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Attenuation of hyperglycemia and hyperlipidemia in streptozotocin-induced diabetic rats by chloroform extract of fruits of *Ferocactus latispinus* and *Ferocactus histrix*

[Atenuación de la hiperglucemia y la hiperlipidemia en ratas diabéticas inducida por estreptozotocina por el extracto de cloroformo de los frutos de *Ferocactus latispinus* y *Ferocactus histrix*.]

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Abstract

The hypoglycemic effects of hexane, chloroform and methanol extracts from fruits of *Ferocactus latispinus* and *Ferocactus histrix* were evaluated by oral administration to normoglucemic and streptozotocin-induced severe diabetic rats (SD). The anti-diabetic effect was examined by blood glucose, triglycerides, lipid peroxidation, total cholesterol levels in the serum, glycogen content of liver and skeletal muscles, superoxide dismutase (SOD) catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GSHPx) levels. The most active extracts were obtained with chloroform. Chloroform extracts from *F. latispinus* and *F. histrix* increased activities of SOD, GR, GSHPx and CAT, hepatic glycogen content, glucose-6-phosphatase (G6Pase) and the plasma insulin levels. They also, decreased glucokinase (GK) and TBAR (thiobarbituric acid assay). Of the two plants studied *F. latispinus* showed better antihyperglycemic and antihyperlipidemic effects that *F. histrix.* In conclusion *F. latispinus* and *F. histrix* possesses significant antihyperglycemic properties after 4 h after a single oral dose. It can also improve hyperlipidemia and hypoinsulinemia in streptozotocin-induced diabetic. These results demonstrated that *F. latispinus* and *F. histrix* typically used as a health food, has strong antidiabetic effects *in vivo*, thus, it may have beneficial properties in the prevention of diabetes.

Keywords: *Ferocactus latispinus, Ferocactus histrix,* antihyperlipidemia, antihyperglycemia

Resumen

Los efectos hipoglucemiantes de extractos obtenidos con hexano, cloroformo y metanol a partir de frutos de *Ferocactus latispinus* y *Ferocactus histrix* fueron evaluados por la administración oral a ratas normales y con diabetes severa (SD) inducida por estreptozotocina. Los extractos más activos fueron obtenidos con cloroformo el cuál incrementa los niveles de SOD, GR, GSHPx y el CAT, el contenido de glucógeno hepático, la glucosa-6-fosfatasa (G6Pase) y los niveles de insulina plasmática. También producen disminución de la glucoquinasa (GK) y TBARS. De las dos plantas estudiadas la *F. latispinus* presento mayor actividad antihiperglicemiante y antihiperlipidémicos que la *F. histrix.* En conclusión *F. latispinus* y *F. histrix* pueden mejorar la hiperlipidemia y la hipoinsulinemia en animales diabéticos inducida por estreptozotocina. Estos resultados demostraron que *F. latispinus y F. histrix* utilizadas normalmente como un alimento saludable, tiene fuertes efectos antidiabéticos *in vivo*, por lo tanto, pueden tener propiedades beneficiosas en la prevención de la diabetes.

Palabras Clave: *Ferocactus latispinus, Ferocactus histrix,* antihiperlipidémicos, antihiperglicemia

