International Journal of Histology and Cytology Vol. 1 (2), pp. 010-019, February, 2014. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Relative therapeutic potentials of formulated herbal extract and acarbose on diabetic rats

Matisse Berkan and Mehmet Eren

Department of Medical Biology, Faculty of Medicine, Dicle University, Diyarbakir, Turkey.

Accepted 02 January, 2014

Oxidative stress plays an important role in chronic complications of diabetes. Acarbose is an alpha-glucosidase inhibitor used for the treatment of diabetes. A commercial herbal formulated extract, which consists of 13 herbal extract (F13), is also described to have a potential antidiabetic action. The aim of this study was to determine the comparative effects of acarbose and F13 on type 2 diabetic rats. Three to five weeks after induction of diabetes by single dose systemic administration of streptozotocin and nicotinamide (STZ-NA), diabetic rats were treated with acarbose and F13 for two weeks. After the treatment period, the blood glucose, hemoglobin A1c (HbA_1c), triglyceride, cholesterol and nitric oxide synthases (NOS) levels, as well as liver and erythrocyte superoxide dismutase (SOD), malondialdehyde (MDA), catalase (CAT) and glutathione peroxidase (GPx) levels were determined. Renal filtration changes were determined by measuring urine creatinine, plasma creatinine and creatinine clearance. Histological analyses were also performed in liver and kidney. The rats in diabetic groups had significantly higher blood glucose levels than control groups. Induction of diabetes was confirmed by histological analyses of liver and kidney tissues. High blood glucose level in diabetic rats results in peroxidative reactions in lipids, thus MDA levels were increased in diabetic control while acarbose and F13 treatment reduced MDA production. Also, increased SOD levels were found in STZ-NA diabetic rat liver. Both acarbose and F13 treatment, however, showed similar improving effects on diabetic complication in diabetes. Our results, therefore, support the validity of this herbal extract on the management of diabetes as well as diabetes-induced liver and renal complications.

Key words: Acarbose, type 2 diabetes mellitus, free radicals, herbal preparation, histology, rats.

INTRODUCTION

Diabetes mellitus is a syndrome characterized by abnormal insulin secretion, derangement in carbohydrate and lipid metabolism, and is diagnosed by the presence of hyperglycemia. Diabetes is also a risk factor for chronic renal disease. It is a major worldwide health problem and once it occurs, chronic renal failure and end-stage renal disease increases the mortality in type 2 diabetic patients (Atkins, 2005; Atalay and Laaksonen,2002; Ritz and Orth, 1999, Akyuz et al., 2012). According to previous studies, oxidative stress playing an important role in chronic complications of diabetes is postulate to be associated with increased lipid peroxidation. In addition, enhanced oxidative stress and lower antioxidant capacity might be related to etiology of diabetic complications (Pitkanen et al., 1992; Elangovan et al., 2000; Bukan et al., 2003).

Experimental diabetic models could be developed by using certain genetic, chemical and surgical methods. In the new rat model characterized with reduction of 40%

*Corresponding author. E-mail: Dr_berkan@yahoo.co.uk

beta islets in pancreas; 290 mg/kg of nicotinamide (NA) is injected intraperitoneally (i.p) 15 min before the administration of 60 mg/kg of streptozotocin (STZ) (i.p). The main basis of this model is to supply nicotinamide stores consumed by the body and to protect beta cells via nicotinamide.

As a result, this model imitates the characteristics of human type 2 diabetes both histologically and metabolically (stable mild hyperglycemia and glucose intolerance, etc.) (Novelli et al., 2001, 2004; Akyuz et al., 2012).

Acarbose is an alpha-glycosidase inhibitor and an antidiabetic drug used to treatment of diabetes. Acarbose shows its effect by inhibiting intestinal enzymes (alpha-glycosidases), thereby interfering the catabolism of disaccharides, oligosaccharides and polysaccharides in intestines.

Thus, digestion of carbohydrate is delayed dependent on dose, and more importantly, liberation of glucose and its presence in the blood slows down. Moreover, both fluctuations in daily blood sugar and average blood sugar level decrease as a result of this delayed glucose intake through intestines with acarbose. Acarbose also reduces abnormal high concentrations of glycosylated hemoglobin (Wright et al., 1998; Kawamura et al., 1998; Hwua et al., 2003).

Nowadays, drugs are expensive and have many side effects during the treatment of any disorders. Therefore, many herbs have been used for a long time for claimed health benefits, and the potential of the health promoting and disease preventing properties of plant-derived compounds has received increased attention from researchers in recent years (Nasri et al., 2012; Asgarpanah and Ramezanloo, 2012; Alam et al., 2012). Different plants have been used alone or in formulations for the treatment of diabetes and its complications. An example of such herbal formulation under trade name is F13 that is obtained from Karkim (F13®, Karkim Co., Turkey) and traditionally used for the treatment of diabetes in Turkey.

This formulation has the potential to act as antidiabetic. The herbal extract F13 consists of 13 plant parts (*Thymbra spicata*, *Folium myrti, Folium eucalypti globuli, Folium olivarum, Folium sorbi domesticae, Folium juglandis, Lavandula stoechas, Semen foenugraeci, Herba fumariae, Alchemilla vulgaris, Herba millefolii, Folium salviae* and *Herba chamaedrys*) and it is prepared with steam distillation and extraction method by the manufacturer. Most of the herbs in this formulation are used in alternative medicine as antidiabetic, anti-cholesterolemic, antioxidant, diuretic, free radicals cleaner, antiseptic, antihypertensive, antimicrobial and antiviral both in Turkey and in other parts of the world (Buckle, 2001; Inanc et al., 2007; Gunes et al., 1999; Sabu and Kuttan, 2002; Modak et al., 2007; Seeff et al., 2001). The aim of this study was to evaluate the antihyperglycemic, antioxidant and histoprotective effects of acarbose and herbal formulation (F13) on STZ-NA induced type 2 diabetic rats.

MATERIALS AND METHODS

Experimental animals

In this study, three-month-old Sprague-Dawley breed male rats weighing 200 to 250 g and grown in the Department of Medical Biology, Eskisehir Osmangazi University, Medical Faculty, were used. The animals were housed in individual cages at room temperature and left for 1 week for acclimatation before the start of experiment. This study was approved by the local ethical committee of Eskisehir Osmangazi University (affirmation number: 09/21/122).

