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Modulatory effects of *Aloe vera* leaf gel extract on oxidative stress in rats treated with streptozotocin

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Abstract

Oxidative stress is currently suggested as a mechanism underlying diabetes and diabetic-related complications. Oxidative stress results from an imbalance between radical-generating and radical-scavenging systems. Many secondary plant metabolites have been reported to possess antioxidant activity. This study was designed to evaluate the potential antioxidative activity of the ethanolic extract from *Aloe vera* leaf gel in the plasma and pancreas of streptozotocin (STZ)-induced diabetic rats. Glibenclamide was used as a standard reference drug. Oral administration of ethanolic extract at a concentration of 300 mg kg^{-1} body weight for 21 days resulted in a significant reduction in fasting blood glucose, thiobarbituric acid reactive substances, hydroperoxides and alpha-tocopherol and significant improvement in ascorbic acid, reduced glutathione and insulin in the plasma of diabetic rats. Similarly, the treatment also resulted in a significant reduction in thiobarbituric acid reactive substances, hydroperoxides, superoxide dismutase, catalase and glutathione peroxidase and significant improvement in reduced glutathione in the pancreas of STZ-induced diabetic rats when compared with untreated diabetic rats. The ethanolic extract appeared to be more effective than glibenclamide in controlling oxidative stress. Thus, this study confirms the ethnopharmacological use of *Aloe vera* in ameliorating the oxidative stress found in diabetes.

Introduction

Free radicals are continually produced in the body as a result of normal metabolic processes and interaction with environmental stimuli. They are considered to be of great importance as the cause of many disorders, and of diabetes in particular. Mammalian cells are equipped with both enzymic and non-enzymic antioxidant defences to minimize the cellular damage caused by interaction between cellular constituents and oxygen free radicals (Halliwell & Gutterridge 1994). Impairment of the oxidant–antioxidant equilibrium in favour of the former provokes a situation of oxidative stress and generally results from hyperproduction of reactive oxygen species (Durackova 1999). In diabetes, glucose autoxidation and protein glycation may generate increased free radicals, which in turn catalyse lipid peroxidation (Wolff & Dean 1987). In addition to the increased generation of free radicals in diabetes, impaired generation of naturally occurring antioxidants also result in increased oxidative stress (Giugliano et al 1996). Lipid peroxidation of cellular structures, a consequence of free radical activity, seems to play a vital role in aging, atherosclerosis and late complications in diabetes (Kesavulu et al 2001).

Attention has been focused on possible interventions to decrease levels of oxidative stress, such as improved glycaemic control, and drug or antioxidant therapy (Sharma et al 2000). Hence, hypoglycaemic and antioxidative properties would be useful in antidiabetic agents. 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, and it has been suggested that an antioxidant action may be an important property of plant medicines associated with diabetes (Larson 1988). Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes (Pushparaj et al 2000) but only a few have received scientific scrutiny.

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Aloe vera has been used in herbal medicine in many cultures. Aloes are members of the Liliaceae family and are mostly succulent with a whorl of elongated pointed leaves (Klein & Penneys 1988). Taxonomists now refer to *Aloe barbadensis* as *Aloe vera* (Coats & Ahola 1979). The central bulk of the leaf contains the colourless mucilaginous pulp, made up of large thin-walled mesophyll cells containing the *Aloe vera* gel itself. Despite its wide use as a folk remedy over a long period of time, the biochemical details of its action on physiological/pathophysiological functions have not been systematically worked out. Our previous experimental results revealed the glucose lowering effect of *Aloe vera* gel extract in streptozotocin (STZ)-induced diabetic rats and its lack of effect on blood glucose in normoglycaemic rats (Rajasekaran et al 2004).

This study aimed to evaluate the antioxidant potency of *Aloe vera* gel extract in the plasma and pancreas of streptozotocin-induced diabetic rats. The results were compared with glibenclamide, a known hypoglycaemic drug.

