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BENEFICIAL EFFECTS OF *ALOE VERA* LEAF GEL EXTRACT ON LIPID PROFILE STATUS IN RATS WITH STREPTOZOTOCIN DIABETES

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SUMMARY

1. The effect of diabetes mellitus on lipid metabolism is well established. The association of hyperglycaemia with an alteration of lipid parameters presents a major risk for cardiovascular complications in diabetes. Many secondary plant metabolites have been reported to possess lipid-lowering properties. The present study was designed to examine the potential antihyperlipidaemic efficacy of the ethanolic extract from *Aloe vera* leaf gel in streptozotocin (STZ)-induced diabetic rats.

2. Oral administration of *Aloe vera* gel extract at a dose of 300 mg/kg bodyweight per day to STZ-induced diabetic rats for a period of 21 days resulted in a significant reduction in fasting blood glucose, hepatic transaminases (aspartate aminotransferase and alanine aminotransferase), plasma and tissue (liver and kidney) cholesterol, triglycerides, free fatty acids and phospholipids and a significant improvement in plasma insulin.

3. In addition, the decreased plasma levels of high-density lipoprotein–cholesterol and increased plasma levels of low-density lipoprotein–and very low-density lipoprotein–cholesterol in diabetic rats were restored to near normal levels following treatment with the extract.

4. The fatty acid composition of the liver and kidney was analysed by gas chromatography. The altered fatty acid composition in the liver and kidney of diabetic rats was restored following treatment with the extract.

5. Thus, the results of the present study provide a scientific rationale for the use of *Aloe vera* as an antidiabetic agent.

Key words: *Aloe vera*, diabetes mellitus, lipid profile, streptozotocin.

INTRODUCTION

Diabetes mellitus is a multifactorial disease that has a significant impact on the health, quality of life and life expectancy of patients, as well as on the health care system. Worldwide, the number of people with diabetes is expected to double over the 13 year period from 1997 to 2010, so that it is expected that there will be over 221 million people with diabetes worldwide by 2010.[1] Diabetes is characterized by hyperglycaemia together with biochemical alterations of glucose and lipid metabolism.[2] These traits are hypothesized to be responsible for the damage to cell membranes, which, in turn, results in an elevated production of reactive oxygen species (ROS).[3] The elevated generation of ROS and the simultaneous decline in antioxidative defence mechanisms observed in diabetic patients could promote the development of late complications.[4] To reduce the risk of late complications and negative outcomes of diabetes mellitus, such as blindness, renal failure and limb amputation, the control not only of blood glucose levels, but also lipid levels is necessary.[5]

From the beginning of the last century, evidence of the lipid-lowering properties of medicinal plants has accumulated.[6] Many scientists have demonstrated the role of medicinal plants in the control of hyperlipidaemia. Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes,[7] but only a few have received scientific scrutiny. Among these plants, *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.[8] Taxonomists now refer to *Aloe barbadensis* as *A. vera*.[9] The central bulk of the leaf contains colourless mucilaginous pulp, made up of large, thin-walled mesophyll cells containing the *A. 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 investigated. Previous experimental results were highly encouraging, because they revealed that the blood glucose level in streptozotocin (STZ)-induced diabetic rats was significantly lower after the oral administration of an ethanolic extract of *A. vera* gel.[10]

The aim of the present study was to evaluate the effects of an *A. vera* gel extract on circulatory and tissue lipids in rats with STZ-induced diabetes. The results obtained with *A. vera* were compared with glibenclamide, a known hypoglycaemic drug.

METHODS

Preparation of *A. vera* gel extract

Aloe vera powder was prepared from *A. vera* leaf gel according to a previously published procedure,[11] with slight modifications. Mature, healthy and fresh leaves of *A. vera*, with an approximate length of 0.762–0.914 m were removed and washed with fresh water. The thick epidermis was selectively removed. The inner colourless, mucilaginous pulp was homogenized and centrifuged at 6400 *g* at 4°C for 15 min to remove the fibres. The resultant supernatant was lyophilized immediately. The lyophilized sample was extracted with 95% ethanol. The filtrate was collected and evaporated to dryness under reduced pressure of 250 mmHg in a rotary evaporator. A known amount of solvent-free extract was suspended in sterilized water fresh each time and administered intragastrically. The dosing schedule used was once per day.