Treatment

Glucose levels were measured from tail vein at the beginning of the experiment and also once a week during the experiment. The rats were divided into six groups as shown in Table 1. Diabetes was induced by a single intraperitoneal injection of 290 mg/kg body weight NA (Sigma Chemical, St. Louis, Mo., USA) dissolved in saline 15 min before a single intraperitoneal injection of 60 mg/kg STZ (Sigma Chemical, St. Louis, Mo., USA) dissolved in saline immediately before use. Subsequently, the animals were treated with the extract of the herbal formulation (F13®, Karkim Co., Turkey) during two weeks to test possible antidiabetic and antioxidative effects. Moreover, to determine whether the inhibition of alpha-glycosidase has any effect on free radical formation in the type 2 diabetes, acarbose was administrated (Sigma Chemical, St. Louis, Mo., USA) 5 to 8 weeks after the induction of the diabetes, and the effects of administration of F13 were determined by comparing with acarbose. Doses of the given substrates are summarized in Table 1. Subsequently, the rats were sacrificed after the treatment period, and blood, urine, liver and kidney tissue samples were collected under ether anesthesia.

Biochemical analysis

Fasting and postprandial blood glucose was measured every week during the experiments with an Accu-Chek® Go Glucometer (Roche Diagnostics, Mannheim, Germany) in all animals. After determining the volume and pH (pH meter; inoLab® pH 720, WTW Laboratory, Germany) of urine samples collected, urine creatinine and plasma creatinine activity were measured spectrophotometrically by Jaffe's reaction. Creatinine clearance was also calculated from urine creatinine, plasma creatinine and the 24-h urinary excretion volume as described by Gunes et al. (1999). All spectrophotometric measurements were performed by Shimadzu UV-1601 digital spectrophotometer (Schimadzu Corp., Kyoto, Japan). Hemoglobin A1c (HbA_{1c}) levels of groups were measured by Boehringer-Mannheim 911 Hitachi Automatic Analyzer and triglyceride and cholesterol levels were determined by Roche Diagnostic Modular System.

Antioxidant and free radical assays

The method described by Sun et al. (1988) was used to prepare erythrocyte hemolysate. Superoxide dismutase (SOD) activity was determined by SOD determination kit (FLUKA, St. Louis, MO, Cat. No: 19160) based on water-soluble tetrazolium salt (WST) reaction. Malondialdehyde (MDA) reaction was applied based on color reaction of MDA, one of the final products from lipid peroxidation,

Table 1. The substrates given to control and experimental groups.

Groups		n	Administrated substrates
	I	7	(Control) Water + CSD
Control	II	7	Acarbose [5 mg/kg per day for 14 days, i.g.] + Water + CSD
	III	7	F13 [5 ml/kg per day for 14 days, i.g.] + Water + CSD
	IV	7	(Diabetic Control) [60 mg/kg STZ + 290 mg/kg NA i.p.] + Water + CSD
Diabetic	V	7	(60 mg/kg STZ + 290 mg/kg NA i.p.)+ Acarbose (5 mg/kg per day for 14 days, i.g.)+ Water + CSD
	VI	7	(60 mg/kg STZ + 290 mg/kg NA i.p.) + F13 (5 ml/kg per day for 14 days, i.g.) + Water + CSD

CSD, Commercial standard diet; i.g, intragastric.

with thiobarbituric acid (TBA) (Uchiyama and Mihara, 1978). Nitric oxide synthase (NOS) activity, catalase (CAT) activity and glutathione peroxidase (GPx) activity were determined by using nitric oxide synthase assay kit (Bioxytech®, Oxis International Inc, Portland, OR, USA, Cat No: 22113), ammonium molybdate-hydrogen peroxide reaction with manual assay (Goth, 1991) and glutathione peroxidase assay kit (Calbiochem®, EMD Biosciences,. Inc., San Diego, CA, cat. No: 354104), respectively.

Histopathological examination

All tissues were collected from rats, immediately fixed in 10% neutral formalin solution, embedded in paraffin, and then stained with haematoxylin and eosin.

Statistical evaluation

The obtained data were expressed as mean ± standard deviation (S.D.) and analyzed using analysis of variance (ANOVA). Tukey's test was used to test for differences among means when ANOVA indicated a significant difference. Differences were considered statistically significant if $P < 0.05$.

RESULTS

Effects acarbose and F13 treatment on blood glucose levels

The blood glucose levels were determined every week during the experiment. The levels of fasting and post-prandial blood glucose at the beginning of the experiment, and at 5th and 7th weeks are given in Tables 2 and 3, respectively. The diabetic animals exhibited gradually increased hyperglycemia. Acarbose and F13 treatment caused a decrease in the elevated blood glucose levels in STZ-NA diabetic rats. The fasting blood glucose levels were similar in controls and experiment groups before STZ+NA injection (P>0.05). Meanwhile, the glucose levels increased gradually in STZ+NA treated groups (IV, V and VI). The blood glucose levels of groups IV and V were higher in the 5th week when compared to controls (P< 0.05, P< 0.001, respectively). Moreover, at the 7th week, fasting blood glucose levels of acarbose and F13 treated groups decreased significantly (P<0.001) when compared to diabetic control (Tables 2 and 3). The postprandial blood glucose levels were similar in controls and experimental groups before STZ+NA injection (P>0.05). The glucose levels increased gradually in STZ+NA treated groups (IV, V and VI). At the 7th week, after the acarbose and F13 treatment, postprandial blood glucose levels of only acarbose treated group decreased significantly (P< 0.05) when compared to diabetic control (group IV) (Tables 2 and 3). Postprandial blood glucose levels of diabetic control (group IV) and F13 treated diabetic group (group VI) were significantly higher (P<0.01, P<0.001, respectively) when compared to control. At the 7th week, fasting blood glucose levels of acarbose treated group decreased significantly (P< 0.05) when compared to diabetic control.

Biochemical analysis

Triglyceride levels were significantly higher in the acarbose and F13 treated diabetic groups (P<0.05, P<0.001); meanwhile there was no significant difference in HbA_1c and cholesterol levels between controls and other experimental groups (P>0.05) (Table 4).