Materials and Methods

Preparation of *Aloe vera* gel extract

Aloe vera powder was prepared from *Aloe vera* leaf gel according to the published procedure (Grieve 1975) with slight modifications. Mature, healthy and fresh leaves of *Aloe vera* having a length of approximately 2.5–3 feet were removed and washed with fresh water. The thick epidermis was selectively removed. The inner colourless mucilaginous pulp was homogenized and centrifuged to remove the fibres. The resultant homogenate was immediately lyophilized. The lyophilized sample was subjected to different solvent extractions using n-hexane, chloroform, petroleum ether and 95% ethanol. The filtrate was collected and evaporated to dryness under reduced pressure in a rotary evaporator. The phytochemical analysis of individual solvent-free extracts was qualitatively carried out by the method of Harborne (1973). Phytochemical screening revealed the presence of relatively more active ingredients in the ethanolic extract. A known amount of ethanol-free extract was suspended in sterilized water freshly each time and administered intragastrically. The dosing schedule used was once per day.

Animals

Male albino Wistar rats, about 160–200 g, were used in this study. The rats were housed in individual cages in an air-conditioned room (25 ± 2°C) and acclimatized for a period of 7 days. The rats were maintained on standard chow diet and were allowed free access to water. The experiments were designed and conducted according to ethical norms approved by the Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (Approval no. 360/01/A/CPCSEA).

Induction of experimental diabetes

The rats were fasted for 16 h before induction of diabetes by intraperitoneal injection of 55 mg kg^{-1} (based on bodyweight) of streptozotocin (STZ) (Sigma, St Louis, MO) freshly dissolved in 0.1 M cold sodium citrate buffer, pH 4.5 (Chattopadhyay et al 1997). Control rats received an equivalent amount of buffer intraperitoneally. The rats were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycaemia. Hyperglycaemia was confirmed one week after induction via blood glucose level measurements after a 16-h fast. Rats with a fasting blood glucose level greater than 250 mg dL^{-1} were considered as diabetic and included in this study.

Experimental procedure

The rats were divided into four groups (10 rats 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^{-1}) in aqueous solution daily using an intragastric tube for 21 days; Group IV, diabetic rats given glibenclamide (600 μg kg^{-1}) in aqueous solution daily using an intragastric tube for 21 days.

After 21 days of experimental period, the 16-h fasted rats were sacrificed by cervical decapitation. Blood was collected in tubes containing potassium oxalate and sodium fluoride. Plasma was separated and used for the estimation of glucose (Sasaki et al 1972), vitamin C (Omaye et al 1979) and vitamin E (Desai 1984), and insulin assay was carried out by using radio immunoassay (Linco research Inc., USA). The pancreatic tissue was excised, rinsed in ice-cold physiological saline and homogenized in 0.1 M Tris-HCl buffer (pH 7.4) using a tissue homogenizer with a Teflon pestle at 4°C.

The tissue homogenate and plasma were used for the following estimations: TBARS (thiobarbituric acid reactive substances) was estimated by the method of Okhawa et al (1979); hydroperoxides were assayed by the method of Jiang et al (1992); reduced glutathione was estimated by the method of Ellman (1959).

The antioxidant enzymes in the tissue homogenate were assayed: superoxide dismutase (SOD) was estimated using the method of Misra & Fridovich (1972); catalase (CAT) was assayed by the method of Takahara et al (1960); glutathione peroxidase (GPx) was assayed by the method of Rotruck et al (1973). Total protein present in the tissue homogenate was estimated by the method of Lowry et al (1951).

Statistical analysis

All the grouped data were statistically evaluated with SPSS/7.5 software. Hypothesis testing methods included one-way analysis of variance followed by least significant difference (LSD) test. $P < 0.05$ was considered to indicate statistical significance. All the results were expressed as mean $\pm$ s.d. for six rats in each group.

Results

The preliminary phytochemical screening of the ethanolic extract of *Aloe vera* revealed the presence of phenols, alkaloids, flavonoids, sterols, triterpenoids, saponins, carotenoids, glycosides and anthroquinones.

The diabetic rats showed a significant increase in blood glucose level and a significant decrease in plasma insulin level compared with control rats (Table 1). Upon oral administration of *Aloe vera* gel extract and glibenclamide, the levels were found to be similar to those of normal rats with the effect being more pronounced in the group of rats treated with *Aloe vera*.

A marked increase in the levels of plasma TBARS and hydroperoxides was observed in diabetic rats when compared with control rats (Table 2). Treatment with *Aloe vera* gel extract and glibenclamide reversed these levels to near normal values and the effect was more pronounced in the group of rats treated with *Aloe vera*.

The level of reduced glutathione in the plasma and pancreas of diabetic rats was significantly lower than in control rats (Table 3). Upon oral administration of *Aloe vera* gel extract and glibenclamide, the level was found to be similar to that in normal rats and the effect was more pronounced in the group of rats treated with *Aloe vera*.

