Antioxidant and Free Radical-Scavenging Properties of *Stevia rebaudiana* (Bertoni) Extracts and L-NNA in Streptozotocine-Nicotinamide Induced Diabetic Rat Liver

Streptozotosin-Nikotinamid ile İndüklenen Diyabetik Sıçan Karaciğerinde *Stevia rebaudiana* (Bertoni) Ekstresi ve L-NNA'nın Antioksidan ve Serbest Radikal-Toplayıcı Özellikleri

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ABSTRACT Objective: Free radical production is implicated in the pathogenesis of diabetes mellitus, in which several pathways and different mechanisms are shown to involve in the pathophysiology of the complications. Through the administration of nicotinamide(NA) and streptozotocin(STZ), a model of human type II diabetes can be induced in rats. The aim of this study was to determine the effects of Stevia rebaudiana (SrB) and L-NNA (N-nitro-L-arginine) in the formation of free radicals in STZ-NA induced type II diabetic rats. **Material and Methods:** In this study, rats were treated with SrB and L-NNA 5-8 weeks after the induction of diabetes. The levels of glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA) were determined in the liver homogenate and erythrocyte hemolysate. The nitric oxide synthase (NOS) levels were also measured in the liver homogenate and serum. To study the histological changes in diabetes, the liver tissue samples stained with hematoxylein-eosin and were investigated under light microscope. **Results:** Although fasting and postprandial blood sugar levels were high in diabetic groups, blood sugar levels were significantly reduced in diabetic treated groups. While the erythrocyte MDA levels of stevia-treated diabetic group decreased, lipid peroxidation increased with L-NNA treatment in both control and diabetic groups. There was no significant difference for the tissue CAT, NOS, and erythrocyte SOD and CAT activities between. Liver samples in control group had a normal structure. Normal histological arrangement was noticed in the livers of control group. In hepatocytes of the diabetic control group, necrotic cells with pycnotic nucleus and eosinophylic cytoplasm as well as sinusoidal dilatation were seen. The hepatocyte structure was more protected in diabetic LNNA group than in diabetic group. The maximum protection was seen in hepatocytes of diabetic stevia L-NNA group. **Conclusion:** SrB and L-NNA reduced blood glucose levels in diabetic rats and had some beneficial effects on oxidative and histological changes. However, a NOS inhibitor, L-NNA was less effective than SrB treatment in type II diabetes.

Key Words: Diabetes mellitus, type 2; stevia; nitroarginine; nitric oxide synthase; free radicals

ÖZET Amaç: Serbest radikal üretimi diyabet patogenezinde rol oynar, bu nedenle serbest radikaller diyabet komplikasyonlarının çeşitli yolları ve farklı mekanizmaların patofizyolojisinde etkilidir. Sıçanlara nikotinamid (NA) ve streptozotozin (STZ) uygulanması ile insan tip II diyabetinin bir modeli oluşturulabilir. Bu çalışmanın amacı Stevia rebaudiana Bertoni (SrB) ve L-NNA'nın (N-nitro L-arginin) STZ-NA-indüklenmiş tip II diyabetik sıçanlardaki serbest radikal oluşumu üzerine olan etkilerini belirmekti. **Gereç ve Yöntemler:** Bu çalışmada, diyabet indüklendikten 5-8 hafta sonra sıçanlar SrB ve L-NNA ile tedavi edildi. Karaciğer homojenatlarında ve eritrosit hemolizatlarında glutatyon peroksidaz (GPx), süperoksit dismutaz (SOD), katalaz (CAT) ve malondialdehit (MDA) seviyeleri belirlendi. Aynı zamanda karaciğer homojenatında ve serumda nitrik oksit sentaz (NOS) seviyeleri ölçüldü. Diyabetteki histolojik değişiklikleri incelemek için karaciğer doku örnekleri hematoksilen-eozin ile boyandı ve ışık mikroskopu ile incelendi. **Bulgular:** Açlık ve tokluk kan şekerleri diyabetik gruplarda yüksek olmakla birlikte tedavi edilen diyabetik gruplarda kan şekeri düzeyleri anlamlı oranda azalmıştır. Stevia ile tedavi edilen diyabetik grubun eritrosit MDA seviyeleri düşmüş olmakla birlikte, L-NNA tedavisi hem kontrolde hem de L-NNA ile tedavi edilmiş diyabetik grupta lipid peroksidasyonunu artırmıştır. Kontrol ile karşılaştırıldığında doku CAT, NOS ve eritrosit SOD ve CAT aktiviteleri yönünden herhangi bir farklılık belirlenmemiştir. Kontrol grubunun karaciğer örneklerinde normal histolojik yapılanma gözlenirken, diyabetik kontrol grubunun karaciğer dokularında piknotik nükleus ve eozinofilik sitoplazmalı nekrotik hücreler ve sinuzoidal dilatasyon belirlenmiştir. Diyabetik kontrol grubu ile karşılaştırıldığında diyabetik L-NNA grubunda hepatosit yapısı normaldi. Bununla birlikte SrB ve L-NNA tedavisinin hepatositlerde yüksek oranda koruma sağladığı belirlendi. **Sonuç:** Bulgularımız, diyabette SrB ve L-NNA tedavisinin kan glukoz seviyelerini düşürdüğünü, oksidatif ve histolojik değişiklikler üzerine bazı olumlu etkilere sahip olduğunu, bununla birlikte bir NOS inhibitörü olan L-NNA'nın SrB ile karşılaştırıldığında tip II diyabet üzerine daha az etkili olduğunu göstermiştir.