Urine and plasma creatinine and creatinine clearance

We found significantly higher urine creatinine levels in control groups treated with acarbose and F13 when compared to the control (P<0.01) (Table 5). However, no significant difference in plasma creatinine was found between the control and experimental groups (P>0.05) (Table 5). In addition, creatinine clearance was different in the F13 treated control and diabetic control groups compared to the control group (P<0.01 and P<0.05, respectively) (Table 5). When groups II and III with group I were compared; creatinine clearance level of F13 treated control group (III) was significantly higher (P < 0.05). Further, when groups V and VI with group IV were compared, creatinine clearance levels of acarbose and F13 treated diabetic groups was reduced (P<0.05, P<0.01). No statistical differences were found when

Table 2. The fasting blood glucose levels of control and diabetic groups.

Groups		n	Before injection	5th week (beginning of the treatment)	7th week (final of the treatment)
	I	7	82.42 ± 2.4	75.42 ± 3.0	81.14 ± 2.3
Control	II	7	81.00 ± 1.3	83.42 ± 1.4	84.85 ± 1.9
	III	7	81.42 ± 1.7	84.28 ± 2.1	80.28 ± 1.8
	IV	7	78.00 ± 1.5	89.28 ± 4.1*	103.57 ± 4.1***
Diabetic	V	7	75.42 ± 1.3	93.42 ± 2.6**	78.42 ± 1.83^{+++}
	VI	7	78.85 ± 1.7	87.71 ± 4.1	77.85 ± 4.0^{+++}

* P<0.05 ** P<0.01 *** P<0.001 (Compared to the control) $^{+++}$ P<0.001 (Significance between the diabetic groups when compared to group IV).

Table 3. The postprandial blood glucose levels of control and diabetic groups.

Groups		n	Before injection	5th week (beginning of the treatment)	7th week (final of the treatment)
	I	7	107.85 ± 2.7	102.00 ± 2.7	109.57 ± 3.8
Control	II	7	98.14 ± 3.5	110.00 ± 2.6	111.00 ± 4.5
	III	7	98.71 ± 3.9	111.57 ± 1.7	114.85 ± 5.9
	IV	7	113.14 ± 3.2	154.00 ± 12.4	169.28 ± 6.0***
Diabetic	V	7	102.14 ± 3.8	134.42 ± 7.5	137.28 ± 4.2^{+}
	VI	7	100.85 ± 4.0	162.00 ± 29.8*	149.42 ± 14.3**

* P<0.05, ** P<0.01, *** P<0.001 (compared to the control); $^{+}$P<0.05 (significance between the diabetic groups when compared to group IV).

Table 4. The HbA_1c, triglyceride and cholesterol levels of controls and experimental groups (mg/dl).

Groups		n	HbA_1c (mg/dl)	Triglyceride (mg/dl)	Cholesterol (mg/dl)
	I	7	4.58 ± 0.30	24.14 ± 4.05	46.14 ± 3.23
Control	II	7	4.92 ± 0.77	29.14 ± 6.22	33.28 ± 4.64
	III	7	4.31 ± 0.13	27.14 ± 3.71	43,00 ± 5.85
	IV	7	4.85 ± 0.58	28.85 ± 6.91	44.42 ± 11.19
Diabetic	V	7	4,58 ± 0.51	46.14 ± 15.74**	44.57 ± 16.52
	VI	7	4.25 ± 0.12	60.71 ± 10.35***	42.71 ± 6.82

** P<0.01, *** P<0.001 (compared to the control).

Table 5. The Urine pH, urine volume, urine creatinine, plasma creatinine and creatinine clearance levels of controls and experimental groups.

Groups		n	Urine pH	Urine volume (ml)	Urine creatinine (mg/dl)	Plasma creatinine (mg/dl)	Creatinine clearance (ml/min)
	I	7	8.0 ± 0.3	3.4 ± 0.5	139 ± 6	1.90 ± 0.05	0.168 ± 0.02
Control	II	7	7.7 ± 0.2	3.9 ± 0.3	345 ± 35**	1.91 ± 0.02	0.475 ± 0.04
	III	7	7.6 ± 0.3	4.0 ± 0.3	344 ± 7**	1.84 ± 0.01	0.528 ± 0.04*
	IV	7	8.3 ± 0.3	7.0 ± 0.4**	236 ± 53	1.86 ± 0.03	0.637 ± 0.15**
Diabetic	V	7	8.8 ± 0.7	2.6 ± 1.0	191 ± 38	1.84 ± 0.02	0.149 ± 0.06^{++}
	VI	7	8.6 ± 0.7	3.7 ± 0.7	240 ± 7	2.03 ± 0.09	0.286 ± 0.05^{+}

* P<0.05, ** P<0.01 (compared to the control); $^{+}$P<0.05, $^{++}$ P<0.01 (significance between the diabetic groups when compared to group IV).

groups V and VI were compared with groups II and III.

Effects on antioxidant and free radical levels

Erythrocyte MDA, SOD, CAT, GPx and serum NOS levels are presented in Table 6. There were no significant differences in the SOD, CAT and GPx activities between control and diabetic groups. Erythrocyte MDA levels significantly decreased in the F13 treated diabetic groups ($P<0.001$). On the other hand, increased serum NOS levels in acarbose treated diabetic groups were obtained. MDA, SOD, CAT and GPx levels of liver homogenates were presented are Table 7. The results obtained indicated no significant differences in the CAT and GPx activities between control and diabetic groups. In addition, SOD and MDA levels of the untreated diabetic groups (diabetic control) were significantly increased ($P<0.001$).

Histopathological findings

In this study, binuclear hepatocytes, sinusoidal dilatations, nuclear hypertrophy, necrotic cells with pyknotic nucleus and eosinophilic cytoplasm were observed in diabetic control groups (Figures 1 and 2). In the acarbose and F13 treated diabetic group, hepatocytes and sinusoidal structures showed nearly normal histology and binuclear hepatocytes were observed in some areas (Figures 3 and 4). In addition, normal liver histology was seen in control groups. Moreover, the kidney samples of control groups were histologically normal (Figure 5), whereas glomerular basement membrane thickening, the distal tubular epithelium, cytoplasmic clear cell change and medial thickening of small arteries were observed in diabetic control (Figure 6). The kidney samples of acarbose treated diabetic groups showed almost normal histological structure, although glomerular basement membrane thickening were observed in some areas (Figure 7). In F13 treated diabetic groups, glomerular basement membrane thickening was also observed in some areas (Figure 8).