Table 1 Levels of blood glucose and plasma insulin in control and experimental groups of rats

Group	Blood glucose ($mg\,dL^{-1}$)	Plasma insulin ($\mu U\,mL^{-1}$)
Normal control	85.81 ± 5.20	15.86 ± 1.38
Diabetic control	332.27 ± 20.80^{a}	5.12 ± 0.68^{a}
Diabetic + *Aloe vera*	96.80 ± 5.30^{b}	14.12 ± 1.48^{b}
Diabetic + glibenclamide	$118.46 \pm 6.56^{c,d}$	12.52 ± 0.69^{c}

Values are given as mean ± s.d. for groups of 6 rats each. Values are statistically significant at $P < 0.05$: [a]diabetic control vs normal control; [b]diabetic + *Aloe vera* vs diabetic control; [c]diabetic + glibenclamide vs diabetic control; [d]diabetic + glibenclamide vs diabetic + *Aloe vera*.

Table 2 Levels of thiobarbituric acid reactive substances (TBARS) and hydroperoxides in plasma of control and experimental groups of rats

Group	TBARS ($nM\,mL^{-1}$)	Hydroperoxides ($10^{-5}\,mM\,dL^{-1}$)
Normal control	2.80 ± 0.11	8.82 ± 0.16
Diabetic control	6.90 ± 0.15^{a}	13.68 ± 0.17^{a}
Diabetic + *Aloe vera*	3.15 ± 0.12^{b}	10.56 ± 0.15^{b}
Diabetic + glibenclamide	$3.99 \pm 0.14^{c,d}$	$11.87 \pm 0.56^{c,d}$

Values are given as mean ± s.d. for groups of 6 rats each. Values are statistically significant at $P < 0.05$: [a]diabetic control vs normal control; [b]diabetic + *Aloe vera* vs diabetic control; [c]diabetic + glibenclamide vs diabetic control; [d]diabetic + glibenclamide vs diabetic + *Aloe vera*.

Table 3 Levels of reduced glutathione (GSH) in plasma and pancreas of control and experimental groups of rats

Group	Plasma GSH ($mg\,dL^{-1}$)	Pancreas GSH (mM/100 g wet tissue)
Normal control	24.66 ± 0.55	21.76 ± 2.14
Diabetic control	15.34 ± 0.53^{a}	15.16 ± 1.45^{a}
Diabetic + *Aloe vera*	22.65 ± 0.45^{b}	19.86 ± 2.12^{b}
Diabetic + glibenclamide	$21.22 \pm 0.19^{c,d}$	18.15 ± 2.18^{c}

Values are given as mean ± s.d. for groups of 6 rats each. Values are statistically significant at $P < 0.05$: [a]diabetic control vs normal control; [b]diabetic + *Aloe vera* vs diabetic control; [c]diabetic + glibenclamide vs diabetic control; [d]diabetic + glibenclamide vs diabetic + *Aloe vera*.

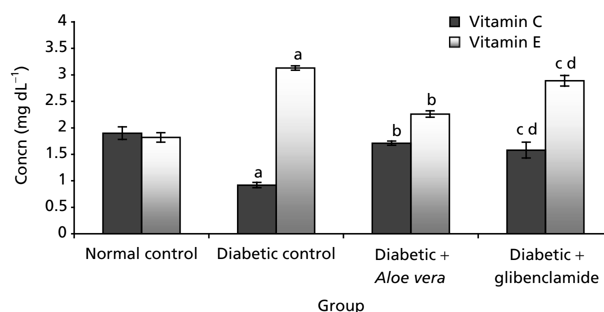

Figure 1 Levels of vitamin C and vitamin E in the plasma of control and experimental groups of rats. Values are given as mean ± s.d. for groups of 6 rats each. Values are statistically significant at $P < 0.05$: [a]diabetic control vs normal control; [b]diabetic + *Aloe vera* vs diabetic control; [c]diabetic + glibenclamide vs diabetic control; [d]diabetic + glibenclamide vs diabetic + *Aloe vera*.

The changes in the levels of ascorbic acid and α-tocopherol in the plasma of all the experimental groups and control rats are shown in Figure 1; the level of α-tocopherol increased markedly while the vitamin C level decreased significantly in diabetic control rats. Treatment with *Aloe vera* gel extract and glibenclamide reversed these levels to near normal values and the effect was more pronounced in the group of rats treated with *Aloe vera*.