Anahtar Kelimeler: Diabetes mellitus, tip 2; stevya; nitroarjinin; nitrik oksid sentaz; serbest radikaller

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Type II diabetes is a chronic metabolic disorder that results from reduced first phase insulin secretion, beta-cell dysfunction with relative glucagon excess, and insulin resistance. It is well established that the control of hyperglycemia is an important factor in preventing diabetic macrovascular complications.[1,2]

A novel experimental model of type II diabetes in adult rats is shown to be established by the combined injection of streptozotocin (STZ) and a partially protective dose of nicotinamide (NA).[3] This model is characterized with a 40% reduction in beta-cell mass, which in turn generates a moderate, stable hyperglycemia and glucose intolerance. The beta-cells still have the ability to respond to glucose though it is altered. Therefore, this model shares a number of similarities with human type II diabetes.[3,4]

Oxidative stress, an imbalance between the generation of reactive oxygen species and antioxidant defence capacity of the body, is closely associated with aging and a number of diseases including cancer, cardiovascular ailments, diabetes and diabetic complications. Several mechanisms may cause oxidative insult in diabetes, although their exact contributions are not entirely clear. Accumulating evidence points to many interrelated mechanisms that increase production of reactive oxygen and nitrogen species or decrease antioxidant protection in diabetic patients.[5,6]

Hyperglycemia-induced auto-oxidation of lipids and glycation of protein/glucose, result in formation of free radicals of oxygen (ROS) and nitrogen (RNS). Mitochondrial leakage of these ROS is the primary reason for oxidative damage.[11] Oxidative atmosphere in cells is also created by the impairment in functioning of endogenous antioxidant enzymes namely superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). GPx, CAT, SOD and glutathione reductase (GR) are known to be inhibited in diabetes mellitus as a result of nonenzymatic glycosylation and oxidation.[11,18]

Thereby, all aerobic organisms including humans have antioxidant defence mechanisms that protect against oxidative damage. However, natural antioxidant defence mechanisms can be insufficient. Hence, dietary intake of antioxidants is important and recommended. Medicinal plants became important in this generation for the treatment of numerous diseases.[19]

Stevia rebaudiana Bertoni (SrB) is a perennial shrub of the Asteraceae (Compositae) family native to certain regions of South America (Paraguay and Brazil).[8,13] The extracts of the leaves of the plant, have been used for many years in the traditional treatment of diabetes in South America. Oral intake of SrB extracts slightly suppresses plasma glucose during an oral glucose tolerance test in healthy and diabetic persons.[7,8] A 35% reduction in blood glucose is also observed in diabetic rats after an oral intake of SrB extracts.[1]

Nitric oxide synthases (NOS) are the family of enzymes that synthesize NO and citrulline from L-arginine. Three major subtypes have been identified; two of them, neuronal NOS and endothelial NOS are constitutively expressed and Ca+2/calmodulin-dependent, whereas the cytokine-inducible isoform is calcium-independent.[9] Recent studies have focused on the possible role of NOS enzymes in diabetes. If these enzymes are affected, they may fail to produce nitric oxide or may produce both nitric oxide under oxidative stress conditions and superoxide, which is likely to result in peroxynitrite formation and contribute to the complications.[10]

L-NNA is a nitric oxide synthase inhibitor, as an analog of L- arginine. The slow reversibility of L-NNA-mediated inhibition provides a degree of selectivity for nNOS and eNOS in longer term studies (for example, with cells or in vivo). None of the compounds described to date can be used to target a specific isoform (for example, L-NNA shows only twofold selectivity for nNOS over eNOS).[9,11]

No detailed studies focused on the effect of *Stevia rebaudiana* Bertoni on lipid peroxidation and antioxidants in STZ-induced diabetic rats. Hence, the present study was planned to evaluate the effect of *Stevia rebaudiana* Bertoni on lipid peroxidation, blood glucose and antioxidant activities in plasma and liver of the STZ-NA induced diabetic rats.