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Animals used

Male albino rats of the Wistar strain, weighing approximately 160–200 g, were used in the present study. Rats were acclimatized to the laboratory conditions for at least 1 week before any experimental work was undertaken. Rats were fed *ad libitum* with a normal laboratory pellet diet and water. The experiments were designed and conducted according to ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and the Institutional Animal Ethics Committee Guidelines (IAEC No. 01/034/04).

Induction of experimental diabetes

Rats were fasted for 16 h prior to induction of diabetes by intraperitoneal injection of 55 mg/kg bodyweight STZ (Sigma, St Louis, MO, USA) freshly dissolved in 0.1 mol/L cold sodium citrate buffer, pH 4.5.[12] Control rats received equivalent amounts of buffer intraperitoneally. Animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycaemia. Hyperglycaemia was confirmed 1 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 diabetic and included in the present study.

Experimental procedure

Rats were divided into four groups, with six rats in each group, as follows: (i) group I, control rats; (ii) group II, STZ-induced diabetic control rats; (iii) group III, diabetic rats given *A. vera* leaf gel extract (300 mg/kg) in aqueous solution daily via an intragastric tube for 21 days; and (iv) group IV, diabetic rats given glibenclamide (600 μg/kg) in aqueous solution daily via an intragastric tube for 21 days.

After 21 days, 16 h-fasted rats were killed by cervical dislocation. Blood was collected in tubes containing heparin. Plasma was separated and used for the estimation of glucose[13] and an insulin assay was performed using a radioimmuno assay (Linco Research, St Charles, MO, USA). The liver and kidney were dissected out, washed immediately in ice-cold saline and homogenized in Tris-HCl buffer, pH 7.4 (0.1 mol/L) with a Teflon homogenizer. Total lipids were extracted from the tissue homogenate according to the method of Folch *et al*.[14]

Analytical methods

The cholesterol content in plasma, liver and kidney was estimated according to the method of Parekh and Jung,[15] triglycerides were estimated according to the method of Rice[16] and free fatty acids were determined according to the method of Itaya.[17] Total phospholipid content was estimated according to the method of Bartlette[18] after digestion with perchloric acid and the phosphorus liberated was estimated as described by Fiske and Subbarow.[19] High-density lipoproteins (HDL), low-density lipoproteins (LDL) and very low-density lipoproteins (VLDL) were separated from the plasma using the dual-precipitation technique[20] and the cholesterol content of the lipoproteins was estimated.

Analysis of the fatty acid composition in the lipid extract was performed using gas chromatography according to the method of Morrison and Smith.[21] Fatty acid analysis was performed using a Tracer 540-gas chromatograph (Hewlett-Packard, USA) equipped with flame ionization with a detector temperature of 220°C. The separating column was 2 cm long with a 2 mm internal diameter and was packed with 10% cilar or chromosorb W, 80/100 mesh. The fatty acids separated were identified by comparison of retention times with those obtained for the separation of a mixture of standard fatty acids. An electronic integrator was used to measure peak areas and for data processing. Individual fatty acids were expressed as a percentage of total fatty acids in 100 mg tissue.

Liver tissue homogenate was also used to assay the activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT).[22]

Statistical analysis

All grouped data were evaluated statistically with SPSS/7.5 software (SPSS, Chicago, IL, USA). Hypothesis testing methods included one-way analysis of variance (ANOVA) followed by the least significant difference (LSD) test. $P < 0.05$ was considered significant. All results are expressed as the mean±SD for six animals in each group.

RESULTS

Table 1 gives the levels of blood glucose and plasma insulin in the control and experimental groups of rats. Diabetic rats showed a significant increase in blood glucose and a significant decrease in plasma insulin compared with corresponding control rats. Following oral administration of *A. vera* extract and glibenclamide, blood glucose and plasma insulin levels reverted back to those seen in control rats.

Table 2 shows the levels of plasma cholesterol, triglycerides, phospholipids, free fatty acids and lipoproteins in the control and experimental groups of rats. The levels of plasma cholesterol, triglycerides, phospholipids, free fatty acids, LDL–cholesterol (LDL-C) and VLDL–cholesterol (VLDL-C) were significantly increased, whereas levels of HDL–cholesterol (HDL-C) were significantly decreased, in diabetic rats compared with corresponding control rats. Oral administration of *A. vera* extract and glibenclamide to diabetic rats significantly reversed all these changes to near normal levels.

Figure 1 and Table 3 show levels of cholesterol, triglycerides, phospholipids and free fatty acids in the liver and kidney of the control and experimental groups of rats, respectively. There was a significant increase in tissue cholesterol, triglycerides, phospholipids and free fatty acids during diabetes compared with levels in corresponding control rats. Following the oral administration of *A. vera* extract and glibenclamide, the levels were found to be similar to those in control rats.