The changes in the levels of TBARS and hydroperoxides in the pancreas of all the experimental groups and control rats are shown in Figure 2; a marked increase in the levels of pancreatic TBARS and hydroperoxides were observed in diabetic rats when compared with control rats. Upon oral administration of *Aloe vera* gel extract and glibenclamide, the levels were found to be similar to those in normal rats and the effect was more pronounced in the group of rats treated with *Aloe vera*.

The change in the activity of pancreatic superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in all the experimental and control rats is summarized in Table 4. A marked increase in the activity of these

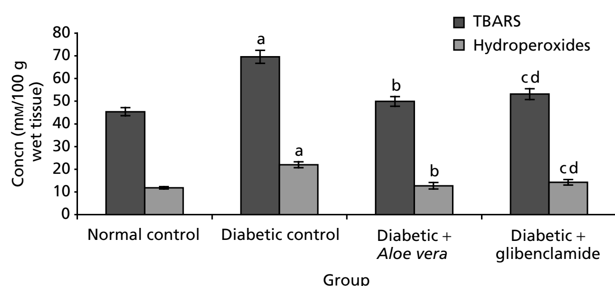

Figure 2 Levels of thiobarbituric acid reactive substances (TBARS) and hydroperoxides in the pancreas of control and experimental groups of rats. Values are given as mean ± s.d. for groups of 6 rats each. Values are statistically significant at $P < 0.05$: [a]diabetic control vs normal control; [b]diabetic + *Aloe vera* vs diabetic control; [c]diabetic + glibenclamide vs diabetic control; [d]diabetic + glibenclamide vs diabetic + *Aloe vera*.

Table 4 Activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) in the pancreas of control and experimental groups of rats

Groups	SOD	CAT	GPx
Normal control	3.22 ± 0.52	15.24 ± 1.30	5.80 ± 0.57
Diabetic control	5.18 ± 0.58[a]	26.81 ± 2.18[a]	7.90 ± 0.63[a]
Diabetic + *Aloe vera*	3.81 ± 0.57[b]	17.30 ± 1.20[b]	6.32 ± 0.44[b]
Diabetic + glibenclamide	4.01 ± 0.57[c]	18.60 ± 1.98[c]	6.90 ± 0.62[c]

Activity is expressed as: 50% of inhibition of adrenaline auto-oxidation per min for SOD; μmol of hydrogen peroxide decomposed per min per mg of protein for catalase; μmol of glutathione oxidized per min per mg of protein for GPx. Values are given as mean ± s.d. for groups of 6 rats each. Values are statistically significant at $P < 0.05$: [a]diabetic control vs normal control; [b]diabetic + *Aloe vera* vs diabetic control; [c]diabetic + glibenclamide vs diabetic control; [d]diabetic + glibenclamide vs diabetic + *Aloe vera*.

enzymes was observed in the pancreas of diabetic rats when compared with the pancreas of control rats. Treatment with *Aloe vera* gel extract and glibenclamide reversed these levels to near normal values and the effect was more pronounced in the group of rats treated with *Aloe vera*.

Discussion

Free-radical-induced lipid peroxidation has been associated with a number of disease processes including diabetes. The increase in oxygen free radicals in diabetes could be due to an increase in blood glucose levels. This study was therefore undertaken to assess the antiperoxidative properties of *Aloe vera* gel extract. STZ is a commonly employed compound for induction of type-1 diabetes (Tomlinson et al 1992) and causes diabetes by rapid depletion of β-cells, which leads to reduction in insulin release. There is evidence that STZ-induced diabetes releases free radicals. Further, STZ diabetic animals may exhibit most diabetic complications, namely, myocardial, cardiovascular, gastrointestinal, nervous, vas deferens, kidney and urinary bladder dysfunction mediated through oxidative stress (Ozturk et al 1996). Glibenclamide is often used as a standard drug in STZ-induced moderate diabetic models to compare the antidiabetic properties of a variety of compounds and its effectiveness in insulin stimulation is also reported (Andrade-Cetto et al 2000). The hypoglycaemic effect of plant extracts is generally dependent upon the degree of β-cell destruction (Grover et al 2000). Treatment of moderate STZ-diabetic rats with medicinal plant extract resulted in activation of β-cells and granulation returned to normal, showing an insulinogenic effect (Kedar & Chakrabarti 1982). The anti-hyperglycaemic activity of *Aloe vera* was associated with an increase in plasma insulin level, suggesting an insulinogenic activity of the gel extract. The increased levels of insulin in this study indicate that *Aloe vera* gel extract stimulates insulin secretion from the remnant β-cells or from regenerated β-cells. In this context, a number of other plants have also been reported to have an antihyperglycaemic effect and a stimulatory effect on insulin release (Pari & Latha 2002).