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INTRODUCTION

Hyperglycemia and hyperlipidemia are two important characteristics of type 2 diabetes, an endocrine disorder based disease. Recently, attention has been focused on the relationship between reactive oxygen species (ROS) and several disorders including aging, various inflammatory diseases, carcinogenesis, neurodegenerative diseases, and diabetes. In fact, diabetes is usually accompanied by increased production of ROS and impaired antioxidant defense, indicating a central contribution of ROS to the onset, progression, and pathological consequences of diabetes (Venkatesh et al., 2010). There is considerable evidence that chronic hyperglycemia is the proximate cause of retinopathy, kidney failure, neuropathies, and macrovascular diseases in diabetes. In addition, it has been demonstrated that β cells are particularly susceptible to oxidative damage. Therefore, as hyperglycemia worsens, β cells steadily deteriorate, secrete less insulin, and participate in a downward spiral of loss of pancreatic functions. Diabetes is a major risk factor for premature atherosclerosis, and oxidative stress plays an important role since diabetic monocytes produce increased superoxide anion (O_2^-). Hyperglycemia may lead to increased generation of free radicals *via* multiple mechanisms (Coskun et al., 2005). The chronic presence of high glucose levels enhances the production of reactive oxygen species (ROS) from protein glycation and glucose autoxidation, which in turn catalyze lipid peroxidation. Accordingly, disturbances of antioxidant defense systems in diabetes have been reported; alteration in antioxidant enzymes, impaired gluthathion metabolism, and decreased ascorbic acid levels. Some studies on diabetes reported a significant increase in the products of both plasma and tissue lipid peroxidation compared with control (Fridlyand and Philipson, 2005). These findings resulted in new approaches to the treatment of diabetic patients, and the relevant studies focus on the support of antioxidant systems. Insulin deficiency stimulates lipolysis in the adipose tissue, and gives rise to hyperlipidemia and fatty liver. Accordingly, although diabetes is characterized as a disease of carbohydrate metabolism, abnormalities of lipid and lipoprotein metabolism are commonly observed. Many natural products and medicinal plants have been shown to significantly reduce oxidative stress and increase the antioxidant endogenous system, which represent an important property of plant medicines used for the treatment of several diseases including diabetes (McCune and Johns, 2002).

Ferocactus are plants widely distributed in several regions of Central Mexico and popularly known as 'biznaga' or ' Lengua del demonio'. The fruits of the plant are edible and commonly sold in local markets. The plant have ball-shaped and is consumed as "acitron" which is crystallized biznaga with sugar. In Mexico it has been used with medicinal purposes since pre-Hispanic times (Andrade-Cetto and Heinrich, 2005). After going through the literature available, it seems that no phytochemical characterization and experimental work has been carried out to verify the claims of such anti-diabetic activity. The current investigation is an attempt to study the hypoglycemic effect of fruit extracts on normal and streptozocin induced diabetic, are known as an insulin-deficiency model.

Lista de abreviaciones: STZ- estreptozotocina; G6Pase-Glucose-6-phosphatase; GK-glucokinase; NADPH-β-nicotinamide-adenine dinucleotide phosphate; SOD-superoxide dismutase; CAT-catalase; glutathione reductase (GR) and glutathione peroxidase (GSHPx)

MATERIALS AND METHODS

Plant material

Fresh fruits of *Ferocactus latispinus* (FL) and *Ferocactus histrix* (FH) were collected at Hidalgo State Mexico. A voucher specimen (No. 6922 and 6923 respectively) was deposited in the Herbarium of the Escuela Nacional de Ciencias Biologicas, IPN for further reference.

Animals

Study was conducted in male Wistar strain albino rats of the same age and weight (180-225 g). Before and during experiment, animals were fed with normal laboratory diet and water ad libitium. Procedures involving animals and their care conformed to the international guidelines Principles of Laboratory Animals Care.

Preparation of plant extracts

Fresh mature fruits of *Ferocactus latispinus* (FL) and *Ferocactus histrix* (FH) were cut into small pieces and each were dried in the laboratory at room

temperature and powdered in a mechanical grinder. To prepare the extract, 300 g of the plant powder was extracted with 1 l of hexane, chloroform and methanol consecutively using a soxhlet apparatus. The residue was removed by filtration and the filtrate was evaporated to dryness at 40-50 ^{o}C under reduced pressure in a rotary evaporator for complete removal of solvent. Aqueous suspension extract fruits were prepared using 2% (v/v) Tween-80 and used for oral administration.

Acute toxicity studies

Healthy adult Wistar strain of albino rats weighing between 180-230 g of either sex, they were orally fed with hexane, chloroform and methanol extracts of FL and FH increasing dose levels of 1, 2 and 3 g/kg body weight. Animals were observed continuously for 2 h for behavioral, neurological and autonomic profiles and after a period of 24 and 72 h for any lethality or death (Turner, 1965).

Study on glucose-loaded animals (oral glucose tolerance test)

After overnight fasting, an 0-min blood sample was taken from the diabetic control rat group and diabetic plant extract group. Rats of both groups were loaded with glucose (2 g/kg, p.o.) 30 min after administration of the drugs or vehicle (for control). Blood samples were drawn by retro-orbital puncture. Blood glucose levels were measured at 30, 60, 120 and 180 min after glucose load to assess the effect of different doses of extract on blood glucose levels of the animals.