Changes in the fatty acid composition in the liver and kidney of the control and experimental groups of rats are summarized in Table 4 and Fig. 2, respectively. A marked increase in the levels of palmitic acid (16 : 0), stearic acid (18 : 0) and oleic acid (18 : 1) in the liver and kidney of STZ-induced diabetic rats was found. In contrast, there was significant decrease in linolenic acid (18 : 3) and arachidonic acid (20 : 4) in tissues of diabetic rats. However, following treatment with either *A. vera* extract or glibenclamide, the fatty acid composition was brought back to near normal.

Table 5 shows AST and ALT activities in the liver of the control and experimental groups of rats. A significant elevation in AST and ALT activity in the liver of STZ-induced diabetic rats was observed compared with corresponding control rats. The administration of *A. vera* extract and glibenclamide significantly decreased AST and ALT activity in the liver of diabetic rats.

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

Group	Blood glucose (mg/dL)	Plasma insulin (μU/mL)
Control	85.81 ± 5.20	15.86 ± 1.38
Diabetic		
Control	332.27 ± 20.80*	5.12 ± 0.68*
+ 300 mg/kg *Aloe vera*	96.80 ± 5.30†	14.12 ± 1.48†
+ 600 μg/kg Glibenclamide	118.46 ± 6.56†	12.52 ± 0.69†

Data are the mean±SD for six animals in each group. *$P < 0.05$ compared with control rats; †$P < 0.05$ compared with diabetic control rats.

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Table 2 Plasma cholesterol, triglycerides, phoshpholipids, free fatty acids and lipoproteins concentrations in control and experimental groups of rats

	Control	Diabetic		
		Control	+ 300 mg/kg *Aloe vera*	+ 600 μg/kg Glibenclamide
Cholesterol (mg/dL)	92.6 ± 5.7	228.3 ± 15.1*	98.3 ± 8.5†	106.2 ± 7.0†
Triglycerides (mg/dL)	73.5 ± 5.2	229.3 ± 16.1*	79.2 ± 5.2†	83.4 ± 5.8†
Phospholipids (mg/dL)	80.5 ± 5.7	163.8 ± 11.1*	85.7 ± 5.8†	88.8 ± 6.7†
Free fatty acids (mg/dL)	58.3 ± 3.6	145.2 ± 10.5*	64.7 ± 4.1†	66.1 ± 4.6†
VLDL-C (mg/dL)	19.3 ± 1.2	58.6 ± 4.5*	21.8 ± 1.6†	24.7 ± 1.6†
LDL-C (mg/dL)	45.1 ± 2.9	139.2 ± 10.3*	48.5 ± 3.1†	53.4 ± 3.4†
HDL-C (mg/dL)	26.5 ± 1.7	21.6 ± 1.6*	23.4 ± 1.5†	22.03 ± 1.42†

Data are the mean±SD for six animals in each group. *$P < 0.05$ compared with control rats; †$P < 0.05$ compared with diabetic control rats.
VLDL-C, very low-density lipoprotein–cholesterol; LDL-C, low-density lipoprotein–cholesterol; HDL-C, high-density lipoprotein–cholesterol.

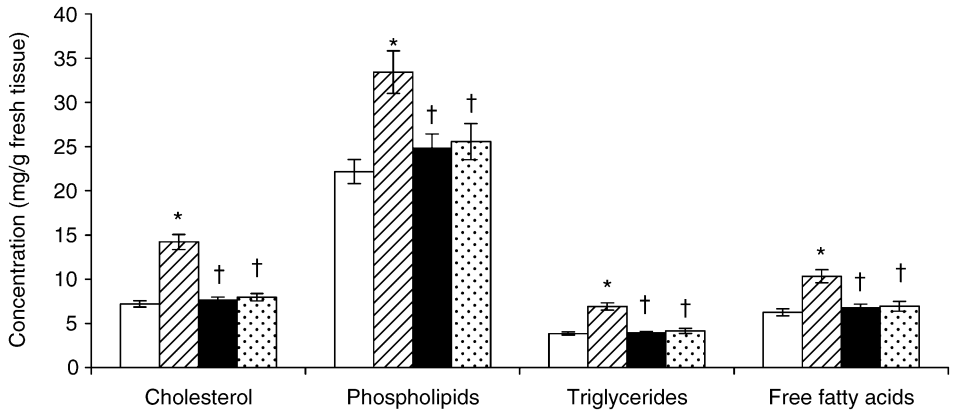

Fig. 1 Cholesterol, phospholipids, triglycerides and free fatty acid levels in livers of control (□) and experimental groups. (▨), diabetic control; (■), diabetic rats treated with 300 mg/kg *Aloe vera*; (⁘), diabetic rats treated with 600 μg/kg glibenclamide. Data are the mean±SD for six animals in each group. *$P < 0.05$ compared with control rats; †$P < 0.05$ compared with diabetic control rats.

