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# Effect of *Aloe vera* leaf gel extract on membrane bound phosphatases and lysosomal hydrolases in rats with streptozotocin diabetes

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Diabetes mellitus is known to promote deterioration of membrane function and impair intra cellular metabolism in the organism. The aim of the present study was to examine the effect of the ethanolic extract from *Aloe vera* leaf gel on membrane bound phosphatases and lysosomal hydrolases in the liver and kidney of streptozotocin (STZ)-induced diabetic rats. The rats treated with STZ showed significant alterations in the activities of membrane bound phosphatases and lysosomal hydrolases in the liver and kidney. Oral administration of *Aloe vera* gel extract at a dose of 300 mg/kg body weight/day to STZ-induced diabetic rats for a period of 21 days significantly restored the alterations in enzymes activity to near normalcy. These results were compared with glibenclamide, a reference drug. Thus, the present study confirms that *Aloe vera* gel extract possesses a significant beneficial effect on membrane bound phosphatases and lysosomal hydrolases.

## 1. Introduction

Diabetes mellitus is recognized by chronic elevation of the glucose in the blood, which results from a deficiency in β-cells of the endocrine pancreas and/or from a subsentivity to insulin in target cells (Jensen et al. 1988). Many evidence have indicated that some biochemical pathways strictly associated with hyperglycemia (non enzymatic glycosylation, glucose autoxidation, polyol pathways) can increase the production of free radicals (Baynes 1991). Thus, the oxygen-derived species that formed during diabetes mediate the cell membrane damage forming lipid hydroperoxides and ultimately leading to the loss of functional integrity of the membrane and cellular metabolism resulting in diseased conditions (Kesavulu et al. 2001). Therefore, crude drugs or natural diet food which possess both hypoglycemic and free radical scavenging activity has become a central focus for research designed to prevent or ameliorate tissue injury and may have a significant role in maintaining health (McCune and Johns 2002). Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes, but only a few have received scientific scrutiny.

*Aloe vera* (L.) has been used in herbal medicine of many cultures. Aloes are members of the Liliaceae family and are mostly succulent with whorl of elongated pointed leaves. Taxonomists now refer to *Aloe barbadensis* as *Aloe vera* (Klein and Penneys 1988). The central bulk of the leaf contains the colorless mucilaginous pulp, made up of large thin-walled mesophyll cells containing the *Aloe vera* gel itself. Though the therapeutic potential of *Aloe vera* has been identified, the biochemical details of its action on physiological/pathophysiological functions have not been systematically worked out.

Our previous experimental results revealed the glucose and lipid lowering properties of *Aloe vera* gel extract in streptozotocin (STZ)-induced diabetic rats (Rajsekaran et al. 2004, 2005a, 2006).

The present study was aimed to evaluate the effect of *Aloe vera* leaf gel extract on the activities of membrane bound phosphatases and lysosomal hydrolases during streptozotocin (STZ)-induced diabetic rats. The results were compared with glibenclamide, a known hypoglycemic drug.

## 2. Investigations and results

### *2.1. Effect of Aloe vera extract on blood glucose level, liver and kidney weight*

The levels of blood glucose, liver and kidney weight of control and experimental groups of rats are shown in Table 1. STZ-induced diabetic rats showed a significant increase in blood glucose level, kidney weight and a significant decrease in liver weight when compared with cor-

**Table 1: Effect of *Aloe vera* extract on blood glucose level, liver and kidney weight in control and experimental groups of rats**

| Groups | Blood glucose (mg/dl) | Liver weight (g) | Kidney weight (g) |
|---|---|---|---|
| Control | $83.75 \pm 5.19$ | $4.19 \pm 0.24$ | $0.95 \pm 0.06$ |
| Diabetic Control | $312.45 \pm 23.74^a$ | $2.72 \pm 0.14^a$ | $1.39 \pm 0.09^a$ |
| Diabetic + *Aloe vera* | $90.18 \pm 5.60^b$ | $4.10 \pm 0.23^b$ | $1.01 \pm 0.06^b$ |
| Diabetic + glibenclamide | $119.24 \pm 7.75^b$ | $3.84 \pm 0.22^b$ | $1.11 \pm 0.07^b$ |