Experimental design

Rats were fasted for 24 h before the induction of diabetes by STZ injection. Experimentally induced SD conditions were developed in a group of normoglycemic male Wistar strain albino rats by single intravenous injection of STZ at the dose of 65 mg/kg body weight/rat in 0.5 ml of physiological saline. This single dose of STZ produced SD or type-I having fasting blood glucose level more than 250 mg/dl after 24 h of STZ injection and this diabetic state was maintained throughout the experimental schedule:

Normal rats: treated with (FL) and (FH) extract 100, 200 or 300 mg/kg body weight.

Severe diabetic: treated with (FL) and (FH) extract 100, 200 or 300 mg/kg body weight.

Animals of control group: received only 2% Tween-80 Solution.

All groups were treated with extract or vehicle for 28 days. The fasting blood glucose was monitored (day 0, at 2, 4, 6, 8, 1 2 hr after extracts administration). Drug solutions or vehicle or standard such as tolbutamide (40 mg/kg body weight) or glibenclamide (0.5 mg/kg body weight) were administered orally by gastric intubations once daily at 9:00 am. On the 28th day, diabetic animals were sacrificed after blood collection, under ether anesthesia and the liver was removed.

Measurement of glucose

Rats were fasted for 18hr and blood samples were collected by puncture of retro-orbital plexus immediately with capillary tube under ether anesthesia into glass vials containing a small quantity of a mixture of potassium oxalate and sodium fluoride as an anticoagulant at 0 hr (before treatment) and after treatment. Plasma blood glucose levels were determined by using GOD-POD method. This colorimetric method is based on the enzymatic reactions of invertase and glucose oxidase-peroxidase (GOD-POD) (Sang-Eun et al., 2007).

Serum lipid profile

Triglycerides content and total cholesterol levels in serum were estimated by Diagnostic Reagent Kit (Genzyme Diagnostics). The level of lipid peroxidation was measured as a thiobarbituric acid reactive substance (TBARS).

Determination of insulin

Serum insulin was measured by a GLAZYME INSULIN-EIA TEST from diabetic rats. 300 mg/kg of extracts were orally administered to the rats for 28 days, blood samples were taken for insulin determination. The level of insulin in serum was expressed in µIU/ml.

Assay of glycogen content

Rats of each group were orally administered 300 mg/kg of extracts for 28 days, they were sacrificed and their livers and skeletal muscle were removed. 100 mg of the liver tissues were homogenized in 5 volumes of an ice-cold 30% (w/v) KOH solution and dissolved in a boiling water-bath (100 ^{o}C) for 30 min. The glycogen was precipitated with ethanol, washed, and resolubilized in distilled water. Thereafter, hepatic glycogen content was determined by the anthrone-

reagent method (Seifer et al., 1950). The amount of blue compound generated by the reaction was assayed by a spectrophotometer at 620 nm. The glycogen content was expressed as mg/g wet tissue.

Assay of G6Pase and protein

The hepatic G6Pase activity was assayed by the method of Trinder et al (1969). In brief, the glucose-6-phosphate in the liver extract was converted into glucose and inorganic phosphate. The inorganic phosphate liberated was determined with ammonium molybdate; ascorbic acid was used as the reducing agent. Excess molybdate was removed by the arsenite-citrate reagent, so that it could no longer react with other phosphate esters or with inorganic phosphate formed by acid hydrolysis of the substrate. The amount of phosphate liberated per unit time, determined as blue phosphor-molybdenum complex was measured by spectrophotometer at 700 nm, it shows the glucose-6-phosphatase activity (Trinder et al., 1969). Protein content of liver extract was quantified with Bradford reagent (Bradford, 1976). Glucose-6-phosphatase activity (mU) was expressed as mmol of phosphate released/min/ mg of protein.

Assay of GK activities in liver

GK (glucokinase) activity was measured using a method previously described by Panserat et al., (2001). β-nicotinamide-adenine dinucleotide phosphate (NADPH) generated by GK was measured using a spectrophotometer at 340 nm. GK activity was estimated by standard method, i.e. subtracting the rate of NADPH formation in presence of 0.5 mM glucose from that obtained in presence of 100 mM glucose. Protein concentration was quantified with Bradford reagent and one unit of enzyme activity (mU) was defined as mmol of substrate molecules converted by 1 mg protein per minute.