Table 3 Cholesterol, triglycerides, phospholipids and free fatty acids in kidneys of control and experimental groups of rats

	Control	Diabetic		
		Control	+ 300 mg/kg *Aloe vera*	+ 600 μg/kg Glibenclamide
Cholesterol (mg/g fresh tissue)	5.73 ± 0.24	8.72 ± 0.48*	6.25 ± 0.28†	6.81 ± 0.36†
Triglycerides (mg/g fresh tissue)	3.17 ± 0.13	5.81 ± 0.28*	3.31 ± 0.13†	3.43 ± 0.20†
Phospholipids (mg/g fresh tissue)	12.98 ± 0.77	18.82 ± 1.37*	13.32 ± 0.82†	14.01 ± 0.96†
Free fatty acids (mg/g fresh tissue)	6.29 ± 0.28	10.71 ± 0.59*	6.70 ± 0.30†	7.18 ± 0.42†

Data are the mean±SD for six animals in each group. *$P < 0.05$ compared with control rats; †$P < 0.05$ compared with diabetic control rats.

Table 4 Fatty acid composition of the liver of control and experimental groups of rats

	Fatty acid (%/100 mg tissue)				
Group	Palmitic acid (16 : 0)	Stearic acid (18 : 0)	Oleic acid (18 : 1)	Linolenic acid (18 : 3)	Arachidonic acid (20 : 4)
Control	22.64 ± 1.49	13.01 ± 0.81	8.87 ± 0.71	7.12 ± 0.48	21.23 ± 1.34
Diabetic					
Control	29.35 ± 2.14*	20.14 ± 1.45*	14.76 ± 1.12*	2.91 ± 0.15*	14.17 ± 1.03*
+ 300 mg/kg *Aloe vera*	23.27 ± 1.58†	13.27 ± 0.84†	9.21 ± 0.64†	6.34 ± 0.32†	19.20 ± 1.42†
+ 600 μg/kg Glibenclamide	24.17 ± 1.81†	13.63 ± 0.98†	9.53 ± 0.71†	6.03 ± 0.24†	18.13 ± 1.26†

Data are the mean±SD for six animals in each group. *$P < 0.05$ compared with control rats; †$P < 0.05$ compared with diabetic control rats.

DISCUSSION

Streptozotocin is a compound commonly used for the induction of type 1 diabetes in experimental rats.[23] Streptozotocin causes diabetes by rapid depletion of β-cells, which leads to a reduction of insulin release. It is well established that glibenclamide produces hypoglycaemia by increasing the secretion of insulin from the existing pancreatic β-cells and this compound is active in moderate STZ-induced diabetes, whereas it is inactive in intense STZ diabetes (in which nearly all β-cells have been destroyed).[24] Because our results showed that glibenclamide reduced blood glucose levels in hyperglycaemic animals, the state of diabetes in the animals used in the

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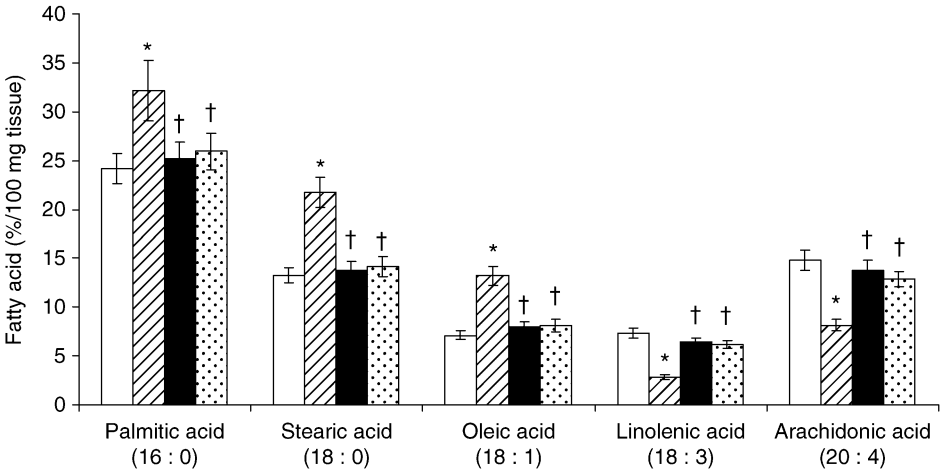

