes. The increased free radicals produced may react with polyunsaturated fatty acids in cell membranes leading to lipid peroxidation. Lipid peroxidation will in turn result in the elevated production of free radicals [26]. Lipid peroxide-mediated damage has been observed in the development of type I and type II diabetes mellitus. Insulin secretion is also closely associated with lipoxygenase-derived peroxides [51]. Low levels of lipoxygenase peroxides stimulate the secretion of insulin, but when the concentration of endogenous peroxides increases, it may initiate uncontrolled lipid peroxidation leading to cellular infiltration and islet cell damage in type I diabetes [31]. The most commonly used indicator of lipid peroxidation are TBARS [29]. The increased lipid peroxidation in the tissues of diabetic animals may be due to the observed remarkable increase in the concentration of TBARS and hydroperoxides in the liver

and kidney of diabetic rats [47]. Cooperative defense systems that protect the body from free radical damage include the antioxidant nutrients and enzymes. Oxidative stress in diabetes coexists with a reduction in the antioxidant capacity, which can increase the deleterious effects of free radicals. Glutathione is a tripeptide normally present at high concentrations intracellularly, and constitutes the major reducing capacity of the cytoplasm [28]. Glutathione is known to protect the cellular system against toxic effects of lipid peroxidation [34]. Decreased level of GSH in the liver and kidney during diabetes represents its increased utilization due to oxidative stress [2]. In the present study, a significant elevation of GSH level was observed in the extract-treated diabetic rats. This indicates that the extract can either increase the biosynthesis of GSH or reduce the oxidative stress leading to less degradation of GSH, or have both effects.

SOD scavenges the superoxide radical by converting it to H_2O_2 and molecular oxygen [30]. The activity of SOD was found to be lower in diabetic control rats. The observed decrease in SOD activity could result from inactivation by H_2O_2 or by glycation of the enzyme, which have been reported to occur in diabetes [46]. CAT is a heme protein, which catalyzes the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals [7]. This decrease in CAT activity could result from inactivation by glycation of the enzyme [53]. Further, an increase in the SOD activity may protect CAT against enzyme inactivation by superoxide radical as these radicals have been shown to inactivate CAT [24]. Thus, the increase in SOD activity may indirectly play an important protective role in preserving the activity of CAT. The reduced activities of SOD and CAT in the liver and kidney have been observed during diabetes and this may result in a number of deleterious effects due to the accumulation of superoxide radicals and hydrogen peroxides.

GPx, an enzyme with selenium, and GST work together with glutathione in the decomposition of H_2O_2 (or) other organic hydroperoxides to non-toxic products at the expense of the GSH [5]. Reduced activities of GPx may result from radical-induced inactivation and glycation of the enzyme [16]. Further, insufficient availability of GSH may also reduce the activity of GPx [17]. The decreased activity of GST observed in diabetic state may be due to the inactivation caused by reactive oxygen species [1]. Reduced activities of GPx and GST in the liver and kidney have been observed during diabetes and this may result in a number

of deleterious effects due to the accumulation of toxic products. In this context, other researchers also reported a decrease in the activity of these antioxidant enzymes (SOD, CAT, GPx and GST) in the liver and kidney of diabetic rats [2, 47]. In diabetic rats treated with the ethanolic extract, a significant increase in activity of these enzymes was observed. This might reflect the antioxidant potency of the ethanolic extract, which by reducing blood glucose levels prevented glycation and inactivation of enzymes. Similar kinds of effect, i.e. prevention of potential glycation of antioxidant enzymes and the ensuing decrease in activity, have been reported with other plants, such as *Gongronema latifolium* and *Eugenia jambolana* well known for their antidiabetic activity [50, 40].

In conclusion, the effect of the ethanolic extract of *Aloe vera* gel on tissue antioxidants is due to reduction in blood glucose level in diabetic rats, which prevents excessive formation of free radicals through various biochemical pathways and also reduces the potential glycation of the enzymes.

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