Measurements of SOD, CAT, GR and GSHPx

The livers were homogenized with a homogenizer (Ultra Turrax T25, Rose Scientific Ltd., Edmonton, Canada) in 10 volumes of a 50 mM sodium phosphate buffer (PH 7.4) at 4 °C. Homogenates were centrifuged (Beckman, U.S.A.) at 15000Xg for 10 min, and the supernatant obtained was used for the following antioxidant enzyme measurements. Antioxidant enzyme activities in the liver were assayed using commercial kits superoxide dismutase (SOD) assay kit Bioxytech SOD-525 for SOD activity (Oxis International), catalase assay kit for catalase activity (Cayman Chemical), glutathione peroxidase assay kit Bioxytech GPx-340 for GSHPx activity (Oxis International), and glutathione reductase assay kit Bioxytech GR-340 for GR activity, (Oxis International).

Statistical analysis

Data are shown as mean ± S.E.M. and statistical analyses were performed by means of the Student's t-test or by one-way ANOVA, followed by Dunnett´s multiple-comparison test (DMRT) using SPSS software when appropriate. The statistical significance was assumed at p levels less than 0.05. Concentrations to produce 50% of the maximal responses (EC_{50}) were calculated by a nonlinear curve fitting procedure using Sigma Plot version 8.0.

Results

Acute toxicity was tested up to a high concentration of 3 g/kg. Extracts test did not show any mortality up to a dose of 3 g/kg body weight. Even at this high dose there was no gross behavioral changes, and did not exhibit any sign of toxicity.

Table 1 shows changes in the levels of blood glucose in normal, diabetic control and experimental groups after oral administration of glucose (2 g/kg). Diabetic rats showed a significant increase in blood glucose at 60 min and 90 min. In animals treated with chloroform extracts the blood glucose level were significantly ($P<0.05$) decreased after 60 min *F. latispinus* and *F. histrix* treated animals tend to bring values to near normal.

Table1 Effect of chloroform extracts of *Ferocactus latispinus* (FL) and *Ferocactus histrix* (FH) on oral glucose tolerance

Groups (300 mg/kg)	Blood glucose levels (mg/dl) 0 min	30 min	60 min	90 min	120 min
Normal	94.12 ± 0.99	182.43 ± 2.09	163.23 ± 1.13	132.89 ± 1.39	101.67± 1.24
Diabetic control	269.57 ± 6.34	345.36 ± 7.63	401.73 ± 4.48	372.48 ± 5.12	340.11 ± 5.13
Diabetic + FL	248.30 ± 7.65	327.16 ± 7.59 *	302.53 ± 8.24*	164.29 ± 2.39*	143.72 ± 2.26*
Diabetic + FH	258.59 ± 6.52	347.09 ± 6.86*	326.46 ± 2.74*	202.62 ± 3.09 *	184.81 ± 2.87*
Diabetic + Tolbutamide (40 mg/kg)	272.41 ± 4.83	342.37 ± 6.49*	302.81 ± 7.56*	162.51 ± 2.84*	127.76 ± 2.58*

Values are mean ± S.E.M. for 6 animal each group. *p<0.05 when compared with diabetic controls

Table 2 Effect of chloroform extracts of *Ferocactus latispinus* (FL) and *Ferocactus histrix* (FH) on fasting blood glucose level in no-diabetic rats

