Original Article

A Study on the Protective Effect of *Cynodon dactylon* Leaves Extract in Diabetic Rats

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Abstract

Objective To investigate the antidiabetic, antioxidant and hypolipidemic efficacy of *Cynodon dactylon* in diabetic rats.

Methods The experimental rats were randomly divided into three groups: Group I: control; Group II: Alloxan diabetic, untreated; and Group III: Alloxan diabetic treated with ethanolic extract of *C. dactylon* leaves (450 mg/kg·bw). Experimental diabetes was induced by alloxan in a single dose of 150 mg/kg·bw.

Results A Significant diminution of fasting blood sugar level was observed and also significant increase in HDL and decrease (P<0.05) in cholesterol, triglyceride, LDL and VLDL were observed after 15 days of treatment. The investigation also revealed, the activities of AST, ALT, ALP, AP, LDH, and CPK (P<0.05) were decreased in the extract-supplemented group. The significant decrease in protein content and SOD, CAT, GPx, and GSH (P<0.05) activity and increase in LPO in plasma were found to be ameliorated after treatment.

Conclusion Our result supports the fact that administration of extract of *C. dactylon* leave is able to reduce hyperglycemia and hyperlipidemia risk and also reduced the oxidative stress in diabetic rats.

Key words: *Cynodon dactylon*; Ethanolic extract; GC-MS; Alloxan diabetes; Antidiabetic; Antioxidant; Lipid profile; Enzyme profile

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INTRODUCTION

Diabetes mellitus is a serious, complex chronic condition, which is a major health concern worldwide. This metabolic disorder is characterized by hyperglycemia and disturbances of carbohydrate, protein and fat metabolisms[1]. The number of people affected with diabetes worldwide has increased dramatically over recent years. Indeed, by 2015 it has been estimated that the diabetic population will increase to 221 million around the world[2]. The Diabetes Control and Complications Trial (DCCT) Research Group (1993) stated that tight control of blood glucose is an effective strategy in reducing clinical complications of diabetes mellitus significantly and even optimal control of blood glucose can not prevent complications suggesting that an alternative approaches is needed. Ethnobotanical field studies revealed that, a number of plant remedies were used to alleviate the symptoms of diabetes. However, only a few have been evaluated scientifically to confirm the claimed activity[3].

Hyperglycemia also causes oxidative stress, which in turn can result in cellular tissue damage. The uncontrolled hyperglycemia can lead to disturbances of the cell structure and functions of organs[4]. Diabetes is associated with the generation of reactive oxygen species (ROS) causing oxidative damage particularly to heart and kidney.

Cynodon dactylon (L.) Pers. (Fam: Poaceae) is commonly known as “Doob” in India (Arugampul: Tamil). It is a weed and possesses varied medicinal properties. Leaf, root and rhizome of the plant have been used in folk medicine in different countries, as anti-inflammatory, anticystitis[5], antihypertensive,

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antiviral, hypolipidemic agent, antihysteria, antipsychotic, and antigonorrheal infection[6]. In India, the plant is used in the treatment of melena, thirst, anorexia, burning sensations in the body, pruritis, miscarriage and erysipelas[7] and its leaf juice with a pinch of common salt has been used orally to treat stomachache. Decoction of whole plant is given orally to cure menstrual problem[8].

The aim of this study is to mainly investigate the effect of ethanolic extract of *C. dactylon* leaves on antidiabetic, hypolipidemic and antioxidant activity in normal and alloxan diabetic rats. In addition, the study is to investigate whether the extract of *C. dactylon* leaves has a protective effect on the liver and heart of alloxan diabetic rats and related toxicological study.

MATERIALS AND METHODS

Plant Material

Fresh leaves of *C. dactylon* were collected from PRIST University area. The plants were taxonomically identified and authenticated by Rev Dr S John Britto SJ, Director, The Rapinat Herbarium and Centre for Molecular Systematics, St Joseph's College (Autonomous), Tiruchirappalli, Tamil Nadu, India. The voucher specimens are deposited at the Rapinat herbarium and its number is RHCD02.

Preparation of Ethanolic Extract of C. dactylon Leaves

Fresh leaves of *C. dactylon* were air dried in shade and powdered. The extraction was carried out by mixing the powdered (550 gm) leaves with 1:2 (w/v) in 70% ethanol (v/v) for 2 days. The resulted extract was filtered and concentrated by rotary evaporator under reduced pressure and low temperature. The yield of extract was 12.2% (w/w) in terms of dried starting material.