Values are given as mean ± SD for groups of six animals in each group
[a] $p < 0.05$ when compared with control rats
[b] $p < 0.05$ when compared with diabetic control rats

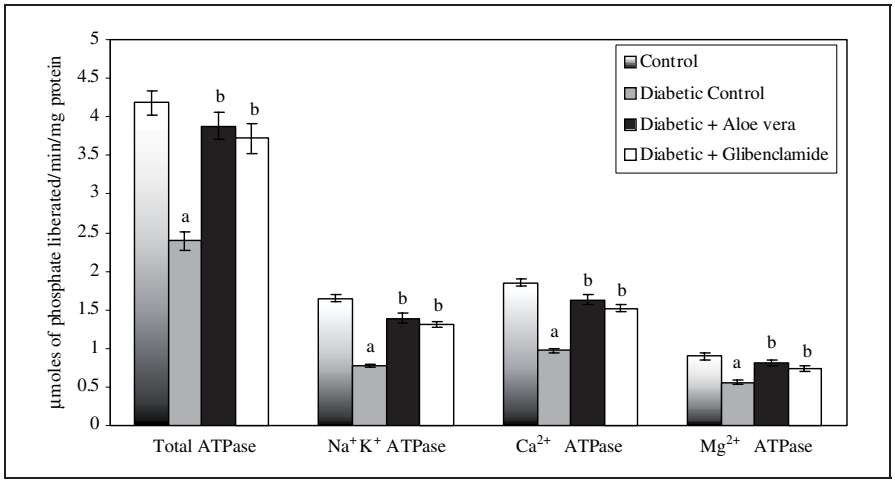


Fig. 1:
Effect of *Aloe vera* extract on membrane bound phosphatases in the liver of control and experimental groups of rats
Values are given as mean ± SD for groups of six animals in each group
[a] $p < 0.05$ when compared with control rats.
[b] $p < 0.05$ when compared with diabetic control rats

**Table 2: Effect of *Aloe vera* extract on membrane bound phosphatases in the kidney of control and experimental groups of rats**

| Parameters | Control | Diabetic control | Diabetic + *Aloe vera* | Diabetic + glibenclamide |
|---|---|---|---|---|
| Total ATPase | 6.33 ± 0.26 | 2.94 ± 0.13[a] | 5.88 ± 0.26[b] | 5.32 ± 0.23[b] |
| $Na^+$-$K^+$ ATPase | 2.61 ± 0.13 | 1.08 ± 0.05[a] | 2.18 ± 0.09[b] | 2.01 ± 0.08[b] |
| $Ca^{2+}$ ATPase | 1.75 ± 0.07 | 0.83 ± 0.03[a] | 1.64 ± 0.08[b] | 1.53 ± 0.09[b] |
| $Mg^{2+}$ ATPase | 2.58 ± 0.11 | 1.29 ± 0.05[a] | 2.31 ± 0.08[b] | 2.14 ± 0.09[b] |

Values are given as mean ± SD for groups of six animals in each group
[a] $p < 0.05$ when compared with control rats
[b] $p < 0.05$ when compared with diabetic control rats
Activity was expressed as: µ mole of phosphate liberated/min/mg protein

responding control rats. Following oral administration of *Aloe vera* extract and glibenclamide, blood glucose level, liver and kidney weight was found to be similar to those in control rats.

### *2.2. Effect of Aloe vera extract on liver and kidney membrane bound phosphatases*

The activity of membrane bound phosphatases in the liver and kidney of control and experimental groups of rats is summarized in Fig. 1 and Table 2, respectively. There was a marked decrease in the activity of membrane bound phosphatases such as Total ATPase, $Na^+$-$K^+$ ATPase, $Ca^{2+}$ ATPase and $Mg^{2+}$ ATPase in the liver and kidney of STZ-induced diabetic rats compared with activities in corresponding control rats. *Aloe vera* extract and glibenclamide administration resulted in the normalization of these enzymes activity towards near normalcy.