DISCUSSION

In the present study, antihyperglycemic, antidiabetic and antioxidative potential effects of the formulated herbal extract were evaluated in a nicotinamide and streptozotocin -induced diabetic rat model. The herbal extract (F13) consisted of *T. spicata*, *F. myrti, F. eucalypti globuli, F. olivarum, F. sorbi domesticae, F. juglandis, L. stoechas, S. foenugraeci, H. fumariae, A. vulgaris, H. millefolii, F. salviae* and *Herba chamaedrys* extracts. The antidiabetic, anti-cholesterolemic, antioxidant, diuretic, free radicals cleaner, antiseptic, antihypertensive, anti-microbial and antiviral effects of these plants have been reported (Buckle, 2001; Inanc et al., 2007; Gunes et al., 1999; Sabu et al., 2002; Modak et al., 2007; Seeff et al., 2001). Likewise, in our study, clear reduction in blood glucose levels was observed in STZ-NA induced diabetic rats treated with F13.

Hemoglobin A1c (HbA1c) is the most common measurement for the determination of glycemic control for patients with diabetes. There is a concern that the measurement of HbA1c may be affected by the severity of kidney dysfunction or the hematological complications of kidney disease (Cavanaugh, 2007). It has been reported that HbA1c values, triglyceride and cholesterol levels in diabetic subject were significantly higher (Kuppusamy et al., 2010). In our study, however, there were no significant differences in HbA_1c and cholesterol levels between control and treated diabetic groups. STZ-NA induced type 2 diabetic model imitates the characteristics of human type 2 diabetes and it is characterized by stable mild hyperglycemia and glucose intolerance (Novelli et al., 2001; Novelli et al., 2004). The stable HbA1c values in our study may be due to stable mild hyperglycemia or short retention time of the experiment (lack of glucose toxicity). Ordinarily, insulin activates the lipoprotein lipase, which hydrolyses triglycerides. However in diabetic condition, lipoprotein lipase is not activated due to insulin deficiency, thus resulting in hypertriglyceridemia (Kuppusamy et al., 2010). Similarly, we found increased triglyceride levels in diabetic acarbose and diabetic F13 groups when compared to control.

The kidneys are important target organs of diabetes and kidney failure often leads to death in diabetes. Diabetes causes glomerular lesions, atherosclerosis of renal veins, pyelonephritis and nephropathy (Prakash et al., 2007; Chen et al., 2007). Increased urine volume and creatinine clearance can also be observed in diabetes (Gunes et al., 1999; Murali et al., 2003). Glomerular filtration rate is determined by measuring creatinine clearance, and decrease in creatinine clearance indicates glomerular degeneration (Murali et al., 2003). In contrast, we found increased creatinine clearance levels in diabetic control. Also, creatinine clearance levels of acarbose and F13 treated diabetic groups were decreased when compared to diabetic control. The assay for creatinine in plasma and urine were defined by Jaffé (1886). However, difficulties with the reaction with respect to its lack of specificity and sensitivity have been discussed. Thus, plasma creatinine based measurements remain the most widely used method to assess renal function in animals (Dunn et al., 2004). In this study, there were no statistical differences in plasma creatinine levels of groups when compared to control, and this may be because STZ-NA diabetes shows mild renal insufficiency depending on the duration of experiment.

Diabetes causes increased oxidative damage through generation of reactive oxygen species (ROS), and free

Table 6. Erythrocyte SOD, MDA, CAT, GPx, and serum NOS levels.

Groups		n	SOD (% inhibition)	MDA (U/gHb)	CAT (kU/L)	Gpx (nmol/min/ml)	NOS (nmol/ml/s)
Control	I	7	53.53 ± 10.2	13.10 ± 2.0	213.13 ± 25.58	4.00 ± 2.00	0.23 ± 0.19
	II	7	46.65 ± 5.30	25.50 ± 4.5	208.57 ± 21.29	5.09 ± 2.07	0.21 ± 0.10
	III	7	52.97 ± 4.26	19.11 ± 4.6	207.30 ± 30.33	3.63 ± 1.36	0.27 ± 0.04
Diabetic	IV	7	48.58 ± 1.87	35.27 ± 12.6***	209.48 ± 26.05	5.45 ± 3.09	0.17 ± 0.06
	V	7	58.01 ± 14.52	23.27 ± 12.2	215.56 ± 45.17	3.42 ± 1.20	0.38 ± 0.09^{++}
	VI	7	44.79 ± 3.76	15.47 ± 1.9^{+++}	218.50 ± 20.64	3.74 ± 1.23	0.10 ± 0.03

*P<0.05, ** P<0.01, *** P<0.001 (compared to the control); $^{+}$P<0.05 , $^{+++}$ P<0.001 (significance between the diabetic groups when compared to group IV).

Table 7. Liver SOD, MDA, CAT and GPx levels.

Groups		n	SOD (%inhibition)	MDA (U/wet tissue)	CAT (kU/ml protein)	Gpx (nmol/min/ml)
Control	I	7	50.14 ± 3.80	2.57 ± 0.12	3.25 ± 0.20	11.31 ± 1.98
	II	7	50.57 ± 3.50	2.57 ± 0.18	3.21 ± 0.40	12.01 ± 1.01
	III	7	48.85 ± 7.81	2.49 ± 0.05	3.28 ± 0.14	11.56 ± 0.96
Diabetic	IV	7	63.28 ± 3.90***	3.27 ± 0.38***	3.43 ± 0.14	11.64 ± 1.27
	V	7	56.71 ± 4.68	2.79 ± 0.10^{++}	3.42 ± 0.14	10.40 ± 1.73
	VI	7	51.28 ± 3.59	2.69 ± 0.19^{+++}	3.19 ± 0.12	12.88 ± 2.35

*** P<0.001 (compared to the control); $^{++}$P<0.01, $^{+++}$P<0.001 (significance between the diabetic groups when compared to group IV).

