Full Length Research Paper

The effects of *Yucca schidigera* on blood glucose and lipid levels in diabetic rats

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The aim of this study was to investigate the effects of *Yucca schidigera* (YSE) on lipid peroxidation and some biochemical parameters in streptozotocin-induced (STZ) diabetic rats. Four groups of seven rats each were used in this study. Supplementation period was three weeks. The groups were: I. control group (C), normal feed; II. diabetic (DM-YSE), normal feed plus YSE 100 ppm/kg; III. diabetic (DM), normal feed; and IV. yucca group (YSE), normal feed plus YSE 100 ppm/kg. malondialdehyde, glutathione, cholesterol, triglyceride and glucose levels were measured by spectrophotometric methods whereas insulin and leptin levels were measured by ELISA. Pancreatic tissues were examined to distinguish histopathological changes. YSE had increased plasma insulin levels in yucca group compared to the diabetics. There was no significant difference in leptin levels between control and diabetic groups while a significant increase was observed in YSE fed groups compared to others. There was no significant difference among four groups in terms of triglyceride or cholesterol levels. No histological anomalies were observed in pancreatic tissues when compared to the control group, however, STZ-DM induced changes were detected. According to the results of the study, YSE does not have a direct effect on glucose levels in diabetics, however it may act through insulin.

Key words: Diabetes Mellitus, Oxidative stress, *Yucca schidigera*, Histopathological changes

INTRODUCTION

Diabetes mellitus (DM) is a multifactorial chronic metabolic disorder characterized by defects in secretion and/or activity of insulin and is associated with impairments in lipid, protein and carbohydrate metabolisms that result in chronic hyperglycemia (Altan et al., 2006; Cariello, 2003; Chakrabarti and Rajagopalan, 2002). DM is one of the most serious health problems and affects many people worldwide (Committee on the Classification of Diabetes, 2003). This disease is among the first five reasons of death in many countries (Green et al., 2003). Long term effects of DM cause specific chronic complications (Ceriello, 2003; Giugliano et al., 1996). Oxidative stress has been reported to be associated with insulin resistance in various human and animal models (Bhor et al., 2004). Studies conducted on type 2 diabetics showed that there was an inverse correlation between oxidative stress and insulin sensitivity (Pessin et al., 2000). The hallmarks of this disease are insulin deficiency and/or insulin resistance. Insulin deficiency or insulin resistance predominates the development of type 2 diabetes. So, antidiabetic agents and insulin are widely used in the management of diabetes (Al-Achi, 2005; Cariello, 2003; Cariello, 2000; Eddouks et al., 2005). Most of these herbs have been studied increasingly because of their hypoglycemic and antioxidative nature (Harris et al., 1998; Li et al., 2004). The animal and human studies in which saponin-rich herbs were used reported (Cheeke, 1999; Francis et al., 2002) that saponins have antibacterial (Cheeke et al., 2006), antioxidant (Sur et al., 2001), anti-cancer (Sparg et al., 2004), antihypertensive effects

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(Bingham et al., 1978), and have a role in decreasing blood cholesterol (Rao and Kendall, 1986; Southon et al., 1988) and glucose levels (Sparg et al., 2004). Although there is a vast amount of literature indicating hypocholesterolemic, antiinflammatory and antibacterial effects of *Yucca schidigera* extract (YSE), none of them investigated the interaction between yucca (Yc) and hormones such as leptin and insulin that play active role in energy metabolism of diabetics.

Also leptin hormone is closely related with neuro endocrine functions (Casanueva et al., 1999). Data obtained in many studies showed that leptin is reduced basal and glucose stimulated insulin secretion. This suggests that leptin is generating a negative feedback on insulin secretion (Andrews, 1998); which appears to be dose-dependent. The present study was structured as a preparatory work in order to establish the effects of YSE on blood glucose, insulin, leptin and some biochemical parameters of diabetic rats.