MATERIAL AND METHODS

TREATMENTS OF RATS

Two to three months old Sprauge Dawley rats were used for the present study (Medical Biology Animal Laboratory, Eskisehir, Turkey). The animals were housed in individual cages at room temperature with a 12-h on/12-h off light schedule, and left for 1 week for acclimitization before the start of the experiment. At the beginning of the experiment, glucose levels were measured from tail vein. The rats were divided into seven groups as described (Table 1).

The rats were intraperitoneally administered 290 mg/kg of nicotinamide, (Sigma Chemical, St. Louis, Mo., USA) dissolved in saline, 15 min before an intravenous injection of 60 mg/kg STZ (Sigma Chemical, St. Louis, Mo., USA), dissolved in saline immediately before use. The animals were treated with SrB extract (NuNaturals, Inc., Eugene. OR, USA), in order to test antidiabetic and possible antioxidative effects of this extract. To determine whether the inhibition of NOS would have any effect on the development of type II diabetes and free radical formation, L-NNA was administrated (Sigma Chemical, St. Louis, Mo., USA) 5-8 weeks after the induction of the diabetes. Doses of the given substrates are summarized in Table 1.

After administration of STZ and NA, the blood glucose levels were determined every week from tail veins of all animals during the next 7-week period using the Accu-Check® Go Glucometer (Roche Diagnostics, Mannheim, Germany). Fasting and postprandial blood glucose levels were measured.

This study was approved by the local ethical committee of Eskisehir Osmangazi University of Animal Experiments.

BIOCHEMICAL ANALYSIS

Erythrocyte hemolysates were prepared as described previously.[26] Liver was extracted by homogenization for the SOD and GPx analysis according to the kit protocols. For the measurement MDA and CAT levels, homogenates were prepared from liver as described.[17]

SOD activity was measured using SOD Determination Kit (FLUKA, St. Louis, MO, Cat. No: 19160).

CAT activity was determined using ammonium molybdate- hydrogen peroxide reaction. MDA was measured by TBA reaction as a lipid peroxidation product, as previously described.[17]

Glutathione peroxidise (GPx) activity was determined using Glutathione Peroxidase Assay Kit (CALBIOCHEM®, EMD Biosciences,. Inc., San Diego, CA, Cat. No: 354104).

NOS activity was measured in the serum sample using Nitric Oxide Synthase Assay Kit (BIOXYTECH®, Oxis International, Inc, Portland, OR, USA, Cat. No: 22113).

HISTOPATHOLOGICAL PROCEDURES

All tissue specimens were obtained from the same region of the right lobe of the liver of the rats. The fragments from tissues were fixed in 10% neutral formalin solution, embedded in paraffin, and then stained with hematoxylin and eosin.

TABLE 1: The substrates given to control and experiment groups.

Groups		Received Substrates
Control Groups		
	I.	(Control) Water + CSD
	II.	SrB [200 mg/kg, intragastric(i.g.)] + Water + CSD
	III.	L-NNA [100 mg/kg, intraperitoneal (i.p.)] + Water + CSD
Diabetic Groups		
	IV.	(Diabetic Control) [60 mg/kg STZ + 290 mg/kg NA i.p.] + Water + CSD
	V.	(60 mg/kg STZ + 290 mg/kg NA i.p.)+ SrB (200 mg/kg, i.g.) + Water + CSD
	VI.	(60 mg/kg STZ + 290 mg/kg NA i.p.) + L-NNA (100 mg/kg, i.p.) + Water + CSD
	VII.	(60 mg/kg STZ + 290 mg/kg NA i.p.) + SrB (200 mg/kg, i.g.) + L-NNA (100 mg/kg, i.p.) + Water + CSD

CSD: Commercial standard diet; SrB: Stevia rebaudiana Bertoni; L-NNA: N-nitro L-arginine; STZ: Streptozotocin; NA: Nicotinamide.

The measurement of nucleus of hepatocyte was then performed in each of the slices using Osiris imaging software (Digital Imaging Unit, University Hospitals of Geneva, Geneva, Switzerland).[27] One of the basic features of this program is its capability to handle sets of static as well as dynamic images directly on a variety of personal computers and workstations. In our study, measurement was performed on nucleus of hepatocyte. We prepared five slices for every rat and measured the dimensions of 10 nuclei of hepatocytes for every slice. Means for measurements were calculated.

STATISTICAL ANALYSIS

The data were expressed as mean ± standard deviation (S.D.), and analyzed using repeated measures of variance. Tukey test was used to test for differences among means when ANOVA indicated significance ($P< 0.05$) and ($P< 0.001$). Differences were considered statistically significant if $P< 0.05$.

RESULTS

The diabetic animals exhibited hyperglycemia consistently. SrB and L-NNA treatment caused a decrease in the elevated blood glucose levels in STZ-NA treated diabetic rats, as measured at the end of the study.