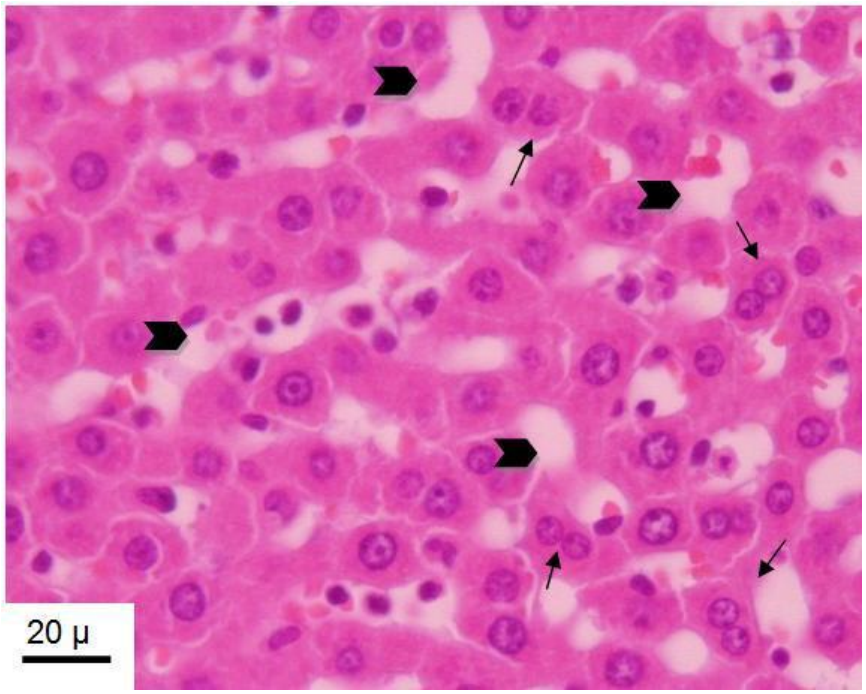

Figure 1. Diabetic groups: binuclear hepatocytes (→) and sinusoidal dilatations ( ), H and E × 100.

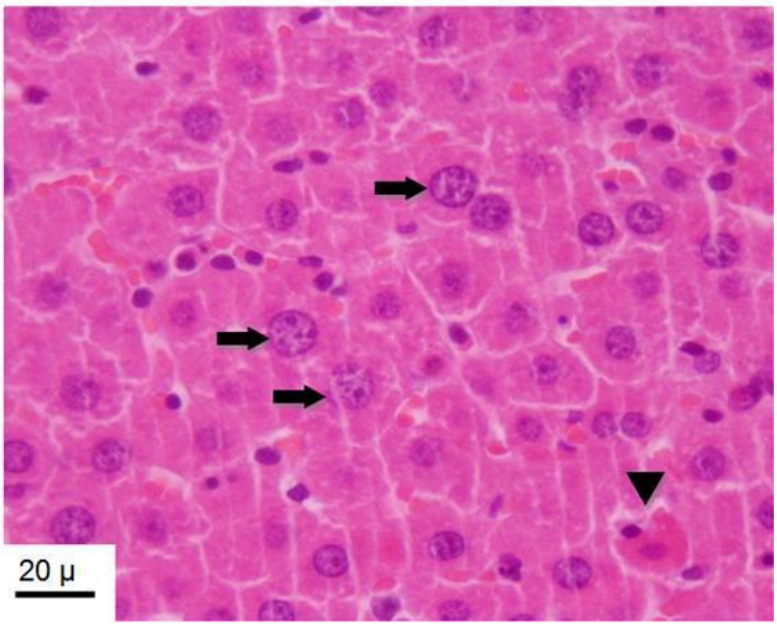

Figure 2. Diabetic group showing nuclear hypertrophy (➡) and the necrotic cells with pyknotic nucleus and eosinophilic cytoplasm (▼), H and E × 100.

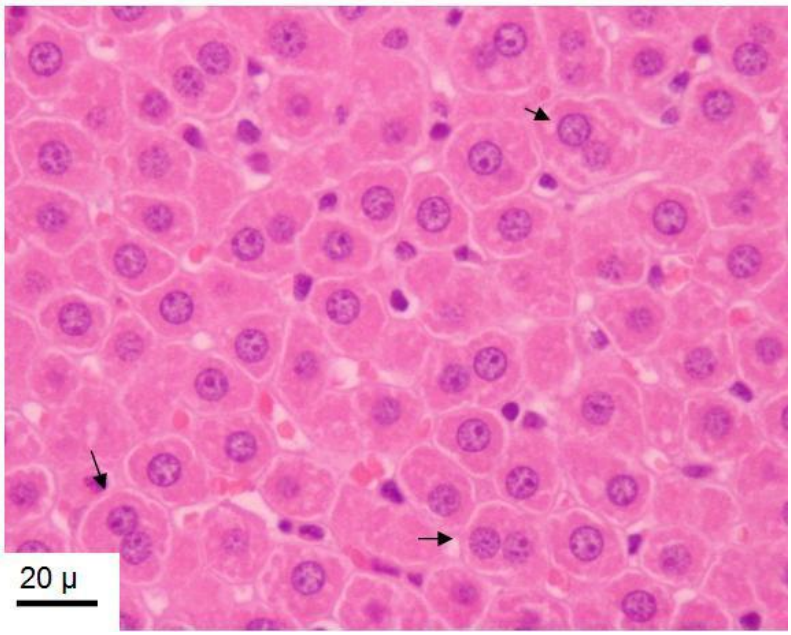

Figure 3. Acarbose treated diabetic group: Hepatocytes and sinusoidal structures in diabetic-acarbose group showed nearly normal histology, while binuclear hepatocytes were observed in some areas (→). H and E × 100.

radical-mediated oxidative stress has an important role in the pathogenesis of various diabetic complications. Antioxidant defense system allows a balance between the generation of oxidants and antioxidants. The range of antioxidant defenses should be adequate to protect against oxidative damage (Pitkanen et al., 1992; Elangovan et al., 2000; Kuppusamy et al., 2010). Overproduction of superoxide takes place when cellular