Fig. 2 Free fatty acid composition in the kidneys of control (□) and experimental groups. (▨), diabetic control; (■), diabetic rats treated with 300 mg/kg *Aloe vera*; (⊡), diabetic rats treated with 600 μg/kg glibenclamide. Data are the mean±SD for six animals in each group. *$P < 0.05$ compared with control rats; †$P < 0.05$ compared with diabetic control rats.

Table 5 Transaminase activity in livers of control and experimental groups of rats

Group	Activity (nmol pyruvate liberated/h per mg protein) AST	ALT
Control	623.41 ± 32.41	931 ± 51
Diabetic		
Control	764.22 ± 46.61*	1083 ± 54*
+ 300 mg/kg *Aloe vera*	630.75 ± 35.95†	949 ± 49†
+ 600 μg/kg Glibenclamide	637.12 ± 38.55†	954 ± 43†

Data are the mean±SD for six animals in each group. *$P < 0.05$ compared with control rats; †$P < 0.05$ compared with diabetic control rats.

AST, aspartate aminotransferase; ALT, alanine aminotransferase.

present study was not severe. The hypoglycaemic effect of plant extracts is generally dependent upon the degree of β-cell destruction. Treatment of moderate STZ-diabetic rats with medicinal plant extract resulted in the activation of β-cells and granulation returning to normal, showing an insulinogenic effect.[25] The antihyperglycaemic activity of *A. vera* was associated with an increase in plasma insulin, suggesting that the antihyperglycaemic activity of *A. vera* could be due to an insulinogenic activity of the gel extract. The increased levels of insulin observed in the present study indicate that the *A. vera* gel extract stimulates insulin secretion from the remnant β-cells and/or from regenerated β-cells. In this context, a number of other plants have also been reported to have antihyperglycaemic and an insulin-releasing stimulatory effect.[26]

In STZ-induced diabetes, the increase in blood glucose levels is usually accompanied by an increase in plasma cholesterol, triglycerides, LDL and VLDL and decreases in HDL.[27] Activation of hormone-sensitive lipase (HSL) during insulin deficiency is accompanied by enhanced release of free fatty acids from adipose tissue.[28] Thus, excess fatty acids in the plasma produced by the STZ-induced diabetes promotes the conversion of excess fatty acids into phospholipids and cholesterol in the liver. These two substances, along with excess triglycerides formed in the liver, may be discharged into the blood in the form of lipoproteins.[29] The observed increase in plasma phospholipids is a consequence of elevated lipoproteins. Therefore, the marked hyperlipidaemia that characterizes the diabetic state may be regarded as a consequence of the uninhibited actions of lipolytic hormones on fat depots. However, treatment with the *A. vera* extract normalized plasma lipid status, which was presumably mediated by a control of lipid metabolism.

The liver is an important insulin-dependent tissue, which plays a pivotal role in glucose and lipid homeostasis and is severely affected during diabetes.[30] Liver tissue participates in the uptake, oxidation and metabolic conversion of fatty acids, the synthesis of cholesterol and phospholipids and the secretion of specific classes of serum lipoproteins. In diabetes, fatty acids are increasingly taken up by the liver and, after esterification with glycerol phosphate, they are deposited as triglycrides. As a result, diabetic liver steatosis develops.[31] Furthermore, the accumulation of triglycerides and long-chain fatty acyl coenzyme A (CoA) in the liver leads to a reduction in insulin-mediated metabolic activity and can cause type 2 diabetes, resulting in metabolic syndrome.[32] 3-Hydroxy-3-methylglutaryl CoA reductase catalyses the rate-limiting step in cholesterol biosynthesis and its activity was found to be significantly increased in the liver of diabetic rats.[28] The increase in liver cholesterol in diabetic rats observed in the present study could be due to increased cholesterogenesis. The present study showed a decrease in liver cholesterol, triglycerides, phospholipids and free fatty acids in diabetic rats after treatment with the *A. vera* extract. This reduction may be attributed to increased clearance and decreased production of the major transporters of endogenously synthesised cholesterol and triglycerides. Furthermore, the increase in ALT activity in diabetes is almost always due to hepatocellular damage and is usually accompanied by an increase in AST activity.[33] Our studies with liver tissues of STZ-diabetic rats indicate a trend towards increased activity of transaminases. Moreover, the AST and ALT activity has been used as an indicator of liver function.[34] The reversal of AST and ALT activity in *A. vera*-treated diabetic rats towards near normalcy is evidence of the prevention of cellular and tissue damage under diabetic conditions, which may further strengthen the optimized lipid metabolism in the liver of diabetic rats.