BLOOD GLUCOSE LEVELS

The blood glucose levels were determined every week during the experiment. The levels of fasting and postprandial blood glucose at the beginning of the experiment, and at 5th and 7th weeks are given in Table 2 and Table 3, respectively.

The plasma glucose levels wero similar in controls and experiment groups before STZ injection ($P>0.05$). The plasma glucose levels increased gradually in STZ+NA treated groups (IV., V., VI. and VII.).

The plasma glucose levels of diabetic groups increased in the 5th week when compared to controls ($P< 0.05$), ($P< 0.001$).

In the 7th week, after the SrB and L-NNA treatment, blood glucose levels of SrB, L-NNA and SrB+ L-NNA treated groups decreased significantly (Table 2 and 3).

BIOCHEMICAL FINDINGS

Erythrocyte MDA, SOD, CAT and GPx levels, and serum NOS levels are presented in Table 4. There was not any significant difference in the SOD and CAT activities between control and diabetic groups. Erythrocyte MDA level significantly decreased in the SrB treated diabetic groups ($P< 0.05$). Serum NOS levels of group II., V., VI and VII significantly decreased (compared the diabetic control IV) ($P< 0.05$, $P< 0.001$). The erythrocyte GPx activity increased in diabetic control group compared to controls ($P< 0.01$).

MDA, SOD, CAT, NOS and GPx levels of liver homogenates are presented in Table 5 (Table 4). There was not any significant difference in the NOS and CAT activities between control and diabetic groups. In addition, SOD and GPx activities of

TABLE 2: Fasting blood glucose levels of control and diabetic groups.

Groups		n	Before injection	5th week (beginning of the treatment)	7th week (final of the experiment)
Control Groups					
	I.	11	81.27 ± 1.4	78.09 ± 2.1	78.73 ± 2.2
	II.	12	82.50 ± 1.9	81.00 ± 1.5	71.92 ± 4.9
	III.	11	81.45 ± 1.4	79.82 ± 1.2	70.10 ± 1.7
Diabetic Groups					
	IV.	11	82.82 ± 0.9	90.30 ± 2.3**	97.56 ± 2.6***
	V.	12	81.17 ± 3.0	99.83 ± 3.0***	88.42 ± 2.0
	VI.	12	80.33 ± 1.8	93.00 ± 2.7***	92.20 ± 1.8*
	VII.	14	78.86 ± 1.7	93.07 ± 2.0***	87.50 ± 1.1

* $P< 0.05$, ** $P< 0.01$, *** $P< 0.001$ (when compared to control group I).

TABLE 3: Postprandial blood glucose levels of control and diabetic groups.

Groups	n	Before injection	5th week (beginning of the treatment)	7th week (end of the experiment)
Control Groups				
I.	11	113.73 ± 2.3	112.55 ± 2.2	110.27 ± 1.7
II.	12	114.58 ± 4.1	103.25 ± 2.3	113.92 ± 2.8
III.	11	118.45 ± 2.8	106.45 ± 2.2	113.10 ± 3.5
Diabetic Groups				
IV.	11	115.73 ± 1.6	130.40 ± 2.1***	150.89 ± 4.2***
V.	12	113.00 ± 2.9	149.58 ± 3.4***	120.83 ± 1.5*
VI.	12	115.25 ± 2.0	150.67 ± 3.8***	132.70 ± 1.6***
VII.	14	114.79 ± 1.6	136.00 ± 2.6***	121.25 ± 1.5*

* P< 0.05, *** P< 0.001 (when compared to control group I).

TABLE 4: The levels of erythrocyte MDA, SOD, CAT and GPx, and serum NOS of control and diabetic groups.

Groups	n	SOD (U/gHb)	MDA (U/gHb)	CAT (KU/l)	Gpx (mU/ml)	NOS (nmol/ml/sec)
Control Groups						
I.	11	7498 ± 427	31.21± 2.2	210.76 ± 17.43	1.98 ± 0.6	0.62 ± 0.13
II.	11	7546 ± 491	36.18 ± 2.7	207.65 ± 6.30	1.98 ± 0.5	0.54 ± 0.06+
III.	11	6766 ± 309	28.32 ± 1.0	208.95 ± 8.11	1.80 ± 0.4	0.60 ± 0.07
Diabetic Groups						
IV.	11	7841 ± 562	39.64 ± 2.2	234.10 ± 13.53	6.12 ± 1.0**	1.01 ± 0.20
V.	11	6769 ± 625	21.48 ± 1.5*	227.28 ± 12.48	1.41 ± 0.2	0.51 ± 0.07+
VI.	11	8834 ± 763	22.48 ± 1.7	225.30 ± 24.48	1.80 ± 0.4	0.39 ± 0.06+++
VII.	11	6628 ± 462	36.61 ± 2.3	230.43 ± 30.23	2.17 ± 0.6	0.23 ± 0.01+++

* P< 0.05, ** P< 0.01 (when compared to control group I), +when compared to group IV, P< 0.05, +++ when compared to group IV, P< 0.001.