Phyto-chemical Analysis

Ethanolic extract of *C. dactylon* was analyzed by gas chromatography equipped with mass spectrometry (GC-MS-QP2010-Shimadzu). The chromatographic conditions were as follows: Column: DB-% ms (length 30.0 m, diameter 0.25 mm, film thickness 0.25 μm). The 1μL DG ethanolic extract was injected into the GC-MS in split less mode at 200 °C. The column oven temperature was held at 45 °C for 1 min, then programmed at 10 different rates up to 280 °C and held for 18 min. Helium carrier gas was maintained at a flow rate of 1.4 mL/min.

Animals

Male albino Wistar rats of the same age group and body weight 130-150 g were selected for all the experiments. The animals were housed in polypropylene cages at an ambient temperature of 25-30 °C and 45%-55% relative humidity with 12 h each of dark and light cycle. Rats were fed with pellet diet and water *ad libitum.* The study was approved by the Institutional Animal Ethical Committee (IAEC).

Induction of Diabetes for Experimental Animals

Albino rats were made diabetic by a single intraperitoneal (i.p.) injection of alloxan dissolved in normal saline at a dose of 150 mg/kg·bw. Animals with a blood glucose concentration >200 mg/dL were considered to be diabetic.

Experimental Design

The animals were randomly divided into three groups: Group I: control animals (normal, nondiabetic animals) (n=6); Group II: alloxan diabetic, untreated animals (n=6); and Group III: alloxan diabetic animals treated with ethanolic extract of *C. dactylon* leaves (n=6). Ethanolic extract of *C. dactylon* leaves was given to experimental animals orally at a dosage of 450 mg/ kg daily for 15 days. After 15 days of treatment, the animals were euthanized and different organs collected for protein content study and collected plasma for measurement for biochemical assays.

Biochemical Assay

Blood samples were collected through the tail vein of the experimental animals on day 15th treatment. Effect on oral glucose tolerance was evaluated by feeding 10 g/kg glucose after the treatment with 450 mg/kg·bw extract. Blood glucose and the specific activities of enzymes namely, lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid phosphatase (AP), and alkaline phosphatase (ALP) were estimated with standard spectrophotometer techniques[9]. Plasma total cholesterol and HDL-cholesterol and plasma triglyceride were determined by spectrophotometric methods[10].

LDL-cholesterol levels were calculated using formula:

LDL-cholesterol = total cholesterol−

$$\frac{\text{HDL} - \text{cholesterol} + \text{triglycerides}}{5}$$

VLDL-cholesterol levels were calculated using formula: VLDL = Triglyceride/5 Atherogenic index (AI) was calculated by using the following formula:

atherogenic index=

$$\frac{\text{total cholesterol} - \text{HDL} - \text{cholesterol}}{\text{HDL} - \text{cholesterol}}$$

The activities of antioxidants like superoxide dismutase (SOD), catalase (CAT), Glutathione Peroxidase (GPx), reduced glutathione (GSH), and lipid peroxidation (MDA)[11] were estimated by spectrophotometer techniques.

Statistical Analysis

The data are expressed as mean ± standard error of the mean (SEM) by using SPSS 14 evaluation. Statistical comparisons were performed by one-way analysis of variance (ANOVA). The results were considered statistically significant if the *P* values were 0.05 or less.

RESULTS

Body Weight and Organs Weight

The body weight and weight of various organs of the experimental rats i.e. alloxan treated diabetic rats and control are tabulated as 1 and 2. The total body weight decreased during diabetes, but the individual organ weight like heart, liver, spleen, and kidney increased in diabetic rats, whereas weight of brain decreased. These changes in organ weight were alleviated to different extents by feeding *C. dactylon* leaves extract. It has been reported that, the weight of the liver increases during diabetes and our results are also in agreement with the same.

Plasma and Organs Protein Profile

Total protein content was significantly reduced, whereas albumin and globulin increased in diabetic rats as compared with control rats. In diabetic rats, increase in the total protein and decrease in the albumin and globulin content were observed due to the *C. dactylon* treatment and values are indicated in Figure 1. The different organ protein content of control, diabetic and treated groups are presented in Tables 3 and 4. A decrease in protein content of diabetic rat liver, spleen, kidney, brain, pancreases, muscle, and adipose tissue but, an increase in heart protein content was observed when compare with control rats. The organ protein contents were restored to normal level after the treatment of ethanolic extract of *C. dactylon* leaves.