MATERIALS AND METHODS

Experimental design

Twenty eight (28) rats (Sprague-Dawley strain with a body weight of 200 to 225 g), fed with standard laboratory chow and water, were used in the study. The study was carried out at the experimental animals laboratory under the control of the Animal Ethics Committee of Afyon Kocatepe University. They were randomly divided into four groups (seven rats per group) and placed in separate cages during the study. The rats which were given yucca extract were placed individually in separate cages and were restricted to consume the same amount of saponin. All animals were fasted overnight. The fasting blood glucose levels were measured at 8 am in all groups. Streptozocin (60 mg/kg dose STZ), an experimental diabetogenic substance, was dissolved in 0.9% cold saline and administered intraperitoneally to the animals in the experimental groups. The fasting blood glucose levels were measured again after two days in those groups. Streptozocin administration resulted in high levels (239.2 ± 41.97 mg/dl) of blood glucose after two days. All animals in the experimental groups were evaluated as experimental streptozocin-induced Diabetes mellitus.

The groups were as follows: Group I: control group (C), (received only the chow); group II: diabetic + yucca (DM-YSE), (100 ppm/kg *Y. schidigera* extract added to the chow); group III: diabetic (DM) (received only the chow); group IV: yucca group (YSE), (100 ppm/kg *Y. schidigera* extract added to the chow). All the four groups were fed on the same hour with the same amount of feed once per day. As the yucca extract was in powder form and it could be mixed up in the feed, all of their feed was powdered in the beginning. The animals which have been given yucca were placed individually to separate cages and 100 ppm/kg yucca extract were added to the standard rat feed. The only difference between the yucca groups and the others was the additional yucca extract in their feed. Yucca extract was purchased by ordering from the USA as ready to use. Supplementation period was three weeks.

Plant material

Y. schidigera extract (Sarsaponin 30® includes > 8% steroidal saponin), incorporated in the trial rations of the study as source of saponin, were provided from Desert King Int. (San Diego, CA, USA).

Biochemical measurements

A drop of blood taken from the tails of rats were used to determine blood glucose levels with Accu-check test strips (Bayer, Germany). The measurement was checked once in two days intervals. At the end of treatments, blood samples were collected before morning feeding from all the rats after the animals were fasted overnight and they were sacrificed by anesthetizing with a combination of ketamine and xylazine HCl (ketalar, 20 mg/kg, i.p.). Blood samples were taken from the heart into tubes with heparin and plasma was obtained by centrifugation at 3000 rpm (+4°C) for 10 min.

Malondialdehyde (MDA), cholesterol, triglyceride, glucose, insulin and leptin levels were determined from the blood samples. MDA was estimated according to the Draper and Hardley method (Draper and Hardley, 1990). Plasma leptin concentration and plasma insulin levels were determined by using commercially available rat/mouse insulin ELISA kit (Linco). The levels of triglycerides and total cholesterol were measured by commercially available assay kits (TECO Diagnostics, CA, USA) by using Shimadzu UV-1601 spectrophotometer.

Histopathological findings

Directly after decapitation, pancreatic tissue samples of the rats were fixed in 10% neutral formal. Routine histological analysis of the tissues was performed. The paraffin-embedded tissue samples were sectioned at 5 μ and scrutinized under light microscope (Nikon Eclipse E600).

Statistical analyses

Statistical analyses of data from the experiment were analyzed with SPSS statistical software (SPSS for Windows; release 10.0.1 standard version) using Duncan's test.

RESULTS

In this study, we found that glucose levels increased significantly (p<0.001) in the diabetic groups (II and III) compared to the control group (I). Also, plasma insulin levels increased significantly, particularly in the yucca group (IV) when compared to DM-YSE (II) and DM (III) groups (p<0.05) and this was statistically important. There was a significant increment in MDA levels in DM-YSE (II) and DM (III) groups compared to the control group (I) (p<0.001), whereas MDA levels decreased in yucca group (IV) compared to the control group (I). No significant difference in terms of leptin levels were detected between the DM-YSE group (II) and the control group (I) (p>0.05), while it was significantly higher in the yucca group (IV) than the other groups (II, III) (p<0.001). It was also found that cholesterol and triglyceride levels increased significantly in the DM group (III) compared to the yucca group (IV) (p>0.05), however, there was a significant decrease in plasma total cholesterol and triglyceride levels (p>0.05) in the yucca group (IV) compared to the control group (I) (Table 1).