Group	Dose (mg/kg)	Blood glucose levels (mg/dl) at different hours 0	2	4	6	8	12
Control	----	105.19 ± 4.32	104.13 ± 0.98 (1.0)	103.57 ± 0.73 (1.5)	103.29 ± 2.31 (1.8)	101.89 ± 0.68 (3.1)	102.13 ± 1.28 (2.9)
FL	100	107.28 ± 5.14	93.75 ± 0.94 (12.6)	90.16 ± 2.23 * (16)	82.36 ± 1.67 * (23.1)	85.26 ± 2.79* (20.5)	99.71 ± 1.46 * (7)
	200	98.87 ± 3.98	84.34± 2.12 * (14.7)	80.75± 1.54* (18)	65.84 ± 4.52* (33.4)	69.48± 2.14 * (30)	87.32 ± 0.87 * (12)
	300	98.17 ± 4.65	80.56 ± 1.98* (18.0)	78.04± 2.14* (20.4)	56.71 ± 3.06* (42.2)	61.80± 2.83* (37.0)	79.69 ± 0.34* (19)
FH	100	106.48 ± 5.03	95.70 ± 1.27* (10.1)	92.51 ± 2.63* (13.1)	85.67 ± 1.83 * (19.5)	90.56 ± 2.56 * (15)	99.73 ± 1.35* (6.3)
	200	98.69 ± 4.87	85.53± 0.76* (13.3)	82.36 ± 1.82* (16.5)	68.12 ± 4.21* (31)	72.52± 1.26* (26.5)	89.90 ± 0.87* (9.0)
	300	96.98 ± 3.86	80.68 ± 1.05 * (17.0)	79.48 ± 1.21* (18.0)	59.61 ± 4.27* (38.5)	63.59 ± 1.30 * (32.3)	82.64± 1.92 * (14)
Tolbutamide	40	99.47 ± 4.61	76.85 ± 1.85 * (22.6)	59.34 ± 0.87* (40.3)	56.23 ± 1.69 * (43.4)	59.10 ± 1.08* (40.5)	71.10 ± 1.04* (28.4)

All values are expressed as Mean ± SEM, n=6; Values given in the parenthesis are percent blood glucose. Reduction; *P<0.05 when compared to control group animals; where the significance was performed by Oneway ANOVA followed by post hoc Dunnett´s test.

Effect of different doses of chloroform extracts of *F. latispinus* and *F. histrix* on blood glucose levels of normal, and STZ induced diabetic rats are shown in Tables 2 and 3 respectively. The maximum hypoglycemic activity on normal and diabetic mice, as

compared to the control group of animals (receiving an equal dose of Tween 80) was observed at 300 mg/kg doses. The maximum percentage blood glucose reduction to normal mice at 6 h with 300 mg/kg doses of chloroform extracts of fruits of *F. latispinus* and *F. histrix* were 43.2 and 38.5% respectively. Tolbutamide 40 mg/kg dose produced 43.47% blood glucose reduction at 6 h in normal mice. The effect of several doses of chloroform extracts of fruits of *F. latispinus* and *F. histrix* on fasting blood glucose levels in STZ induced diabetic mice were assessed at different time intervals (Table 3). The percentage of blood glucose reduction of chloroform extract of fruits FL and FH at dose of 300 mg/kg and a time of 6 h were 43.0 and 39.0% respectively. Tolbutamide (40 mg/kg) produced 45.74% blood glucose reductions at 6 h in diabetic mice. Glibenclamide at doses of 0.5 mg/kg exhibited at 6 h blood glucose reduction of 39.82%. Chloroform extracts showed a marginal reduction in glucose level, which was not significant at dose level of 100 mg/kg. However, at 200 and 300 mg/kg dose progressively reduced glucose level to a significant value ($p<0.05$). Among the tested extracts chloroform from fruits was the most active. The results were comparable with of tolbutamide, which acts by stimulation of insulin release (Hermann et al., 1994).

Table 3 Effect of chloroform extracts of *Ferocactus latispinus* (FL) and *Ferocactus histrix* (FH) on fasting blood glucose level in diabetic rats