Table 1. Effect of Ethanolic Extract of *C. dactylon* on Body Weight in Control and Experimental Animals

Groups	Body Weight (gm)
Before treatment	
Normal control	134.8 ± 8.24
Diabetic induced	135.1 ± 10.2
Diabetic treated[a]	137 ± 9.28

Note. Values are expressed mean ± SEM of six animals. [a] $P<0.05$, as compared to diabetic induced.

Table 2. Effect of Ethanolic Extract of *C. dactylon* on Organs Weight in Control and Experimental Animals

Groups	Organs Weight (gm)				
	Heart	Liver	Spleen	Kidney	Brain
Normal Control	0.42 ± 0.12	3.2 ± 0.32	0.34 ± 0.15	1.12 ± 0.15	1.3 ± 0.19
Diabetic Induced	0.56 ± 0.12	6.0 ± 0.21	0.79 ± 0.28	1.34 ± 0.24	1.15 ± 0.23
Diabetic Treated[a]	0.42 ± 0.19	4.0 ± 0.97	0.59 ± 0.18	0.98 ± 0.12	1.0 ± 0.25

Note. Values are expressed mean ± SEM of six animals. [a] $P<0.05$, as compared to diabetic induced.

Table 3 . Effect of Ethanolic Extract of *C. dactylon* on Organs Protein Profile in Control and Experimental Animals

Groups	Protein Concentration (mg of Protein/gm of Tissue)			
	Heart	Liver	Spleen	Kidney
Normal Control	41.14 ± 5.21	66.91 ± 6.91	129.53 ± 9.61	46.26 ± 5.87
Diabetic Induced	43.51 ± 6.32	61.65 ± 7.21	85.50±7.42	39.35 ± 6.32
Diabetic Treated[a]	33.6 ± 7.23	70.5 ± 8.54	133.9 ± 10.25	56.4 ± 8.52

Note. Values are expressed mean ± SEM of six animals. [a] $P<0.05$, as compared to diabetic induced.

Table 4. Effect of Ethanolic Extract of *C. dactylon* on Organs Protein Profile in Control and Experimental Animals

Groups	Protein Concentration (mg of Protein/gm of Tissue)			
	Brain	Pancreases	Muscle	Adipose Tissue
Normal Control	20.24± 2.31	83.33 ± 8.91	56.41 ± 4.25	8.87 ± 1.24
Diabetic Induced	17.60± 3.89	16.70 ± 4.24	40.28±3.24	6.57 ± 1.32
Diabetic Treated[a]	29.2 ± 4.58	29.8 ± 5.82	45.2 ± 6.32	7.2 ± 1.92

Note. Values are expressed mean ± SEM of six animals. [a] $P<0.05$, as compared to diabetic induced.

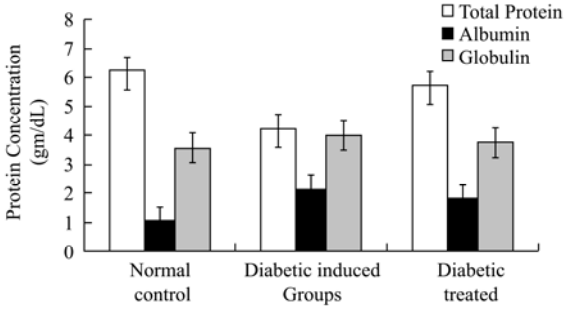

Figure 1. Effect of ethanolic extract of *C. dactylon* on plasma protein profile in control and experimental animals. Values are expressed mean ± SEM of six animals.

Blood Glucose Level

The effect of ethanolic extract of *C. dactylon* leaves on blood glucose levels is shown in Figure 2. A significant difference in the blood glucose levels in all groups were observed at the end of the 15-day of treatment. The blood glucose levels were increased significantly in alloxan diabetic rats as compared with the control rats ($P<0.05$). In diabetic rats, significant decrease in blood glucose levels were observed after the oral administration of ethanolic extract of *C. dactylon* leaves. Blood glucose levels of normal, diabetic rats and treated animals were 101.0±5.2, 210.0±6.9, and 110.5±8.92 mg/dL, respectively ($P<0.05$).

Plasma Lipid Profile (Cholesterol, Triglyceride, HDL, LDL, and VLDL)

Differences in the plasma cholesterol levels of all groups were observed at the end of the 15-day of treatment ($P<0.05$) as indicated in Table 5. Serum cholesterol levels were significantly increased in diabetic rats when compared with controls groups. Oral administration of ethanolic extract of *C. dactylon* leaves had significantly reduced the plasma cholesterol levels in diabetic treated rats. In diabetic rats, a significant elevation of plasma LDL-cholesterol and VLDL-cholesterol was observed as compared with control rats. No change in HDL-cholesterol level was observed. After administration of ethanolic extract of leaves of *C. dactylon*, it was found to reduce LDL-cholesterol ($P<0.05$) and VLDL-cholesterol and increase HDL-cholesterol in diabetic treated rats ($P<0.05$). Triglyceride and atherogenic index in plasma were increased significantly in diabetic rats as compared with normal rats. Administration of leaf extract reversed these effects ($P<0.05$).