MDA: Malondialdehyde; SOD: Superoxide dismutase; CAT: Catalase; Gpx: Glutathione peroxidase; Nos: Nitric oxide synthase.

TABLE 5: The MDA, SOD, CAT, GPx and NOS levels of liver tissues of control and diabetic groups.

Groups	n	SOD (%inhibition)	MDA (U/wet tissue)	CAT (kU/ml protein)	Gpx (nmol/min/ml)	NOS (nmol/ml/sec)
Control Groups						
I.	11	76.88 ± 9.98	0.292 ± 0.05	198.37 ± 74.54	14.85 ± 7.28	1.42 ± 0.65
II.	11	88.17 ± 7.86	0.378 ± 0.06	156.58 ± 67.18	19.00 ± 3.16	2.36 ± 0.93
III.	11	82.08 ± 8.69	1.105 ± 0.29***	113.97 ± 31.84	9.71 ± 1.70	2.91 ± 0.98
Diabetic Groups						
IV.	11	61.77 ± 3.54*	0.820 ± 0.56**	254.07 ± 56.66	3.57 ± 0.97***	1.55 ± 0.96
V.	11	88.47 ± 12.60	0.543 ± 0.19	212.47 ± 85.21	20.42 ± 1.90*	1.82 ± 0.64
VI.	11	90.69 ± 6.48*	1.456 ± 0.23***	168.51 ± 32.43	6.42 ± 2.76***	1.33 ± 0.85
VII.	11	89.11 ± 5.02	0.344 ± 0.02	157.38 ± 58.25	9.14 ± 0.89*	2.51 ± 1.25

* P< 0.05, ** P< 0.01, *** P< 0.001 (when compared to control group I).

MDA: Malondialdehyde; SOD: Superoxide dismutase; CAT: Catalase; Gpx: Glutathione peroxidase; Nos: Nitric oxide synthase.

the untreated diabetic groups (diabetic control) were significantly decreased (P< 0.05, P< 0.001). We found increased MDA levels in diabetic control (P< 0.01) and L-NNA treated (P< 0.001) groups.

HISTOLOGICAL FINDINGS

Hepatocytes were in normal histology in control group in light microscopic analyze (Figure 1). When compared with control group maximum degenera-

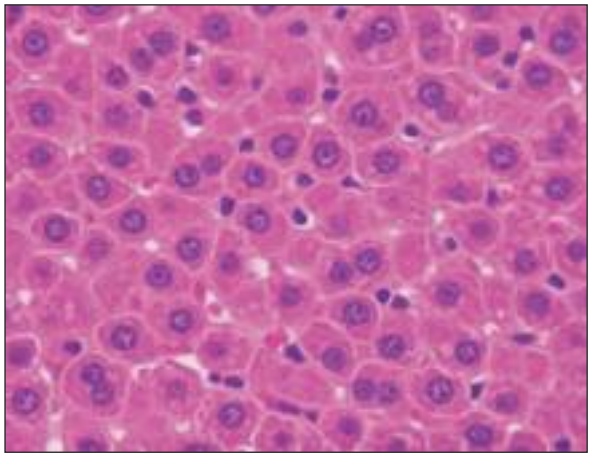
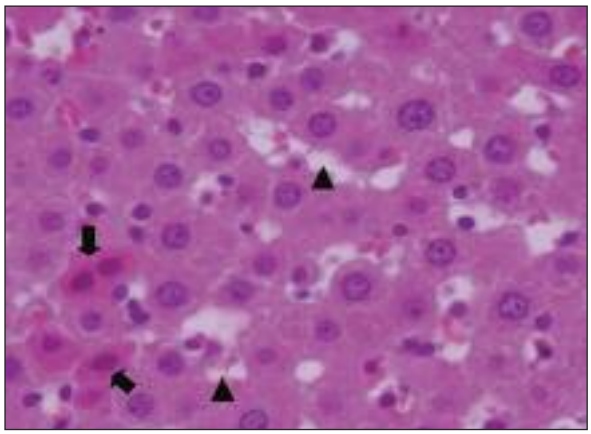
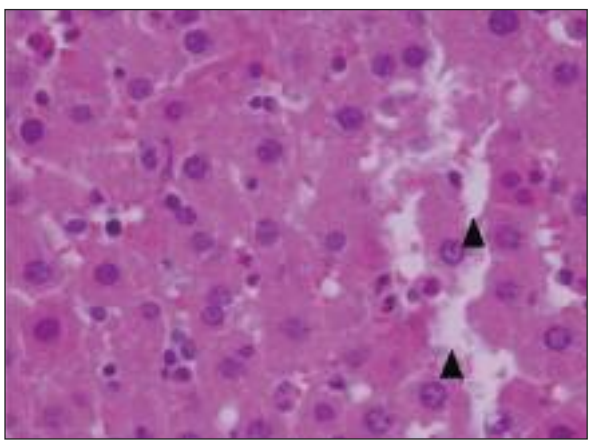


FIGURE 1: Hepatocytes samples in control group: Hepatocytes and sinusoidal structures have normal histology in the control group, H&E x 100.