### *2.3. Effect of Aloe vera extract on hepatic lysosomal hydrolases*

The activity of hepatic lysosomal hydrolases in control and experimental groups of rats is shown in Table 3. A significant elevation in the activity of hepatic lysosomal enzymes such as β-D-glucuronidase, β-D-*N*-acetyl glucosaminidase, β-D-galactosidase, cathepsin-D and acid phosphatase in STZ-induced diabetic rats was observed when compared with corresponding control rats. Administration of *Aloe vera* extract and glibenclamide significantly reduced the activity of these enzymes in the liver of diabetic rats.

### *2.4. Effect of Aloe vera extract on renal lysosomal hydrolases*

The activity of renal lysosomal hydrolases in control and experimental groups of rats is shown in Fig. 2. A significant increase in the activity of β-D-glucuronidase, β-D-*N*-acetyl glucosaminidase, acid phosphatase and cathepsin-D in the kidney of STZ-induced diabetic rats was found. In contrast, there was a significant decrease in the activity of β-D-galactosidase was observed in the kidney of diabetic rats. However, following treatment with either *Aloe vera* extract or glibenclamide, the activity of these enzymes was brought back to near normal.

## 3. Discussion

Diabetes is characterized by a severe derangement of intracellular metabolism and functional alterations of cell

**Table 3: Effect of *Aloe vera* extract on lysosomal hydrolases in the liver of control and experimental groups of rats**

| Parameters | Control | Diabetic control | Diabetic + *Aloe vera* | Diabetic + glibenclamide |
|---|---|---|---|---|
| β-D-Glucuronidase | 20.09 ± 0.92 | 35.41 ± 1.94[a] | 22.80 ± 1.08[b] | 24.18 ± 1.19[b] |
| β-D-*N*-Acetyl glucosaminidase | 44.92 ± 2.83 | 62.18 ± 4.48[a] | 47.17 ± 3.16[b] | 49.81 ± 3.34[b] |
| β-D-Galactosidase | 38.28 ± 2.29 | 49.15 ± 3.34[a] | 40.12 ± 2.68[b] | 41.26 ± 2.26[b] |
| Cathepsin-D | 20.84 ± 1.29 | 31.79 ± 2.38[a] | 21.95 ± 1.47[b] | 22.75 ± 1.57[b] |
| Acid phosphatase | 110.81 ± 8.31 | 160.53 ± 14.12[a] | 121.76 ± 9.50[b] | 132.05 ± 10.34[b] |

Values are given as mean ± SD for groups of six animals in each group
[a] $p < 0.05$ when compared with control rats
[b] $p < 0.05$ when compared with diabetic control rats
Activity was expressed as: µmole of p-nitrophenol liberated/h/mg protein for β-D glucuronidase, β-D-*N*-acetyl glucosaminidase and β-D-galactosidase; µmole of tyrosine released/h/mg protein for cathepsin-D; µmole of phenol liberated/h/100 mg protein for acid phosphatase

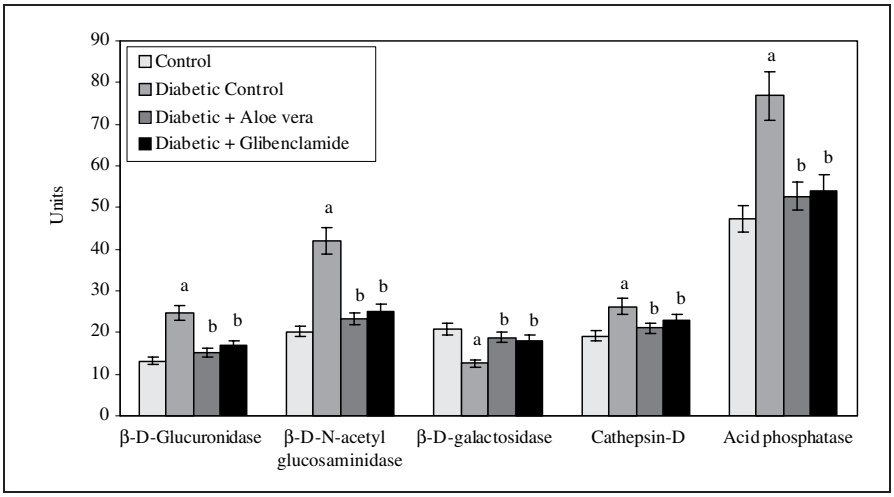


Fig. 2:
Effect of *Aloe vera* extract on lysosomal hydrolases in the kidney of control and experimental groups of rats
Values are given as mean ± SD for groups of six animals in each group
[a] $p < 0.05$ when compared with control rats.
[b] $p < 0.05$ when compared with diabetic control rats
Activity was expressed as: μmole of p-nitrophenol liberated/h/mg protein for β-D-glucuronidase, β-D-*N*-acetyl glucosaminidase and β-D-galactsidase; μmole of tyrosine released/h/mg protein for cathepsin-D; μmole of phenol liberated/h/100 mg protein for acid phosphatase