Group	Dose (mg/kg)	Blood glucose levels (mg/dl) at different hours					
		0	2	4	6	8	12
Diabetic control	----	341.14 ± 1.42	350.21 ± 0.96	347.15 ± 1.93	358.91 ± 2.48	353.11 ± 1.15	369.425± 4.46
FL	100	348.49 ± 1.49	320.28 ± 0.14* (8.0)	316.26 ± 5.01* (9.2)	309.30 ± 3.12* (11.2)	315.36± 0.97 * (10.0)	319.45 ± 3.64* (8.3)
	200	360.31 ± 4.56	317.43 ± 0.19* (12.0)	299.52 ± 4.93* (17.0)	242.27± 2.84 * (33.0)	258.32 ± 1.96* (28.3)	314.62 ± 4.19* (13.0)
	300	363.28 ± 2.98	306.61 ± 1.86* (16.0)	241.20 ± 4.48* (33.6)	206.79 ± 1.99* (43.0)	220.71 ± 1.17* (39.2)	299.08 ± 1.71 * (18.0)
FH	100	350.62 ± 3.85	328.34± 0.12* (6.3)	323.75 ± 6.01* (8.0)	315.13 ± 1.78* (10.1)	319.46± 2.48 * (9.0)	326.53 ± 2.59* (7.0)
	200	349.28 ± 1.98	314.51 ± 0.31* (10.0)	303.06 ± 5.25* (13.2)	241.53± 2.63 * (31)	252.39± 1.21 * (28.0)	312.64 ± 4.56* (10.5)
	300	353.17 ± 3.72	301.47 ± 1.60* (15.0)	281.44 ± 5.21* (20.3)	216.27 ± 3.50* (39.0)	227.47± 1.45 * (35.6)	299.16 ± 1.65* (15.2)
Tolbutamide	40	348.43 ± 4.37	275.11 ± 2.67* (21.0)	213.23 ± 2.59* (39.0)	185.6 ± 1.58 * (46.0)	209.50 ± 2.43* (40.0)	244.27± 3.46* (30.0)
Gibenclamide	0.5	336.62 ± 2.49	269. 51± 1.56* (20.0)	194.48 ± 2.77* (42.2)	202.6 ± 2.11 * (40.0)	214.43± 1.21 * (36.3)	259. 22± 2.81* (23.0)

All values are expressed as Mean ± SEM, n=6; Values given in the parenthesis are percent blood glucose. Reduction; *P<0.05 when compared to control group animals; where the significance was performed by Oneway ANOVA followed by post hoc Dunnett´s test.

The effect of extracts on serum total cholesterol, total triglycerides levels are presented are Table 4. The lowering effect on serum total cholesterol level was found significant ($p<0.05$) with chloroform extracts tested. Table 4 shows TBAR as index of lipid peroxidation in plasma, which increased in diabetic rats during the 28 days of the duration of the experiment. Chloroform extracts from fruits significantly lower these levels as compared to those in diabetic control.

As shown in Table 5 glycogen content was increased by FL and FH as compared with diabetic group. The result in our studies shown that there is an increase in the glycogen content of liver and skeletal muscle of diabetic rats treated with extracts.

G6Pase activity was assessed in all groups (Table 5). Compared with diabetic control, the G6Pase effect was

decreased by FL and FH chloroform extracts. GK activity was increased by FL and FH chloroform extracts. FL and FH extracts decreased G6Pase activity and increased glycogen and GK activity in liver, which indicates increased hepatic glucose uptake and decreased hepatic glucose release. So, both fruits of *F. latispinus* and *F. histrix* enable hypoglycemic activity probably by reducing HGO via decreasing G6Pase activity and increasing GK activity.

Table 4 Effect of chloroform extracts fruits *Ferocactus latispinus* (FL) and *Ferocactus histrix* (FH) on level of serum triglyceride, total cholesterol, TBARS and glycogen content in skeletal muscle in STZ induced diabetic rats

	Mean Concentration (mg/g) ± SEM			
Treatment (300mg/kg)	Triglycerides (mg/dl)	Total cholesterol (mg/dl)	Skeletal muscle glycogen	TBARS (mg/protein)
Normal	91.32 ± 1.29	128.17 ± 2.49	12.57 ± 1.97	0.96 ± 2.01
Diabetic Control	178.15 ± 2.76[a]*	242.89 ± 3.21 [a]*	3.99 ± 2.17 [a]*	1.80 ± 3.47 [a]*
FL	135.48 ± 1.65[b]**	165.43 ± 2.85 [b]**	9.71 ± 1.59 [b]**	1.23 ± 1.26 [b]**
FH	143.31 ± 2.82[b]**	173.07 ± 1.87 [b]**	7.98 ± 2.63 [b]**	1.29 ± 1.68 [b]**
Glibenclamide (0.5)	92.54 ± 1.13 [b]**	129.61 ± 1.76[b]**	12.11 ± 2.41[b]**	1.04 ± 2.20[b]**

All values are expressed as Mean ± SEM, n=6 Values. [a]when compared to normal control group, *P<0.01 [b]when compared to diabetic control group, **P<0.01; where the significance was performed by Oneway ANOVA followed by post hoc Dunnett´s test.