A



B

FIGURE 2: Hepatocytesin the diabetic control group: The necrotic cells with pycnotic nuclei and eosinophilic cytoplasm (➡) **(A)** and sinusoidal dilatation (▲) **(A, B)** in hepatocytes, H&E x 100.

tion was seen in the diabetic group (Figure 2A, B). However degeneration was also detectable in diabetic- stevia group (Figure 3). The hepatocyte structure was maintained more in diabetic LNNA group than in the diabetic group (Figure 4A, B). The maximum protection was seen in the hepatocytes of diabetic stevia + LNNA group (Figure 5). The stevia and the L-NNA control groups' hepatocyte structures were almost normal (Figures 6 and 7).

Necrotic cells with pycnotic nuclei eosinophilic cytoplasm were seen in the diabetic control group (Figure 2A). Sinusoidal dilatations were also observed in diabetic control groups (Figure 2A, B). A significant nuclear hypertrophy was seen in the hepatocytes of diabetic control groups (Table 6) ($P < 0.001$). Hepatocytes of the L-NNA treated diabetic group had almost normal histomorphological structure. However, partial sinusoidal dilatations could be seen (Figure 4A). On the other hand, necrotic cells with pycnotic nuclei and eosinophilic cytoplasm were seen in some areas in hepatocytes in this group (Figure 4B). Severe cell degeneration was seen in hepatocytes of SrB treated diabetic group. In this group, cells were seen empty, and nuclei were seen separated from cytoplasm (Figure 3). Hepatocytes and sinusoidal structures in diabetic-stevia-L-NNA group had almost normal histology (Figure 5). Hepatocytes and sinusoidal structures were normal in control group and L-NNA treated control group (Figures 6, 7). Hepatocytes in stevia treated control group had almost normal histological structure although partial sinusoidal dilatations were seen (Figure 6).

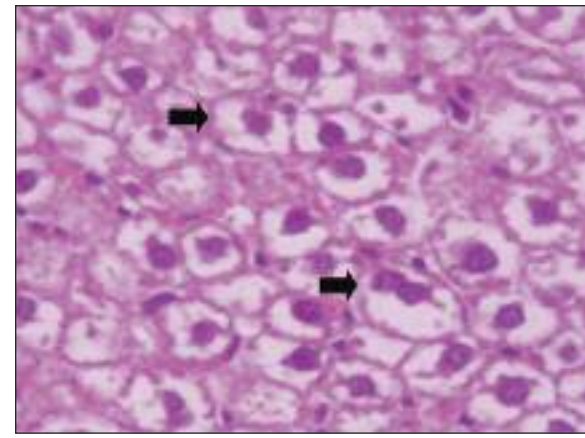
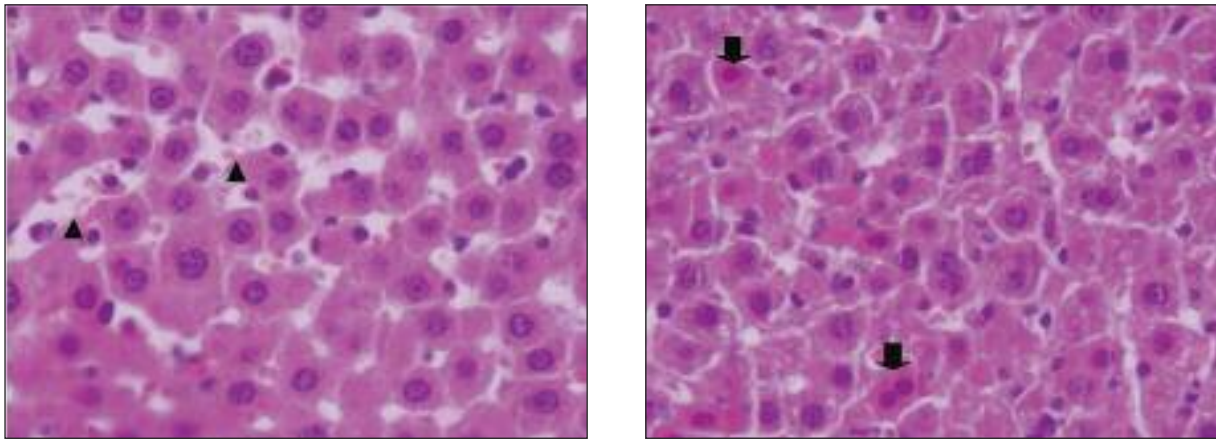


FIGURE 3: Hepatocytes in diabetic stevia group. Cells were seen empty and nuclei were seen separated from the cytoplasm (➡), H&E x 100.