membranes (Parinandi et al. 1990). This study was therefore undertaken to assess the effect of *Aloe vera* gel extract on membrane bound phosphatases and lysosomal hydrolases. The kidney weight increased markedly while the liver weight decreased significantly in diabetic rats. The observed decrease in liver weight may be due to enhanced catabolic processes such as glycogenolysis and proteolysis, which is the outcome of lack of insulin and/or cellular glucose in liver cells (Gupta et al. 1999). There is however, an increase in kidney weight observed is due to thickening of basement membrane. These changes may lead to serious microvascular changes in the pathogenesis of diabetic nephropathy (Meyer et al. 1998). Treatment with *Aloe vera* extract significantly restored the alterations in liver and kidney weight of diabetic rats and this could be resulted from antihyperglycemic effect of the extract (Rajasekaran et al. 2004).

Membrane bound phosphatases are ubiquitous enzymes essential for the maintenance of electrolyte balance and fundamentally involved in the maintenance of ion gradients that drive the co-transport of amino acids and sugars, regulate cell volume and contribute to part of the membrane potential (Sweeney and Klip 1998). Alterations of these transport enzymes are thought to be linked to several complications of diabetes mellitus, for example hypertension (Shahid and Mahboob 2003). $Na^+$-$K^+$ ATPase, a membrane-linked enzyme that catalyzes the hydrolysis of ATP and couples it to the transport of $Na^+$ and $K^+$ across the cell membrane thereby generating the transmembranous $Na^+/K^+$ gradient. This pump is essential for the regulation of cell volume, uptake of nutrients, regulation of neurotransmitters release and contractibility and excitability properties of nerve tissue (Hernandez 1992). Alterations in $Na^+$-$K^+$ ATPase enzyme have been documented in diabetic tissues (Ng et al. 1993). $Na^+$-$K^+$ ATPase is rich in thiol groups, oxidation of thiol groups by reactive oxygen species (ROS) is reported to inhibit enzyme activity in diabetic condition (Thomas and Reed 1990). The other potential explanation for the loss of enzyme activity as a consequence of STZ-induced diabetes may be linked to the lack of insulin, which is a potential stimulator of $Na^+$-$K^+$ ATPase (Gupta et al. 1996). Reduced membrane $Ca^{2+}$ ATPase activity may be responsible for increase in intracellular calcium and consequently, for elevated vascular resistance which is frequently associated with hypertension (Zemel et al. 1990). The decrease in $Ca^{2+}$ ATPase activity in the diabetic state may be due to altered membrane properties including functional and compositional changes (Winegrad 1987). Glycosylation is reported to cause changes in physical and biochemical properties of proteins and the rate of glycosylation depends on the proportion of sugar present in carbonyl form (Means and Chang 1982). It is reported that glycosylation of membrane proteins significantly inhibits $Ca^{2+}$ ATPase activity (Ramanadevi et al. 1997). The activity of $Ca^{2+}$ ATPase is also modulated by cellular thiol status (degree to thiol oxidation and lipid peroxidation) (Bironaite and Ollinger 1997). $Mg^{2+}$ ATPase activity is involved in other energy requiring process in the cell membrane and its acitivity is sensitive to membrane lipid peroxidation. In general, lipid peroxidation and glycosylation of membrane proteins could influence the functions of different ATPases in diabetic condition. Reversal of different ATPases activity upon *Aloe vera* extract treatment to diabetic rats reflects the restoration of the functional integrity of membranes and it may be resulted from free radical scavenging effects of *Aloe vera* gel extract (Rajasekaran et al. 2005b, 2005c), which may preserve the thiol moiety of the ATPases from free radicals attack. In addition to its antioxidant potency, the hypoglycemic effect of the gel extract also plays a major role in preventing potential glycation and inactivation of the enzymes.