Table 5 Effect of chloroform extracts fruits *Ferocactus latispinus* (FL) and *Ferocactus histrix* (FH) after 28 days treatment on plasma insulin, glycogen levels, activities of liver glucose 6-phosphatase and glucokinase in STZ-induced SD

Treatment (mg/kg)	Glycogen content (mg/kg)	Glucose 6-phosphatase activity (mU)	Glucokinase activity (mU)	Plasma insulin (µU/ml)
Normal control	16.21 ± 4.23	0.37 ± 3.21	3.19 ± 0.09	139.0 ± 3.86
Diabetic Control	11.10 ± 1.69[a]*	0.70 ± 2.37[a]*	1.16 ± 0.05 [a]*	54. 8 ± 1.84[a]*
FL	14.79 ± 5.31*	0.47 ± 2.34[b]**	2.23 ± 0.04 [b]**	91.6± 1.91[b]**
FH	13.15 ± 4.62 *	0.50 ± 6.01[b]**	1.97 ± 0.06 [b]**	86.7± 2.46 [b]**

All values are expressed as Mean ± SEM, n=6; [a]when compared to normal control group, *P<0.01 [b]when compared to diabetic control group, **P<0.01; where the significance was performed by Oneway ANOVA followed by post hoc Dunnett´s test. Extracts (300 mg/kg) were orally administered to the rats.

Table 6 shows the result of antioxidant defense system in the liver (Table 6). In untreated STZ-diabetic rat liver the SOD, GSH, GSHPx and CAT level were significantly lower. Both extracts produced a gradual significant increase in SOD, GSH, GSHPx and CAT level restoring them toward the normal levels.

Serum insulin level was significantly decreased in streptozotocin-induced diabetic rats as compared to the control. There was also a significant difference of this parameter between both groups (Table 5). After 28 days of extracts supplementation to SD rats, there was a significant increase in serum insulin level in respect to SD group.

Table 6 Effect of chloroform extracts fruits *Ferocactus latispinus* (FL) and *Ferocactus histrix* (FH) on antioxidant enzyme SOD, CAT, GSH and GSHPx in SD rats

Treatment (300/kg)	SOD	CAT (U/mg protein)	GSHPx	GSH
Normal Control	24.7 ± 2.03	194.1 ± 2.43	26.8 ± 0.37	3.6 ± 2.78
Diabetic control	13.6 ± 1.84 [a]*	155.3 ± 2.90 [a]*	17.9 ± 0.19 [a]*	2.4 ± 1.57 [a]*
FL	21.3 ± 1.32 [b]**	178.4 ± 4.71 [b]**	22.3 ± 0.96 [b]**	3.1 ± 3.39 [b]**
FH	18.9 ± 1.70[b]**	168.9 ± 3.82 [b]**	20.5 ± 1.45 [b]**	2.9 ± 2.28 [b]**

All values are expressed as Mean ± SEM, n=6 values. [a]when compared to normal control group, *P<0.01. [b]when compared to diabetic control group, **P<0.01; where the significance was performed by Oneway ANOVA followed by post hoc Dunnett´s test.

Discussion

Fruit of *F. latispinus* and *F. histrix* used as a food, is claimed to be useful in treating diabetes. In this present investigation, the main focal points were the evaluation of the effectiveness of fruit extracts of *F. latispinus* and *F. histrix* in the correction of SD, where β cell degeneration is dramatic.

Streptozotocin is known to destroy insulin-producing pancreatic β-cells. Therefore, STZ-treated rat model would appear to represent a good laboratory experimental diabetic state, with residual or remnant insulin production by pancreatic β-cells (Adewole and Ojewole, 2007). Acute treatment of the treated control group of rats with distilled water alone did not produce any significant change ($P > 0.05$) in the blood glucose concentrations of either the fasted normal or the fasted STZ-treated diabetic rats. However, FL and FH 1ike glibenclamide, produced significant reductions in the blood glucose levels of STZ-treated diabetic rats. Provided that still remnant or residual insulin in the pancreatic β-cells, the hypoglycaemic and antidiabetic effects of FL and FH would, therefore, appear to be most probably mediated via a mechanism that is similar to that of glibenclamide. The finding that FL and FH increased plasma insulin concentrations of the treated rats supports the hypothesis that the hypoglycaemic effect of the extract is likely to be mediated through a mechanism similar to that of glibenclamide (Chakravarthy et al., 1982). The possibility also exists, however, that FL and FH could mimic and/or improve insulin action at the cellular level. The results of the oral glucose tolerance test study strongly suggest that FL and FH dose-dependently reduces postprandial blood glucose levels. This postprandial hypoglycaemic effect of FL and FH could be due to its ability to facilitate or enhance clearance of postprandial blood glucose in rats. From glucose tolerance test it has been indicated that this extract did not execute the antihyperglycemic effect by modulating the absorption of glucose in the intestine.