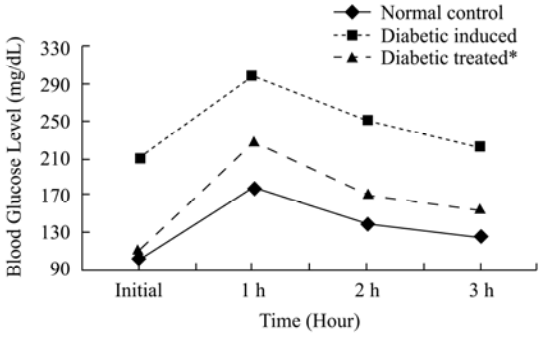

Figure 2. Effect of ethanolic extract of *C. dactylon* on blood glucose level (oral glucose tolerance level after feeding 10 g/kg) glucose in control and experimental animals. Values are expressed mean ± SEM of six animals.

Plasma Enzymes Profile (AST, ALT, ALP, AP, LDH, and CPK)

A significant elevation of plasma ALT and AST activity ($P<0.05$) were observed in diabetic rats as compared with control. Treatment of diabetic rats with ethanolic extract of *C. dactylon* leaves resulted in a significant decrease in the levels of ALT and AST when compared with diabetic control. A significant increase in the levels of ALP, AP, LDH, and CPK ($P<0.05$) were observed in diabetic control when compared with normal control group. A significant level of reduction in plasma-marker enzymes (ALP, AP, LDH, and CPK) were noticed due to the effect of ethanolic extract of *C. dactylon* leaves as indicated in Table 6.

Antioxidant Level

Table 7 illustrates the activities of SOD, CAT, GPx,

GSH, and LPO in plasma of normal and experimental groups. There was a significant reduction in the activities of SOD, CAT, GPx, and GSH during diabetes. Administration of ethanolic extract of *C. dactylon* leaves tends to bring the values to near normal. There was a significant elevation in plasma LPO during diabetes when compared to the corresponding control group. Administration of ethanolic extract tends to bring the values to near normal.

Phyto-Chemical Analysis

GC–MS analysis of *C. dactylon* extract detected molecular peaks with typical retention time of analyzed components, which are shown in total ions chromatogram (TIC) (Figures 3a and 3b). The chemical structures are presented in Figure 4 and Table 8. The GC/MS analysis of plant extract revealed the presence of 6 major compounds. The ethanolic extract of leaves contains the following main components i.e. 2-Propenoic acid (Cinnamic acid) (tR;11.98), β-3,7-dimethyl-1,3,6-octatriene (tR; 12.14), 3-(3,4-Dihydroxyphenyl 2-propenoic acid (tR; 12.96), 2-isopropyl-5-methyl cyclohexyl] ester (tR; 13.25), 4-Ethenyl-2-methoxyphenol (tR; 13.3), and 3,7,11,15-Tetra methyl-2-hexadecen-1-ol (tR; 17.6).

Table 5. Effect of Ethanolic Extract of *C. dactylon* on Plasma Lipid Profile in Control and Experimental Animals

Groups	Plasma Lipid Profile (mg/dL)					
	TC	TG	HDL	LDL	VLDL	AI
Normal Control	31.9 ± 3.21	64.9 ± 4.24	25.8 ± 2.89	11.6 ± 3.98	13.0±1.25	0.23 ± 0.05
Diabetic Induced	42.8 ± 2.75	105.6 ± 6.84	28.6 ± 3.24	19.1 ± 2.58	18.2±2.84	0.49 ± 0.09
Diabetic Treated[a]	28.8 ± 4.23	55.6 ± 5.38	33.5 ± 5.24	12.1 ± 2.85	11.0±1.95	0.13 ± 0.04

Note. Values are expressed mean ± SEM of six animals. [a] $P<0.05$, as compared to diabetic induced.