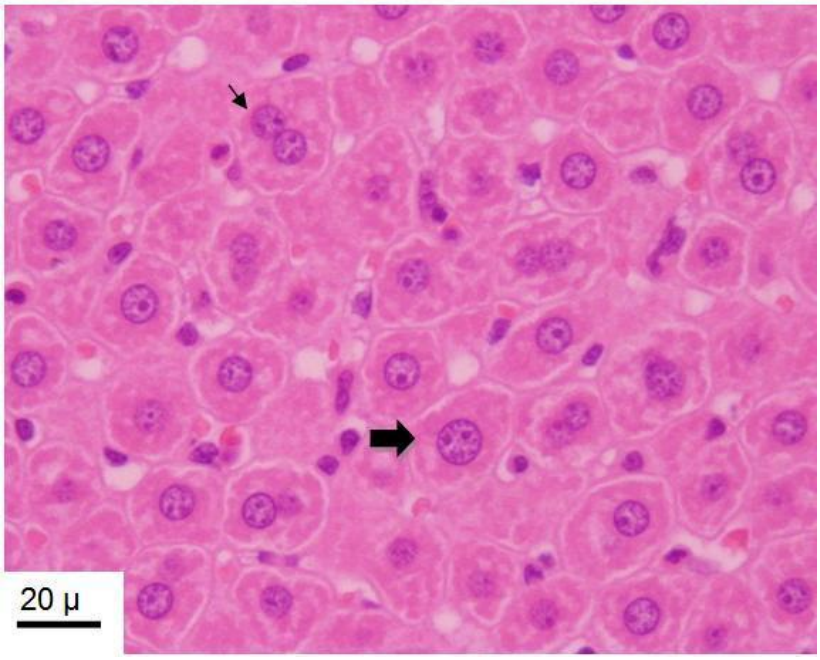

Figure 4. F13 treated diabetic group: Hepatocytes and sinusoidal structures in diabetic-F13 group showed nearly normal histology, binuclear hepatocytes (→), while nuclear hypertrophy (▬►) were observed in some areas, H and E × 100.

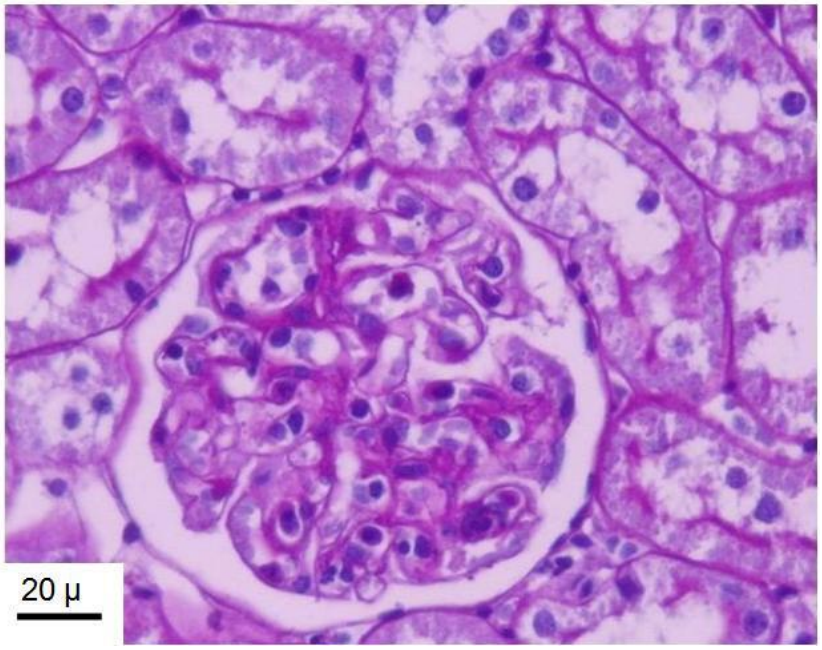

Figure 5. Control group showing histologically normal kidney samples. H and E × 100.

metabolism is destroyed by overproduced glucose, and these results in diabetes complications. Superoxide dismutase is an important antioxidant enzyme and it reduces the increased superoxide by converting it into peroxide and oxygen (Ceriello, 2010; Kuppusamy et al., 2010). Hyperglycemia as demonstrated in this study

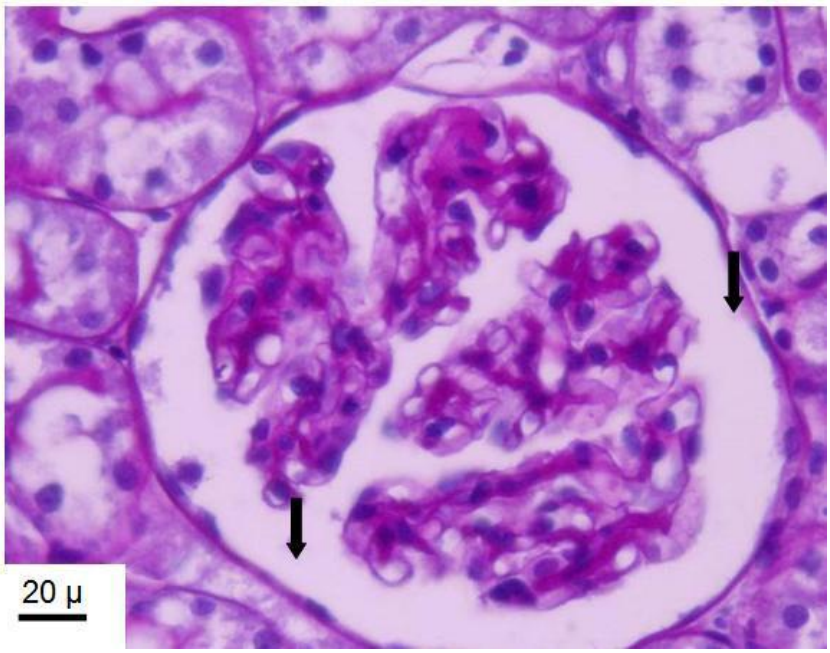

Figure 6. Diabetic control group showing glomerular basement membrane thickening (→). H and E × 100.