A B

FIGURE 4: Hepatocytes in Diabetic L-NNA group: Partial sinusoidal dilatations (▲) **(A)**, necrotic cells with pycnotic nuclei and eosinophilic cytoplasm (➡) **(B)**, H&E x 100.

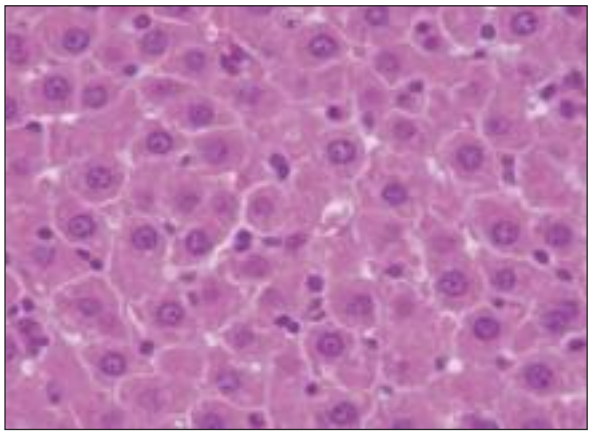


FIGURE 5: Hepatocytes in diabetic-stevia-L-NNA group. Hepatocytes and sinusoidal structures in diabetic-stevia-L-NNA group have almost normal histology, H&E x100.

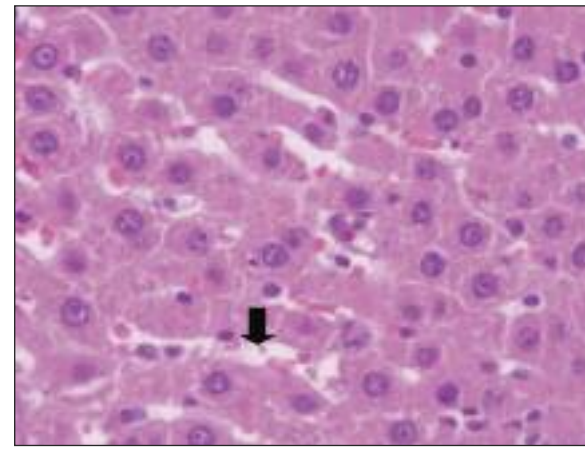


FIGURE 6: Hepatocytes in stevia control group: Partial sinusoidal dilatations (➡), H&E x100.

DISCUSSION

The fundamental mechanism underlying hyperglycemia involves over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues. There are various diabetic animal models which can be used to investigate the pathogenesis and evolution of diabetes, and can be possibly used to screen new anti-diabetic drugs; however, none of them is able to reproduce the complexity seen in human diabetes, especially in type II diabetes mellitus.[12]

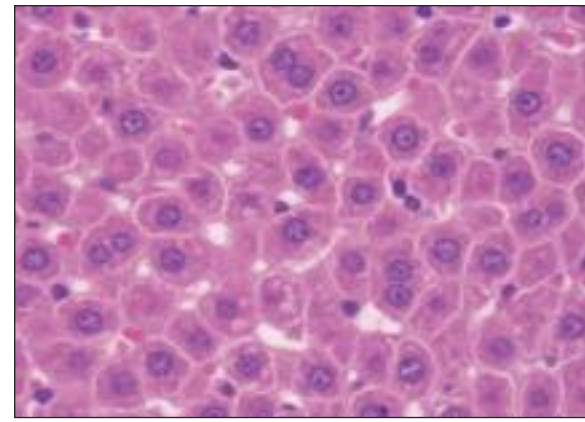


FIGURE 7: Hepatocytes in L-NNA control group: Hepatocytes and sinusoidal structures have normal histology, H&E x100.

Streptozotocin, which is a cytotoxic agent specific for pancreatic β-cells, has been confirmed to have a diabetogenic action, and the intensity of the damage is graded according to the dosage used. Previous studies indicated that by giving 230 mg of

nicotinamide and 60 mg of streptozotocin per kg of body weight to adult rats, a diabetic syndrome with stable metabolic alterations and reduced pancreatic insulin stores could be induced which imitate some features of type II diabetes mellitus.[5,6,12]

THE EFFECTS OF SrB AND L-NNA ON FASTING AND POSTPRANDIAL BLOOD GLUCOSE LEVELS

In the present study, the antihyperglycemic and antidiabetic potential of the leaf extract of *Stevia rebaudiana* Bertoni, a medicinal plant widely used in the traditional medicine for the treatment of diabetes mellitus,[1,7,8,13] was evaluated in a similar nicotinamide and streptozotocin-induced diabetic rat model. Studies using the mild type II diabetic GK rat have also shown a blood glucose-lowering effect of stevioside both when administered intravenously and orally.[8] Likewise, in our study, clear reduction in blood glucose levels similarly observed in STZ-NA induced diabetic rats treated with SrB extract.