Table 6. Effect of Ethanolic Extract of *C. dactylon* Leaves on Plasma Marker Enzyme Profile in Control and Experimental Animals

Groups	Plasma Enzyme Profile (U/L)					
	AST	ALT	ALP	AP	LDH	CPK
Normal Control	50.3 ± 5.21	24.0 ± 2.85	119.0 ± 9.35	33.8 ± 3.51	71.6 ± 7.25	350 ± 32.54
Diabetic Induced	88.6 ± 7.52	40.3 ± 4.28	158.0 ± 8.25	40.0 ± 5.84	112.1 ± 8.71	400 ± 35.21
Diabetic Treated[a]	60.0 ± 6.35	29.0 ± 3.12	115.0 ± 6.28	31.0 ± 4.52	83.5 ± 9.32	362 ±2 8.12

Note. Values are expressed mean ± SEM of six animals. [a] $P<0.05$, as compared to diabetic induced.

Table 7. Effect of Ethanolic Extract of *C. dactylon* on Antioxidant Acitivity in Control and Experimenal Animals

Groups	Antioxidant Activity				
	SOD (U1/mg MDA/ Protein)	CAT (U2/mg mg Protein)	GPx (U3/mg Protein)	GSH (mmol/L of GSH/ Protein)	LPO (nmol/L of mg Protein)
Normal Control	8.90 ± 1.25	31.20±3.21	452 ± 10.25	251 ± 8.24	56 ± 3.25
Diabetic Induced	6.32 ± 0.96	29.70 ± 2.58	308 ± 11.58	210 ± 10.23	76 ± 6.35
Diabetic treated[a]	12.8 ± 2.35	32.5 ± 4.82	392 ± 15.24	225 ± 12.58	60 ± 5.82

Note. Values are expressed mean ± SEM of six animals. [a] $P<0.05$, as compared to diabetic induced. U1/one unit of activity was taken as the enzyme reaction, which gave 50% inhibition of NBT reduction in 1 min; U2/mmol of hydrogen peroxide consumed/minute; U3/mg of glutathione consumed/minute.

Table 8. Identification of Constituents in Ethanollic Extract of *C. dactylon* Leaves by GC-MS

S.no.	Retention Time	Formula	Molecular Weight	Component Name
1	11.98	C9H8O2	148.16	2-Propenoic acid (Cinnamic acid)
2	12.14	C10H16	136.24	β-3,7-dimethyl-1,3,6-octatriene
3	12.96	C9H8O4	180.16	3-(3,4-Dihydroxyphenyl 2-propenoic acid
4	13.25	C12H22O2	198.30	2-isopropyl-5-methylcyclohexyl] ester
5	13.30	C9H10O2	150.18	4-Ethenyl-2-methoxyphenol
6	17.6	C20H40O	296.54	3,7,11,15-Tetramethyl-2-hexadecen-1-ol

DISCUSSION

Diabetes mellitus is one of most common chronic disease and is associated with hyperlipidemia and co-morbidities such as obesity, hypertension. Hyperlipidemia is a metabolic complication of both clinical and experimental diabetes[12]. In this study, we have observed that the ethanolic extract of *C. dactylon* leaves decreases blood glucose level in alloxan induced diabetic rats. The possible mechanism of action of extract could be correlated with the reminiscent effect of the channels, hypoglycemic sulphonylureas, tolbutamide, which promote insulin secretion by closure of K^+-ATP membrane depolarization and stimulation of Ca^+ influx, an initial key step in insulin secretion. In this context, number of other plants has also been reported to have antihyperglycemic and stimulatory effects[13].

The lipid abnormalities accompanied with premature atherosclerosis is the major cause of cardiovascular diseases in diabetic patients. Therefore, an ideal treatment for diabetes in addition to glycemic control should have a favorable effect on lipid profile. Cardiovascular diseases are listed as the cause of death in 65% people suffering from diabetes[14]. The dose of 450 mg/kg of the ethanolic extract not only lowered the TC, TG, VLDL, and LDL levels but also enhanced the cardio-protective lipid HDL after 15 days of treatment. Several studies have shown that an increase in HDL-cholesterol is associated with a decrease in coronary risk and most of the drugs that decrease total cholesterol also decrease HDL-cholesterol. In the present study, the ethanolic extract of *C. dactylon* leaves not only decrease the total cholesterol but also enhances the HDL-cholesterol. High levels of TC and more importantly LDL cholesterol are major coronary risk factors. Administration of leaves extract to diabetic rats for 15 days lowered TC and LDL cholesterol which is the important finding of this experiment as diabetes is associated with coronary complications, and major cause of morbidity and deaths in diabetic subjects. Recent studies suggest that TG itself is independently related to coronary heart disease[15] and most of the antihypercholesterolemic drugs do not decrease TG levels, but extract *C. dactylon* leaves lowered after 15 days of treatment. Its strong effect on diabetic hypertriglyceridemia could be through its control of hyperglycemia. This is in agreement with the fact that (i) the level of glycemic control is the major determinant of very low-density lipoprotein and triglyceride concentrations and (ii) improved glycemic control following sulfonylurea therapy decreases levels of serum VLDL and total triglyceride[16].