The results of the examination of tissues from all groups are given in Figure a, b, c and d. We observed histological features belonging to pancreatic tissue in the

Table 1. Levels of biochemical parameters in all studied groups.

Parameter	C ($\overline{X}$ ±SE)	DM-YSE ($\overline{X}$ ±SE)	DM ($\overline{X}$ ±SE)	YSE	Statistical significance ($\overline{X}$ ±SE)
Glucose (mg/dl)	124.66±3.63*	248.57±25.28*	479±28.98*	66.85±2.72 *	*: II,III-I (p<0.001)
İnsulin (ng/ml)	0.29±0.02*	0.27±0.01*	0.23±0.01*	0.35±0.03*	*: IV-II,III (p<0.05)
MDA(nmol/ml)	2.99±0.10*	3.89±0.17*	4.57±0.31	2.70±0.07*	*: II,III-I (p<0.001)
Leptin (ng/ml)	0.69±0.48*	0.86±0.24*	0.58±0.13	1.44±0.30*	*: IV-other groups (p<0.001)
Cholesterol (mg/dl)	56.84±2.62	51.66±2.18	65.48±3.79	47.37±2.60	No significant difference
Trigliseride (mg/dl)	59.79±3.74	55.81±3.25	70.41±3.68	42.04±4.45	No significant difference

Values are shown mean± S.E. Values with different letters show statistically significant (*p<0.05). C, Control group; DM-YSE, diabet+yucca group; DM, diabet group; YSE, yucca group.
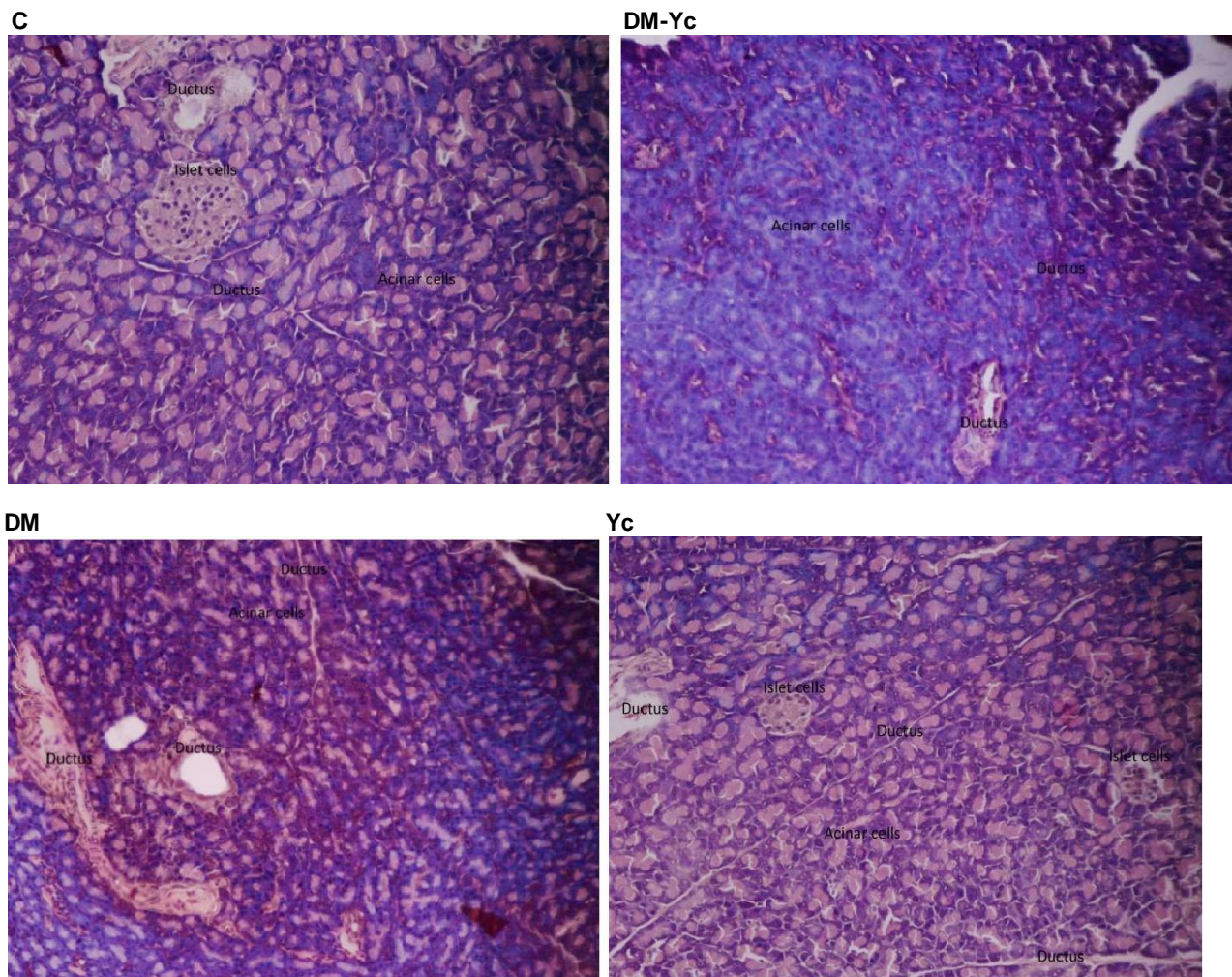