The increase in oxygen free radicals in diabetes could be related to rise in blood glucose levels, leading to auto-oxidation to generate free radicals (Maritim et al., 2003). Increased concentration of TBARS was observed in liver tissue during diabetes. In this study, hepatic TBAR decreased following FL and FH treatment, similar to glibenclamide-treated group. Lipid peroxide-mediated tissue damage has been observed in the development of diabetes mellitus. Elevated level of lipid peroxidation in tissues of STZ-induced diabetic rats is one of the characteristic features of chronic diabetes (Pari and Latha, 2005). In diabetes, it is thought that hypeinsulinemia increases the activity of the enzyme, fatty acyl coenzyme A oxidase, which initiates 13-oxidation of fatty acids, resulting in lipid peroxidation. The increase of lipid peroxidation levels causes functional impairment of membrane by decreasing membrane fluidity and through changing the activity of membrane-bound enzymes and receptors (Manonmani et al., 2005). Lipid peroxidation will in tum result in elevated production of free radicals that are harmful to cells in the body.

The present data also show that STZ-induced diabetes disturbs actions of hepatic antioxidant enzymes (SOD, GSH, GSHPx and CAT). The decreased activities of these enzymes in liver during diabetes mellitus may be due to the production of reactive oxygen free radicals

that can themselves reduce the activity of these enzymes (Kumar et al., 2006). These enzymes could destroy the peroxides and play a significant role in providing antioxidant defenses to an organism. In the enzymatic antioxidant defense system, SOD and CAT are the two important scavenging enzymes that remove superoxide radicals (O_2^-) and hydrogen peroxide, respectively, *in vivo*. GSH plays a main role in minimizing oxidative damage and is known to be involved in the elimination of low H_2O_2 concentrations, whereas CAT is sensitive to higher concentrations of H_2O_2 (Noawaboot et al., 2009). Decrease in SOD, CAT, GSH and GSHPx activities may be due to inadequacy of antioxidant defenses in combating reactive oxygen species (ROS) production. The administration of extracts improves impairments of SOD, GSH, GSHPx and CAT activities in SD. These results suggest that extracts prevents oxidative stress, act as a suppressor against liver cell damage and inhibit the progression of liver dysfunction induced by chronic hyperglycemia (Sowmya and Rajyalakshmi, 1999).

Furthermore, the atenuating effect of this extract on experimental SD has been confirmed here by the testing the glucose-6-phosphatase activity in liver, as well as the quantification of glycogen in liver and skeletal muscle, which are the important indicators of diabetes mellitus. These effects may be due to low activity of cholesterol biosynthesis enzymes and or low level of lipolysis which are under the control of insulin (Boby and Leelamma, 2003). Also the possible mechanism of antihyperglycemic action of this extract appears to be both pancreatic and extra pancreatic, which have been supported here by the serum insulin assay in SD rats. The extra pancreatic effect of this extract has been focused here by the significant recovery of glucose-6-phosphatase activity in liver in SD rats. The extra pancreatic effect may be by the sensitization of insulin receptor in target organ or by inhibiting insulinase activity in both liver and kidney.

Conclusion

In conclusion, *F. latispinus* and *F. histrix* fruit have effects on hyperlipidemia, hypoinsulinemia. Thus, this study provides supporting evidence for the therapeutic potential of fruit of *F. latispinus* and *F. histrix* that may also be helpful to prevent and/or delay the onset of diabetes. We found no toxic effects, therefore, *F. latispinus* and *F. histrix* can be considered as a safe food supplement with potential as an anti-diabetic agent; however the investigation of major active constituents is still in progress.

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