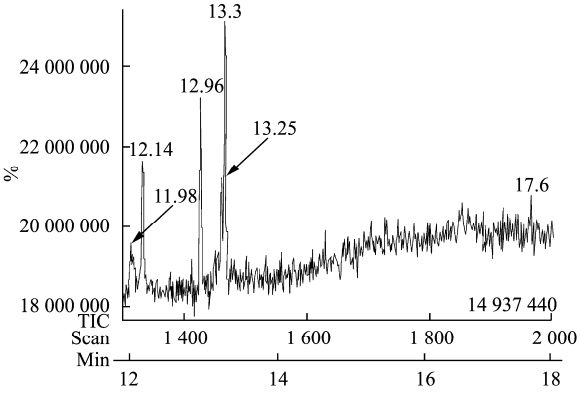

Figure 3a. Total ion chromagram of *C. dactylon* extract from GC-MS analysis and scanned peak of individual components.

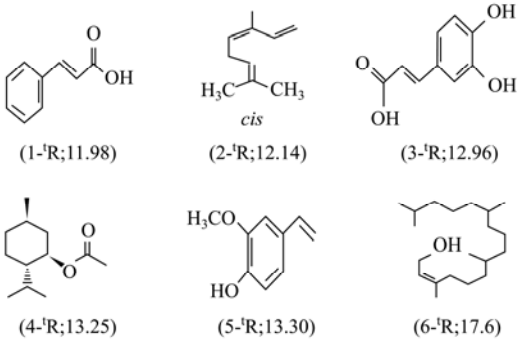

Figure 4. Chemical structure of identified components of ethanolic extract of *C. dactylon* from GC-MS.

Regarding the mechanism of action, insulin plays an important role in lipid metabolism apart from its regulation of carbohydrate metabolism. Insulin is potent inhibitor of lipolysis. During diabetes, activity of lipase enzyme increases lipolysis and release more free fatty acids in the circulation because of lack of insulin[17]. Increase in fatty acid concentration in turn increases the beta-oxidation of fatty acids by increasing the activity of HMG-CoA reductase for producing more cholesterol. Insulin also increases the receptor-mediated removal of LDL-cholesterol and decreased activity of insulin during diabetes causes hypercholesterolemia[18]. The ethanolic extract of *C. dactylon* leaves may enhance