Figure 1. Histopathological changes in pancreatic tissues of experimental groups. a) Control group (C). b) Diabet+Yucca Group (DM-Yc). c) Diabet group (DM). d) Yucca group (Yc) (Nikon Eclipse HEX600).

control group. Pancreatic acinar cells were found in abundance, while zymogen granules in their structure could be clearly distinguished. The islets of langerhans were scattered throughout the pancreas. Yc group presented the same histological features as the control group. No remarkable histological abnormalities in acinar cell structure of DM group were observed. It was detected that although islets of langerhans were not present in DM- YSE

group, acinar cells were of normal appearance.

DISCUSSION

The characteristics of DM are, diminished glucose utilization in insulin dependent tissues such as liver, muscle, adipose tissues and development of hyperglycemia related with increased glyconeogenesis and hepatic glycolgenolysis (Giugliano et al., 1996). In the study, increased fasting plasma glucose levels of the diabetic groups were detected as a characteristic of diabetes. By the second week of the study period, blood glucose levels were observed to decrease in group Yc and remained lower throughout the study when compared to group DM. In parallel to previous studies, our findings reveal that saponins had a decreasing effect on blood glucose level (Lee et al., 1996; Sparg et al., 2004). We demonstrated decreased insulin levels in group DM and group DM-YSE but not in group Yc, compared to the control group (Table 1). Our findings are consistent with the literature suggesting that STZ reduces insulin levels in experimental animals by causing impairment similar to pathogen-nesis of type 1 DM (Bhor et al., 2004; Lee et al., 2000). In diabetic rats fed with Yucca, plasma insulin levels significantly increased compared to group DM. Saponins have been reported to decrease blood glucose levels in different mechanisms such as stimulating insulin release in pancreas, reducing hepatic glucose production, increasing glucose consumption of tissues (Lee et al., 2000) and impairing glucose absorption from gastrointestinal tract (Bhor et al., 2004). Duffy et al. (2001) reported that 200 ppm supplementation of yucca to the diets of rats increased insulin level markedly despite fluctuations in blood glucose level. According to the literature, saponin containing herbs which are suggested to reduce blood glucose levels through distinct mechanisms and steroidal saponin containing yucca may act by stimulating insulin release in pancreas and enhancing insulin activity (Lee et al., 2000). In our study, decrease in blood glucose levels in DM-YSE group may be due to β cells which release off destructive effects of STZ and maintain homeostatic balance resulting in re-institution of insulin release.