Lysosomes are a distinct group of cytoplasmic organelles, known to occur in numerous animal tissues and characterized by their content of a variety of hydrolytic enzymes. Degradation of connective tissue constituents such as collagen and glycoproteins are brought about by enzymes primarily by lysosomal origin. Lysosomes may undergo activation with release of their enzymes, which from latent become free, in catabolic conditions, to degrade cell components (autophagia), or when a macromolecular compound degradable by lysosomal enzymes accumulated in tissues (Arun and Vijayammal 2000). Our studies with liver tissue of STZ-induced diabetic rats indicate the occurrence of a trend toward an increase in the activity of lysosomal enzymes, including β-D-galactosidase. This is in agreement with previous report (Witek et al. 2001). An increase in activity of the investigated lysosomal enzymes in the course of model STZ diabetes probably suggests the vast adaptation processes during hyperglycemia, beginning with labilisation of the membranes of the lysosomal system through an increase in their permeability for particular substrates and intensive degradation processes (Witek et al. 2001). A significant increase in the activities of β-D-glucuronidase, β-D-*N*-acetyl glucosaminidase, acid phosphatase, cathepsin-D and a concomitant decrease in

the activity of β-D-galactosidase was observed in the kidney of STZ-induced diabetic rats. This is in agreement with those reported earlier (Belfiore et al. 1986; Nerurkar et al. 1988). Osicka et al. (2001) suggested that diabetes induced changes in renal lysosomal processing is one of the initial events in the development of diabetic nephropathy. β-D-*N*-acetyl glucosaminidase has been regarded as a possible marker of renal damage (Walter et al. 1992). It has been suggested that the β-D-*N*-acetyl glucosaminidase reflects lysosomal dysfunction of both glomerular and proximal tubular epithelial cells, which may be the cause for poor glycemic control, its reflect brush border damage of proximal tubules which may be caused by diabetic nephropathy (Fushimi and Tarui 1974). Decrease in renal β-D-galactosidase has been reported in chemically induced diabetic rats (Goi et al. 1986). This may be due to increased formation of hydroxyl lysine-linked α-glucosyl-β-galactose disaccharide unit in renal glomerular basement membrane (Beisswenger and Spiro 1970), which leads to thickening of basement membrane and the development of microangiopathy. The altered activity of lysosomal enzymes in diabetic liver and kidney were normalized by insulin treatment by the virtue of its normalization of glucose metabolism (Gorog and Pearson 1985). Thus, the beneficial effect of *Aloe vera* extract on lysosomal enzymes is due to its hypoglycemic nature.

In conclusion, diabetes induces disturbances in the activities of membrane bound phosphatases and lysosomal hydrolases.

This study demonstrates that in the STZ-induced diabetic model, *Aloe vera* extract administration ameliorated the changes in the activities of membrane bound phosphatases and lysosomal hydrolases. The mechanism by which *Aloe vera* extract improves these alterations in diabetic rats is probably by its hypoglycemic and free radical scavenging properties. Preliminary phytochemical investigations in our laboratory revealed the presence of phenols, sterols, triterpenoids, carotenoids, anthroquinones and glycosides as biologically active constituents in the gel extract (Rajasekaran et al. 2005b). Hence it may be concluded that the antidiabetic and antioxidative action of *Aloe vera* gel extract may be due to the presence of these active principles. There is an ongoing research to isolate and characterize the bioactive compound(s) responsible for the antidiabetic/antioxidative action in these crude extract, and to use the (se) compound(s) in a bioassay-directed experiment.

## 4. Experimental

### *4.1. Animals*

Male albino rats of Wistar strain weighing about 160–200 g were used in this study. They were acclimatized to the laboratory conditions at least for one week before carrying out any experimental work. The rats were fed *ad libitum* with normal laboratory pellet diet and water. The experiments were designed and conducted according to the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines (IAEC No. 01/034/04).

### *4.2. Plant material*

*Aloe vera* (L.) plants were maintained and collected from our university campus. The taxonomic identification of the *Aloe vera* plant was confirmed by a senior plant taxonomist, Prof. V. Kaviyarasan, Ph.D., at the Center for Advanced Studies in Botany, University of Madras and a voucher specimen (No. 1070) was deposited in the herbarium.