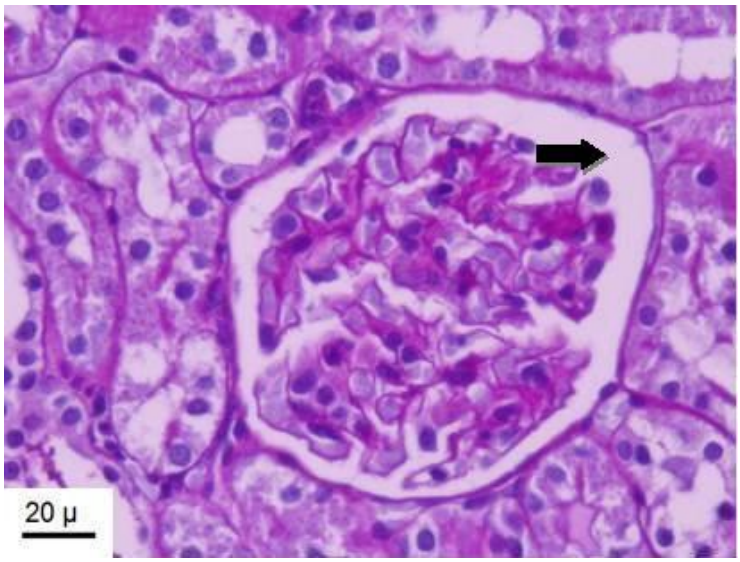

Figure 7. Diabetic acarbose group: nearly normal histological structure, glomerular basement membrane thickening (→) was observed in some areas. H and E × 100.

might be associated with the oxidative stress. In several studies, contradictory SOD activities were reported as decreased, unchanged or increased in type 2 diabetes (Tas et al., 2007). In our study, no difference was found in erythrocyte SOD activities, whereas liver SOD activities significantly increased in diabetic control group. Decreased superoxide dismutase in liver tissue indicated an increased oxidative stress in diabetic control.

Furthermore, high blood glucose level in diabetic patients leads to formation of reactive oxidants causing oxidative damage (Atalay, 2002). In diabetes mellitus, hyperglycaemia induces the peroxidative reactions in lipids,

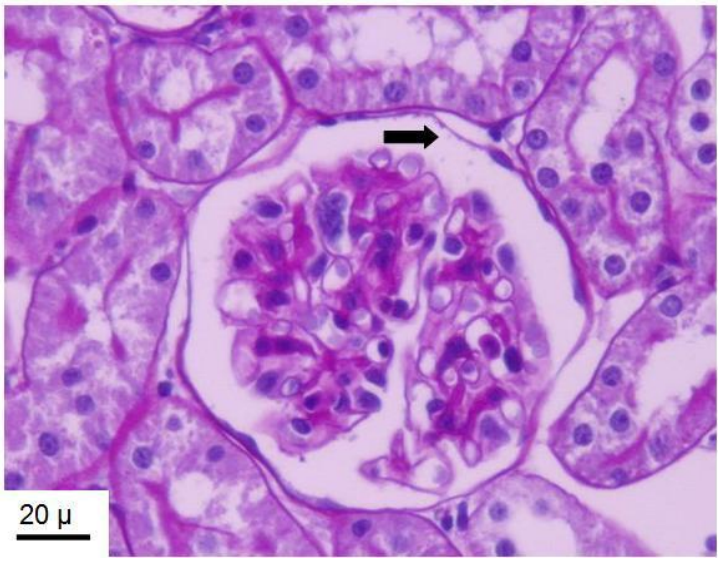

Figure 8. Diabetic F13 group showing glomerular basement membrane thickening (→) as observed. H and E × 100.

thus the product of lipid peroxidation (MDA) increases in diabetes. It was also reported that diabetes causes increased oxidative stress in many organs, especially in liver (Yilmaz et al., 2004). Similarly, we found increased erythrocyte and liver MDA levels in diabetic control group, whereas erythrocyte MDA levels reduced in diabetic group treated with F13. Also, liver MDA levels of acarbose and F13 treated diabetic groups were significantly decreased when compared to diabetic control. However, acarbose and F13 treatment resulted in normalization of MDA levels which may contribute to the beneficial effect on liver lipid peroxidation.

Considering the NOS activities of the groups, no statistically significant differences were found between the control and the other groups. In addition, significantly lower NOS levels were observed in diabetic group treated with acarbose compared to diabetic control group. In diabetic patients, oxidative stress produced in tissues as a result of hyperglycemia leads to overproduction of NO. This overproduction results in various complications in a number of organs such as eye, kidney and cardiovascular system in type 1 and type 2 diabetes (Kawamura et al., 1998; Hwua et al., 2003). In our study, we found that acarbose, an antidiabetic agent, affects diabetes in a positive way by reducing NOS levels and decreasing NO production. Meanwhile, no significant differences were found between control and experimental groups regarding GPx and catalase activities. It has been reported that the levels of antioxidant enzymes such as erythrocyte GPx and catalase of type 1 and type 2 diabetic patients were decreased (Atalay, 2002).

In various studies, it has been reported that diabetes developed by STZ causes histological changes in liver (Gunes et al., 1999). In our study, nuclear hypertrophy and binuclear hepatocytes were observed in liver tissues of diabetic control groups, whereas normal formations were seen in liver histology of control groups and treated diabetic group. As a result, we observed that the herbal formulation (F13) obtained from Karkim shows antidiabetic and antioxidative properties in diabetes complications, and acarbose cured partially the defects of antioxidant enzymes and histological structure caused by diabetes by reducing blood sugar. Hence, there is need for conducting clinical research in herbal drugs and formulation, developing simple bioassays for biological standardization, pharmacological and toxicological evaluation, and developing various animal models for toxicity and safety evaluation. It is also important to establish the active components from these plant extracts.

ACKNOWLEDGEMENT

This study was supported by a grant of the Research Foundation of University of Eskisehir Osmangazi, Turkey (Grant No.200411017).

REFERENCES

Akyuz F, Tekin N, Aydın O, Temel HE, Isıklı B (2012). The effect of metformin and exercise on serum lipids, nitric oxide synthase and liver nitric oxide levels in streptozotocin-nicotinamide induced diabetic rats. Afr. J. Pharm. Pharmacol. 6(5):336-342.

Alam M, Ghiasuddin, Sadat A, muhammd N, Khan AA, Siddiqui BS (2012). Evaluation of *Viburnum grandiflorum* for its *in-vitro* pharmacological screening. Afr. J. Pharm. Pharmacol. 6(22):1606-1610.

Asgarpanah J, Ramezanloo F (2012). Chemistry, pharmacology and

medicinal properties of *Peganum harmala* L. Afr. J. Pharmacy and Pharmacol. 6(22):1573-1580.

Atalay M, Laaksonen DE (2002). Diabetes, oxidative stress and physical exercise. J. Sports Sci. Med. 1:1-14.

Atkins RC (2005). The changing patterns of chronic kidney disease: The need to develop strategies for prevention relevant to different regions and countries. Kidney Int. 68:83-85.

Buckle J (2001). Aromatherapy and diabetes. Diabetes Spectrum 14 (3):124-126.