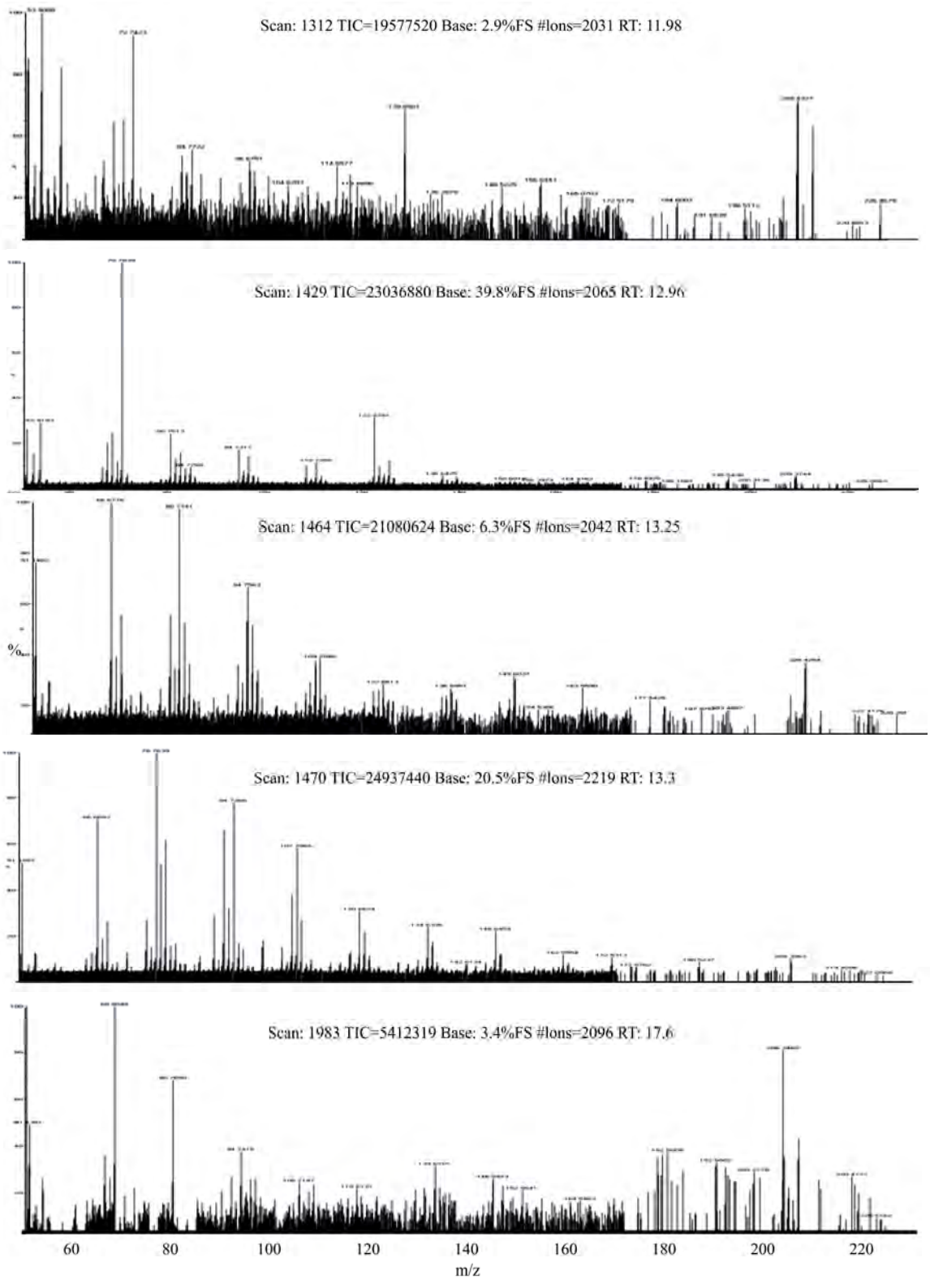

Figure 3b. GC-MS analysis and scanned peak of individual components with retention time.

insulin secretion, activity of enzymes involved in bile acid synthesis and its excretion and this may have caused decrease in plasma cholesterol, TG, LDL, VLDL, and enhanced the HDL level. Plant extract reduce triglyceride in plasma of alloxan-induced diabetic rats and prevent the progression of coronary heart disease.

Supplementation of ethanolic extract improved the function of liver by decreasing the plasma enzymes levels in diabetic treated rats. The increase in AST and ALT will increase the incidence of heart and liver diseases. AST is an enzyme found primarily in the cells of the liver, heart, skeletal muscles, kidneys, and pancreas to a lesser extent, in red blood cells. Its plasma concentration is in proportion to the amount of cellular leakage or damage. It is released into plasma in larger quantities when any one of these tissues is damaged. Its increased levels are usually associated with cardiac arrest or liver disease[19]. The ethanolic extract of *C. dactylon* leaves decreased the AST and ALT level, which is an indication of the protective effect on liver and heart. Measurement of enzymic activities of acid phosphatases (AP) is of clinical and toxicological importance as changes in their activities are indicative of tissue damage by toxicants or in disease conditions. Acid phosphatase activity of plasma of diabetic control rats was found to be increased. At the dose of 450 mg/kg for 15 days, ethanolic extract is reported to inhibit acid phosphatase activity and reduced AP level in plasma.

The significant increase in LDH is mainly due to leakage of this enzyme into the blood because of alloxan toxicity in liver. Higher activity of glucose-6-phosphatase provides H^+ which binds with $NADP^+$ to form NADPH which is helpful in the synthesis of fats from carbohydrates. When glycolysis slows down because of cellular activity, pentose phosphate pathway that is still active in liver provides NADPH, which converts acetyl radicals into long chain fatty acids during diabetes mellitus. Similar results were reported by other researchers in experimental diabetes[20]. However, treatment of alloxan diabetic rats with ethanolic extract of *C. dactylon* leaves for 15 consecutive days could restore the normal metabolism by shifting the balance from lipids metabolism to carbohydrate metabolism. It is well known that diagnosis of cardiac enzymes is important for cardio vascular disease. Plasma CPK activity is a more sensitive indicator in early stages of myocardial ischemia and its peak rises in myocardial tissue damage[21]. The results in diabetic treated animals in this experiment shows a protective effect of ethanolic extract of *C. dactylon* leaves on the heart of experimental animals. Moreover, the significantly lowered activities of CPK, scientifically suggest that the leaf extract of *C. dactylon* have the potential of reducing the factors that produce myocardial infarction. This is so because of the metabolism of alloxan-induced infarct myocardium which might be studied by assessing the level of marker enzyme in the plasma.