### *4.3. Preparation of plant extract*

*Aloe vera* powder was prepared from *Aloe vera* leaf gel according to a published procedure with slight modifications (Grieve 1975). Mature, healthy and fresh leaves of *Aloe vera* having a length of approximately 75 to 90 cm were removed and washed with fresh water. The thick epidermis was selectively removed. The inner colorless mucilaginous pulp was homogenized and centrifuged to remove the fibers. The resultant supernatant was immediately lyophilized. The lyophilized sample was extracted with 95% ethanol. The filtrate was collected and evaporated to dryness under reduced pressure in a rotary evaporator. Known amount of solvent free extract was suspended in sterilized water freshly each time and administered intragastrically. The dosing schedule used was once per day.

### *4.4. Induction of experimental diabetes*

The rats were fasted for 16 h prior to induction of diabetes by intraperitoneal (i.p.) injection (55 mg/kg body weight) of streptozotocin (STZ) (Sigma, St. Louis, MO, USA) freshly dissolved in 0.1 M cold sodium citrate buffer, pH 4.5 (Rakieten et al. 1963). Control rats received equivalent amounts of buffer intraperitoneally. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. Hyperglycemia was confirmed one week after induction via blood glucose level measurements after a 16 h fast. Animals with a fasting blood glucose level greater than 250 mg/dl were considered as diabetic and included in this study.

### *4.5. Experimental design*

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

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

### *4.6. Analytical methods*

On completion of 21 days of experimental period, the 16 h fasted rats were sacrificed by cervical decapitation. The blood sample was collected with potassium oxalate and sodium fluoride solution for the estimation of glucose by the O-toluidine method (Sasaki et al. 1972). Immediately after sacrifice, the liver and kidney were dissected out and immediately washed in ice-cold saline. Blotted with a filter paper, weighed and a portion of the tissues were homogenized in Tris-HCl buffer, pH 7.4 (0.1 M) with a Teflon homogenizer. The homogenate was used for the assays of total ATPase by the method of Hokins et al. (1973), which was modified from the method of Evans (1969), $Na^+$-$K^+$ ATPase by the method of Bonting (1970), $Ca^{2+}$ ATPase by the method of Hjerten and Pan (1983) and $Mg^{2+}$ ATPase by the method of Ohnishi et al. (1982).

Lysosomal fraction was isolated by the method of Wattiaux (1977). The activity of lysosomal enzymes was assayed by the following methods. β-D-Glucuronidase activity was measured according to Hultberg et al. (1976). β-D-*N*-acetyl glucosaminidase activity was measured using the method of Moore and Morris (1982), β-D-galactosidase by the method of Conchie et al. (1967). The activity of cathepsin-D and acid phosphatase was assayed according to Sapolsky et al. (1973), and King (1965), respectively. Protein content in the tissue homogenate was measured by the method of Lowry et al. (1951).

### *4.7. 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 Mean ± S.D. for six animals in each group.

### References

Arun OS, Vijayammal PL (2000) Effect of 2-deoxy-D-glucose on metabolism of glycosaminoglycons in normal and hypercholesterolemic rats. Indian J Exp Biol 38: 31–35.

Baynes JW (1991) Role of oxidative stress in development of complications in diabetes. Diabetes 40: 405–412.

Beisswenger PG, Spiro RG (1970) Human glomerular basement membrane: chemical alteration in diabetes mellitus. Science 168: 596-598.

Belfiore F, Rabuazzo AM, Iannello R, Campione, Vasta D (1986) Anabolic response of some tissues to diabetes. Biochem Med Meta Biol 35: 149–155.

Bironaite D, Ollinger K (1997) The hepatotoxicity of rhein involves impairment of mitochondrial functions. Chem Biol Inter 103: 35–50.

Bonting SL (1970) Biobehavioural base of coronary heart disease. In: Dembroski TM, Schmidt TH, Blumchen G (eds.) Membrane and ion transport, Vol. 1., London, p. 257.