Histopathological examination showed normal pancreatic tissue structure in yucca supplemented groups, similar to that of control group. Islets of langerhans were scattered throughout normal apparent pancreatic acinar cells with abundant zymogen granules. Acinar cells in tissues of the diabetic group had normal structure. The data obtained from histological investigation supports that yucca has enhancing effect on insulin release. Hypertriglyceridemia, hypercholesterolemia and other characteristic features of diabetes were detected in diabetic groups. Total cholesterol and triglyceride levels in the diabetic groups were significantly higher than the other groups, as seen in Table 1. Plasma total cholesterol and triglyceride levels of DM - YSE group and yucca group

decreased significantly. Insulin activates fatty acid uptake into adipose tissue and enables them to be stored as triglyceride (Gurbilek et al., 2004). As a result of insulin deficiency (that is, type 1 DM) and insulin resistance (that is, type 2 DM) a large amount of free fatty acids exist in the circulation. The increase of the free fatty acids in the circulation and the presence of excess amount of free fatty acid in the liver, lead to a decrease in insulin sensitivity and causes a vicious circle (Cefalu, 2002). It was reported that increase of plasma cholesterol levels may be a result of metabolic changes in adipose tissue (Gurbilek et al., 2004). It has been known that saponin containing herbs or saponin extracts affect lipid metabolism (Lee et al., 2000). Southon et al. (1988), Rao and Kendall (1986) and Sidhu and Oakenfull (1986) reported that saponins reduce blood cholesterol levels in rats. It was also reported that saponins reduce cholesterol absorption and increase fecal excretion of neutral sterols such as plant sterols, coprostanol, cholesterol and bile acid (Guclu et al., 2004; Malinow et al., 1981). Hyperglycemia increases oxidative stress over free radical formation and antioxidant system becomes insufficient, ultimately (Dandona et al., 1996). Free radicals are held responsible for the pathogenesis of several diseases such as DM, atherosclerosis, cell damage, cancer, myocardial infarction, hemolytic diseases and autoimmune diseases (Dincer e al., 2003; Wever et al., 1998). Lipid peroxidation (LP) is defined as the effect of free radicals on lipids. Besides from hyperglycemia, LP is generated via lipoxygenase pathway from activated prostaglandins as a result of extensive vascular inflammation. In the present study, the higher increase in LP products of DM group compared to the control group is found to be consistent with the literature (Gurbilek et al., 2004). Only in yucca group, MDA level was below the control group (Table 1).

Insulin secretion is stimulated after oral intake, which, in turn, stimulates leptin synthesis. By the increase of leptin levels in blood, insulin and cortisol levels are diminished to normal levels which establish a new homeostatic balance (Houseknecht et al., 1998). Leptin has effects on regulating appetite and energy use (Fei et al., 1997). Thus, the interaction of cortisol, insulin and leptin may play an important role in adaptation period from fed to starved stage (Bornstein et al., 1997). The addition of 100 ppm YSE to rat's diet led to over two-fold increase in plasma leptin levels compared to control group. As seen in Table 1, yucca was found to increase plasma leptin levels markedly. In a study on supplementation of Persimmon leaf powder, Lee et al. (1996) reported that flavonoids increased leptin levels, and these effects could be related to rich fiber and phenolic compound of herbs. In our study, yucca consisted of phytochemicals such as phenolic materials, fiber, resveratrol and stilbenes (Cheeke, 1999; Francis et al., 2002; Price et al., 1987).

Our study shows that, yucca regulates metabolic disturbances (that is, impaired lipid metabolism) in diabetic rats

and reinstitutes the homeostatic balance. Since it achieves moderate glucose reductions, we suggest that yucca can be used as a supportive agent in blood glucose regulation of diabetics.

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