CAT is a hemeprotein which catalyses the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals[22]. Therefore, reduction in the activity of these enzymes (SOD, CAT) may result in a number of deleterious effects due to the a ccumulation of superoxide anion radicals and hydrogen peroxide. Administration of ethanolic extract of *C. dactylon* leaves increased the activities of SOD and CAT in diabetic rats. The activities of GPx also decreased significantly in diabetic rats. GPx is an enzyme with selenium, catalyses the reduction of hydrogen peroxide and hydroperoxides to non-toxic products. The depletion in the activity of this enzyme may result in the involvement of deleterious oxidative changes due to the accumulation of toxic products. In this context, other workers also reported a decrease in the activities of these antioxidant enzymes (SOD, CAT and GPx) in plasma of diabetic rats[23]. As the alterations produced in the antioxidant activities indicate involvement of deleterious oxidative changes, increased activities of these enzymes in defense system would therefore be important in protection against radical damage. Administration of ethanolic extract of *C. dactylon* leaves have shown to increase the activities of GPx in diabetic rat in this experiment.

GSH, being the most important biomolecule against chemically induced toxicity, can participate in the elimination of reactive intermediates by reduction of hydroperoxides in the presence of GPx. GSH also functions as free radical scavenger and repair of radical caused biological damage[24]. We have observed a decrease in the level of GSH in plasma during diabetes. Administration of ethanolic extract of *C. dactylon* leaves increased the content of GSH in plasma of diabetic rats. Decreased activity of antioxidant enzymes in uncontrolled diabetes is due to decreased GSH formation, which requires NADPH and glutathione reductase. The reduced availability of NADPH could be due to reduced synthesis in HMP shunt resulted due to decreased activity of glucose-6-phosphate dehydrogenase as this enzyme plays a very important role to maintain high ratio of

NADPH/$NADP^+$ in the cell and plays a crucial role in the regeneration of GSH from GSSG. The normal activity of glucose-6-phosphate dehydrogenase possibly attributes to enhanced synthesis of NADPH/$NADP^+$. The NADPH generated, consequently could increase the concentration of GSH observed in this study, which in turn is utilized by GPx[25].

Tremendous increase in lipid peroxidation in plasma which was observed in diabetic rats is attributed to chronic hyperglycemia. Diabetes causes increased production of reactive oxygen species (ROS) dueto the auto-oxidation of monosaccharides, which leads to the production of superoxide and hydroxyl radicals[26], which in turn cause tissue damage by reacting with polyunsaturated fatty acids in membranes. Lipid peroxide mediated damage has been observed in the development of both type I and type II diabetes mellitus. It has been observed that insulin secretion is closely associated with lipoxygenase derived peroxides. 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[27]. A marked increase in the concentration LPO is observed in plasma of diabetic rats. Administration of ethanolic extract of *C. dactylon* leaves significantly decreased the level of LPO in alloxan diabetic rats.

CONCLUSIONS

The ethanolic extract of *C. dactylon* leaves contains a range of active pharmacological agents as analysed by GC-MS, includes alkaloids, steroids and tannins. As from the literature, alkaloids, steroids and tannins are known to reduce blood glucose level in diabetic condition[28]. In general, there is very little biological knowledge on the specific modes of action in the treatment of diabetes such as glycosides, alkaloids, terpenoids, flavonoids, ets. Those are frequently implicated as having antidiabetic effects. It is known that certain alkaloids and flavonoids exhibit hypoglycemic activity and is also known for their ability of beta cell regeneration in pancreas[29]. Tannins have also shown to decrease blood sugar level in experimental animal models. Thus, the significant antidiabetic effect of ethanolic extract of *C. dactylon* leaves may be due to the presence of more than one antihyperglycemic principle and/or their synergistic effects.

In summary, the ethanolic extract exhibited strong hypoglycemic activity in addition to hypolipidemic activity in diabetic animals. This has clinical implications that the relatively nontoxic *C. dactylon* extract, if used as a hypoglycemic agent, may also reverse dyslipidemia associated with diabetes and prevent the cardio vascular complications that are very prevalent in diabetic patients. The present investigation has also opened avenues for further research especially with reference to the development of potent phytomedicine for diabetes mellitus from *C. dactylon* leaves.

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