Conchie J, Gelman AL, Levry GA (1967) Inhibition of glycosidases by aldonolactones of corresponding configuration. The C-4 and C-6 specifi-

city of beta-glucosidase and beta-galactosidase. Biochem J 103: 609–615.
Evans DJ (1969) Membrane adenosine triphosphatase of Escherichia coli: Activation by calcium ion and inhibition by monovalent cations. J Bacteriol 100: 914–916.
Fushimi H, Tarui S (1974) Kidney and serum beta-N-acetylglucosaminidase activities in streptozotocin diabetic rats and their responses to insulin and glucagon. J Biochem (Tokyo) 76: 225–227.
Goi G, Fabi A, Lorenzi R, Lombardo A, Tettamanti G, Burlina AB, Pinelli L, Gaburro D (1986) Serum enzymes of lysosomal origin as indicators of the metabolic control in diabetes: comparison with glycated hemoglobin and albumin. Acta Diabetol Lat 23: 117–125.
Gorog P, Pearson JD (1985) Sialic acid moieties on surface glycoproteins protect endothelial cells from proteolytic damage. J Pathol 146: 205–212.
Grieve M (1975) *Aloe vera*. In: Leyel CF (ed.) A Modern Herbal, London, p. 26–29.
Gupta S, Phipps K, Ruderman NB (1996) Differential stimulation of $Na^+$ pump activity by insulin and nitric oxide in rabbit aorta. Am J Physiol 270: H1287–H1293.
Gupta D, Raju J, Prakash J, Baquer NZ (1999) Change in the lipid profile, lipogenic and related enzymes in the livers of experimental diabetic rats: Effects of insulin and vanadate. Diabetes Res Clin Pract 46: 1–7.
Hernandez RJ (1992) $Na^+/K^+$-ATPase regulation by neurotransmitters. Neurochem Int 20: 1–10.
Hjerten S, Pan H (1983) Purification and characterization of two forms of low-affinity $Ca^{2+}$-ATPase from erythrocyte membranes. Biochim Biophys Acta 728: 281–288.
Hokins LE, Dhal JL, Deupree JD, Dixon KF, Hackney JF, Perduce JE (1973) Studies on the characterization of the sodium potassium transport adenosine triphosphatase. J Biol Chem 248: 2593–2605.
Hultberg B, Linsten J, Sjoblad S (1976) Molecular form and activities of glycosidases in culture amniotic fluid. Biochem J 155: 599–605.
Jensen T, Stender S, Deckert T (1988) Abnormalities in plasma concentrations of lipoprotein and fibrinogen in type 1 (insulin-dependent) diabetic patients with increased urinary albumin excretion. Diabetologia 31:142–145.
Kesavulu MM, Kasmeswara Rao B, Giri R, Vijaya J, Subramanyan G, Apparao C (2001) Lipid peroxidation and antioxidant enzyme status in type 2 diabetics with coronary heart disease. Diabetes Res Clin Pract 53: 33–39.
King J (1965) The hydrolases-acid and alkaline phosphatases. In: Van D (ed.) Practical clinical enzymology, London, p. 191–208.
Klein AD, Penneys N (1988) *Aloe vera*. J Am Acad Dermatol 18: 714–720
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with Folin-phenol reagent. J Biol Chem 193: 265–275.
McCune LM, Johns T (2002) Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous peoples of the north american boreal forest. J Ethnopharmacol 82: 197–205.
Means GE, Chang MR (1982) Nonenzymatic glycosylation of proteins: Structural 5and functional changes. Diabetes 31: 1–4.
Meyer C, Stumvoll M, Wadkarni V, Dostou J, Mitrakou A, Gerich J (1998) Abnormal renal and hepatic glucose metabolism in type 2 diabetes mellitus. J Clin Invest 102: 619–624.
Moore JC, Morris JE (1982) A simple automated colorimetric method for determination of *N*-acetyl-β-D-glucosaminidase. Ann Clin Biochem 19: 157–159.
Nerurkar MA, Satav JG, Katyare SS (1988) Insulin-dependent changes in lysosomal cathepsin D activity in rat liver, kidney, brain and heart. Diabetologia 31: 119–122.