Bukan N, Sancak B, Yavuz O, Koca C, Tutkun F, Ozcelikay TA, Altan N (2003). Lipid peroxidation and scavenging enzyme levels in the liver of streptozotocin-induced diabetes rats. Indian J. Biochem. Biophysics 40(6):447-450.

Cavanaugh KL (2007). Diabetes management issues for patients with chronic kidney disease. Clin. Diab. 25(3):90-97

Ceriello A (2010). Hyperglycaemia and the vessel wall: The pathophysiological aspects on the atherosclerotic burden in patients with diabetes. European J. Preventive Cardiol. 17(1):15-19.

Chen G, Adeyemo AA, Zhou J, Chen Y, Doumatey A, Lashley K, Huang H, Amoah A, Agyenim-Boateng K, Eghan BA Jr, Okafor G, Acheampong J, Oli J, Fasanmade O, Johnson T, Rotimi C (2007). A Genome-Wide Search for Linkage to Renal Function Phenotypes in West Africans With Type 2 Diabetes. Am. J. Kidney Dis. 49:394-400.

Dunn SR, Qi Z, Bottinger EP, Breyer MD, Sharma K (2004). Utility of endogenous creatinine clearance as a measure of renal function in mice. Kidney Int. 65:1959-1967.

Elangovan V, Shohami E, Ghati I, Kohea R (2000). Increased hepatic lipid soluble antioxidant capacity as compared to other organs of streptozotocin-induced diabetic rats: A cyclic voltammetry study. Free. Rad. Res. 32:125-134.

Goth L (1991). A Simple method for determination of serum catalase activity and revision of reference range. Clinica Chimica Acta 196: 143-152.

Gunes HV, Degirmenci I, Aydin M, Bozan B, Aral E, Tunalier Z, Ustuner C, Ercakar M, Baser KHC, Basaran A (1999). The effects of *Rumex patientia* L. and *Urtica dioica* L. on some blood and urine parameters, and liver and kidney histology in diabetic rats. Tr. J. Med. Sci. 29: 227-232.

Hwua C, Hob L, Fuh MMT., Chung Siu S, Sutanegara D, Piliang S, Chan JCN (2003). Acarbose improves glycemic control in insulin-treated Asian type 2 diabetic patients: Results from a multinational, placebo controlled study. Diabetes Res. Clin. Practice 60:111-118.

Inanc N, Cicek B, Sahin H, Bayat M, Tasci S (2007). Use of herbs by patients with diabetes in Kayseri, Turkey. Pak. J. Nutr. 6 (4):310-312.

Kawamura T, Egusa G, Fujikawa R, Watanabe T, Oda K, Kataoka S, Takayama S, Kubo. K, Yamamoto S, Noma K, Orita R, Yamakido M (1998). Effect of acarbose on glycemic control and lipid metabolism in patients with non-insulin-dependent diabetes mellitus. Current Therapeutic Res. 59(2):97-106.

Kuppusamy AK, Muthusamy U, Shanmugam SS, Thirumalaisamy SA, Varadharajan S, Ramanathan S (2010). Antidiabetic, hypolipidemic and antioxidant properties of *Asystasia gangetica* in streptozotocin - nicotinamide-induced type 2 diabetes mellitus (NIDDM) in rats. J. Pharmacy Res. 3(10):2516-2520.

Modak M, Dixit P, Londhe J, Ghaskadbi S, Paul A Devasagayam T (2007). Indian herbs and herbal drugs used for the treatment of diabetes. J Clin. Biochem. Nutr. 40(3):163-173.

Murali B, Umrani DN, Goyal RK (2003). Effect of chronic treatment with losartan on streptozotocin-induced renal dysfunction. Mol. Cell Biochem. 249(1-2):85-90.

Nasri S, Anoush M, Khatami N (2012). Evaluation of analgesic and anti-inflammatory effects of fresh onion juice in experimental animals. Afr. J. Pharm. Pharmacol. 6(23):1679-1684.

Novelli M, Fabregat ME, Fernandez-Alvarez J, Gomis R, Masiello P (2001). Metabolic and functional studies on ısolated ıslets in new rat model of type 2 diabetes. Mol. Cellular Endocrinol. 175:57-66.

Novelli M, Pocai A, Lajoix AD, Beffy P, Bezzi D, Marchetti P(2004). Alterations of β-cell constitutive NO synthase activity is involved in the abnormal insulin response to arginine in new rat model of type II diabetes. Mol. Cellular Endocrinol. 219(1-2):77-82.

Pitkanen OM, Martin JM, Hallman M, Akerblom HK, Sariola H, Andersson SM (1992). Free radical activity during development of insulin dependent diabetes mellitus in the rat. Life Sci. 50 (5):335-339.

Prakash J, Lodha M, Singh SK, Vohra R, Raja R, Usha (2007). Diabetic retinopathy is a poor predictor of type of nephropathy in proteinuric type 2 diabetic patients. J. Assoc. Physicians India. 55:412-6.

Ritz E, Orth SR (1999). Nephropathy in patients with type 2 diabetes mellitus. N. Engl. J Med. 341:1127-1133.

Sabu MC, Kuttan R (2002). Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. J. Ethnopharmacol. 81(2):155-160.

Seeff LB, Lindsay KL, Bacon BR, Kresina TF, Hoofnagle JH (2001). Complementary and alternative medicine in chronic liver disease. Hepatol. 34(3):595-603.

Sun Y, Oberley LW, Li Y (1988). A simple method for clinical assay of superoxide dismutase. Clin. Chem. 3413:497-500.

Tas S, Sarandol E, Ayvalik SZ, Serdar Z, Dirican M (2007). Vanadyl sulfate, taurine, and combined vanadyl sulfate and taurine treatments in diabetic rats: Effects on the oxidative and antioxidative systems. Arch. Med. Res. 38(3):276-283.

Uchiyama M, Mihara M (1978). Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Analytical Biochem. 86:279-286.

Wright BE, Vasselli JR, Katovich MJ (1998). Positive effects of acarbose in the diabetic rat are not altered by feeding schedule. Physiol. Behavior 63(5):867-874.

Yilmaz HR, Uz E, Yucel N, Altuntas I, Ozcelik N (2004). Protective effect of caffeic acid phenethyl ester (CAPE) on lipid peroxidation and antioxidant enzymes in diabetic rat liver. J. Biochem. Mol. Toxicol. 18(4):234-238.