Diabetes mellitus affects the kidney and is the leading cause of diabetic nephropathy. In addition to prominent roles played by factors, such as oxidative stress and advanced glycation end-products among others, abnormal lipid metabolism and the renal accumulation of lipids have also been proposed to play a role in the pathogenesis of diabetic nephropathy.[35] Several studies have shown the presence of lipid deposits in the kidney of diabetic human and experimental animals and have proposed that these deposits may play an

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important role in the pathogenesis of diabetic kidney disease.[36] The elevated levels of renal lipid contents observed in the present study are consistent with those reported previously.[37] Sun *et al*.[38] showed increased renal lipid synthesis to be responsible for the elevated level of renal lipid content. They showed a marked increase in sterol regulatory element-binding protein (SREBP)-1 and fatty acid synthase expression in STZ-diabetic rats, resulting in increased renal accumulation and glomerulosclerosis. In the present study, the ethanolic extract of *A. vera* was able to significantly decrease the concentration of these lipids in treated diabetic rats compared with untreated diabetic rats. This reduction could be beneficial in preventing diabetic complications, as well as in improving lipid metabolism in diabetic kidneys.[39]

Fatty acids, an important component of cell membranes, are eicosanoid precursors and are therefore required for both the structure and function of every cell in the body. Many studies have focused on disorders of lipid metabolism, especially alterations in tissue fatty acids in diabetes. In the present study, we observed significant alterations in the fatty acid composition in tissues of diabetic rats. The most consistent findings were diminished levels of linolenic and arachidonic acids and increased levels of oleic, stearic and palmitic acids in the tissues of diabetic rats compared with levels in control rats. These results are in agreement with those reported earlier.[40] The observed increase in the levels of stearic and palmitic acids coincides with previous reports showing that there is a preferential synthesis of stearic acid and total saturated fatty acids in type 1 diabetic patients.[41] Normalization of tissue saturated fatty acids following the administration *A. vera* extract may be attributed to the decrease in plasma lipids caused by the extract, which results in decreased synthesis of fatty acids. Furthermore, normoglycaemia and inhibition of lipolysis may lead to a decrease in the synthesis of saturated fatty acids in diabetic tissues.

One mechanism that may explain the decrease in polyunsaturated fatty acids in diabetics, is the destruction of polyunsaturated fatty acids by free radicals. The double bond in this type of fatty acid makes them highly susceptible to oxidation and their destruction would lead to lesions that are characteristic of diabetic complications.[42] The major cause of the changes in the levels of linolenic and arachidonic acid is thought to be diminished fatty acid desaturation, particularly the diminished activity of desaturase.[43] Reduced activity of Δ^6-desaturase has been reported in STZ-induced diabetic rats and treatment with insulin was found to restore the activity of this enzyme to normal levels.[44] The administration of *A. vera* afforded a significant restoration of the polyunsaturated fatty acid composition, which is presumably mediated by the scavenging of free radicals[45,46] and the control of lipid metabolism.

Conclusions

In conclusion, anti-oxidants commonly present in plants, such as phenolic compounds and saponins, are known to reduce hyperlipidaemia in diabetes.[47] Preliminary phytochemical screening revealed the presence of phenolic compounds and saponins in the gel extract of *A. vera*.[45] Thus, the anti-oxidants present in the *A. vera* extract may be responsible, in part, for the antihyperlipidaemic effect of the gel extract. In addition to the anti-oxidant potential, the hypoglycaemic effect of the gel extract may be implicated as the major reason for the observed antihyperlipidaemic effect of the extract. This is in agreement with the facts that: (i) the level of glycaemic control is the major determinant of total cholesterol, VLDL-C and triglyceride levels;[48] and (ii) improved glycaemic control following sulphonylurea therapy decreases the levels of serum VLDL-C and total triglycerides.[49] There is ongoing research to isolate and characterize the bioactive compound(s) responsible for the antidiabetic/anti-oxidative action in this crude extract and to use the(se) compound(s) in a bioassay directed experiment.

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