Ng YC, Tolerico PH, Book CBS (1993) Alterations in levels of Na, K-ATPase isoforms in heart, skeletal muscle, and kidney of diabetic rats. Am J Physiol 265: E243–E251.
Ohnishi T, Suzuki T, Suzuki Y, Ozawa K (1982) A comparative study of plasma membrane $Mg^{2+}$-ATPase activities in normal, regenerating and malignant cells. Biochim Biophys Acta 684: 67–74.
Osicka TM, Kiriazis Z, Pratt LM, Jerums G, Comper WD (2001) Ramipril and aminoguanidine restore renal lysosomal processing in streptozotocin diabetic rats. Diabetologia 44: 230–236.
Parinandi NL, Thompson EDW, Schmid HOH (1990) Diabetes heart and kidney exhibit increased resistance to lipid peroxidation. Biochem Biophys Acta 1047: 63–69.
Rajasekaran S, Sivagnanam K, Ravi K, Subramanian S (2004) Hypoglycemic effect of *Aloe vera* gel on streptozotocin-induced diabetes in experimental rats. J Med Food 7: 61–66.
Rajasekaran S, Sivagnanam K, Subramanian S (2005a) Mineral contents of *Aloe vera* leaf gel and their role on streptozotocin-induced diabetic rats. Biol Trace Element Res 108: 185–195.
Rajasekaran S, Sivagnanam K, Subramanian S (2005b) Modulatory effects of *Aloe vera* leaf gel extract on oxidative stress in rats treated with streptozotocin. J Pharm Pharmacol 57: 241–246.
Rajasekaran S, Sivagnanam K, Subramanian S (2005c) Antioxidant effect of *Aloe vera* gel extract in streptozotocin diabetes in rats. Pharmac Rep 57: 90–96.
Rajasekaran S, Ravi K, Sivagnanam K, Subramanian S (2006) Beneficial effects of *Aloe vera* leaf gel extract on lipid propile status in rats with streptzotocin diabetes. Clin Exp Pharmacol Physiol 33: 232–237.
Rakieten N, Rakieten ML, Nadkarni MV (1963) Studies on the diabetogenic action of streptozotocin (NSC-37917). Cancer Chemo Ther Rep 29: 91–98.
Ramanadevi ChV, Hemaprasad M, Padmajareddy T, Reddy PP (1997) Glycosylation of Hemoglobin and erythrocyte membrane proteins mediated changes inosmotic fragility of erythrocytes. Indian J Med Sci 51: 5–9.
Sapolsky AL, Altman RD, Howell DS (1973) Cathepsin-D activity in normal and osteoarthritic human cartilage. Fedn Proc 32: 1489–1493.
Sasaki T, Matsy S, Sonae A (1972) Effect of acetic acid concentration on the colour reaction in the O-toluidine boric acid method for blood glucose estimation. Rinsho Kagaku 1: 346–353.
Shahid SM, Mahboob T (2003) Diabetes and hypertension: Role of electrolytes and $Na^+$-$K^+$-ATPase. Pakistan J Biol Sci 6: 1971–1975.
Sweeney G, Klip A (1998) Regulation of the $Na^+/K^+$-ATPase by insulin: Why and how? Mol Cell Biochem 182: 121–133.
Thomas CE, Reed DJ (1990) Radical-induced inactivation of kidney $Na^+$, $K^+$-ATPase: Sensitivity to membrane lipid peroxidation and the protective effect of vitamin E. Arch Biochem Biophys 281: 96–105.
Walter PJ, Flynn MD, Corrall RJM, Pennock CA (1992) Increases in plasma lysosomal enzyme in type 1 diabetes mellitus. Relationship of diabetic complications and glycemic control. Diabetologia 35: 991–995.
Wattiaux R (1977) Mammalian Cell Membrane. Jamieson GA, Robinson DM (eds.), 2nd edn., London, p. 165.
Winegrad AI (1987) Does a common mechanism induce the diverse complications of diabetes. Diabetes 36: 396–406.
Witek B, Krol T, Kolataj A, Ochwanowska E, Stanislawska I., Sewa A (2001) The insulin, glucose and cholesterol level and activity of lysosomal enzymes in the course of the model alloxan diabetes. Neuroendocrinol Lett 22: 238–242.
Zemel MB, Sowers JR, Shehin S, Walsh MF, Levy (1990) Impaired calcium metabolism associated with hypertension in Zucker obese rats. Metabolism 39: 704–708.