Lipid peroxidation is one of the characteristic features of chronic diabetes. Oxidative stress induces the production of highly reactive oxygen species that are toxic to the cell, particularly the cell membrane in which these radicals interact with the lipid bilayer and produce lipid peroxides. The lipid peroxidation of the cell membrane has been associated with a number of pathological phenomena, such as increased membrane rigidity, decreased cellular deformability and lipid fluidity in erythrocytes. The most commonly used indicator of lipid peroxidation is TBARS. Tremendous increase in TBARS and hydroperoxides in the plasma and pancreas of diabetic rats suggests an increase in oxygen radicals that could be due to either their increased production or decreased destruction (Griesmacher et al 1995; Matkovics et al 1998).

Glutathione, a tripeptide normally present in millimolar concentrations in all cells, is known to protect the cellular system against the toxic effects of lipid peroxidation (Lu 1999). A decreased level of reduced glutathione is reported in the plasma and pancreas of the STZ-induced diabetic condition (Garg et al 1996; Krishnakumar et al 1999). Decreased levels of reduced glutathione in diabetes have been considered to be an indication of increased oxidative stress (Matkovics et al 1998). Administration of *Aloe vera* gel extract and glibenclamide increased the content of reduced glutathione in the plasma and pancreas of diabetic rats by decreasing oxidative stress.

Ascorbate has received much attention as a reducing agent since its discovery and it has been recognized as an outstanding plasma antioxidant (Frei et al 1989). Therefore, disturbances in ascorbic acid metabolism might be important in the pathogenesis of diabetic complications. We observed lowered levels of plasma vitamin C in STZ-induced diabetic rats. An elevation in glucose concentration may depress natural antioxidants like vitamin C (Inouye et al 1999) or this may be due to a decrease in GSH level, since GSH is required for recycling of vitamin C (Chatterjee & Nandi 1999). Vitamin E, the

principal lipid-soluble antioxidant in cell membranes, protects critical cellular structures against oxidative damage (Takenaka et al 1999). Elevated levels of vitamin E have been found in plasma and this may be due to increased intake of vitamin E per unit weight or increase in serum lipid levels in diabetic rats, or both (Aoki et al 1992).

SOD scavenges the superoxide radical by converting it to H_2O_2 and molecular oxygen. CAT and GPx are considered biologically essential in the reduction of hydrogen peroxide. Increased activity of SOD, CAT and GPx was observed in diabetic rats. An increase in the activity of these enzymes may result from radical-induced activation (Gonzales et al 1984). These results are in agreement with earlier data (Kakkar et al 1995). The significant increase in activity of antioxidant enzymes in the diabetic pancreas indicates an adaptive mechanism in response to oxidative stress. However, increased activity of these enzymes observed in diabetic rats was significantly regulated by *Aloe vera* therapy, indicating modulation over oxidative stress.

Conclusion

It may be concluded that diabetes induces disturbances in the determined oxidative parameters that could be responsible for several diabetic complications. The increased oxidative stress in diabetic rats was successfully reduced by *Aloe vera* leaf gel extract. Phenolic antioxidants have been found that function as free radical terminators or metal chelators. Lee et al (2000) reported the presence of a phenolic antioxidant, characterized as 8-C-β-D-[2-O-(E)-coumaroyl]glucopyranosyl[2-[2-hydroxy]propyl-7-methoxy-5-methylchromone, in the alcoholic extract of *Aloe vera* leaf gel. It has been reported that agents with the ability to inhibit lipid peroxidation (e.g. plant phenols) also possess a hypoglycaemic effect (Johnson et al 1993). Preliminary phytochemical investigations in our laboratory also revealed the presence of phenolic compounds in the gel extract. Hence, the observed antioxidant property of *Aloe vera* may be due to the presence of phenolic compounds, which could explain its antidiabetic effect. Further characterization of active components in *Aloe vera*, such as phenolics or related analogues, are warranted and studies are in progress to isolate, identify and characterize such active components.

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