It has been reported that L-NNA treatment does not significantly affect the concentrations of blood glucose and serum insulin levels.[18,19] At the end of the study (final week), there was not any statistically significant decrease in the postprandial blood glucose levels of L-NNA treated diabetic groups compared to untreated diabetic groups (group IV). However, L-NNA treatment caused small declines in the fasting blood glucose levels in L-NNA treated diabetic groups. Thus, L-NNA may decrease the glucose-stimulated increase in blood glucose levels (according to group IV).

LIPID PEROXIDATION AND ANTIOXIDANT STATUS IN ERYTHROCYTE HEMOLYSATE

The possible sources of oxidative stress in the pathogenesis of diabetes and diabetic complications have been extensively studied for years based on animal models and on patients. Diabetics and experimental animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia, which thereby depletes the activity of antioxidative defence system and thus promotes de novo free radical generation. Numerous studies have found increased lipid peroxides or reactive oxygen species and oxidative stress (or both) in different animal models of diabetes.[4,14-16,21]

In our study, there were not any considerable differences in the control group compared to other groups concerning erythrocyte SOD, MDA or CAT activities. We know that oxidative stress is caused by the generation of superoxide anion in mitochondria of liver, kidney and other target tissues. On that account, the antioxidant and free radical status might not have changed in erythrocytes.

However, it has been demonstrated that erythrocyte GPx activity decreases in type II diabetic patients when compared to controls.[22] Similarly, we found important difference in GPx activities when compared to diabetic control (IV) group.

LIPID PEROXIDATION AND ANTIOXIDANT STATUS IN LIVER HOMOGENATE

Furthermore, we determined increased MDA levels in livers of diabetic control group. Increased MDA levels were also observed in liver tissues of L-NNA treated control and diabetic groups. SrB treated groups, SrB treated diabetic and control group, and SrB + L-NNA treated diabetic groups showed normal lipid peroxide levels. Therefore, the present results suggest that SrB treatment seems to be effective in decreasing lipid peroxidation in diabetes.

Superoxide dismutase (SOD) and catalase (CAT) are major antioxidant enzymes. Decreased activity of SOD and CAT are found in diabetes.[5] In our study, as expected, there were not any statistically significant differences in experimental groups compared to control groups concerning the liver CAT activities. However, there was an important decrease in diabetic control (IV) group and L-NNA treated diabetic group when SOD activities of experimental groups were compared to control. There are not any statistical differences in SrB treated groups, SrB treated diabetic and control groups, and SrB + L-NNA treated diabetic groups concerning the liver SOD activities. As an antidiabetic agent, SrB increased the SOD levels in the diabetic groups.

TABLE 6: The hepatocyte diameters of control and diabetic groups.

Groups	n	Hepatocyte Diameter (µm)
Control Groups		
I.	7	7.72 ± 0.38
II.	7	6.78 ± 0.23
III.	7	8.47 ± 0.76
Diabetic Groups		
IV.	7	9.81 ± 1.07***
V.	7	7.38 ± 0.62
VI.	7	7.87 ± 0.29
VII.	7	8.43 ± 0.39

*** P< 0.001 (according to control group I).

Decreased GPx levels are reported in diabetes as an antioxidant enzyme.[5,16] We found decreased GPx levels in the diabetic groups as well. On the contrary, SrB treated diabetic groups showed increased GPx levels.

It has been reported that serum nitric oxide levels increased in STZ induced diabetic rats.[6] In our study, there were not any statistically significant differences when the NOS levels were compared.

NOS LEVELS IN SERUM AND LIVER HOMOGENATE

In STZ-induced diabetic rats, serum levels of nitric oxide (NO) is shown to be increased in the diabetic groups compared the controls.[6] Similarly, in this study, serum NOS activities were lower in SrB and L-NNA treated groups compared to the diabetic control group. This might be caused by diabetes-induced oxidative stress.

Increased activity of NOS in the liver of diabetic animals is reported in some studies.[23,24] Nevertheless, we did not find any statistical differences in the liver NOS levels of the experimental groups when compared to control. This finding might be due to the fact that, type II diabetes harms the tissues in the long term and the short duration of the present experiment was not enough to observe changes in NOS levels in the liver of diabetic animals.

HISTOPATOLOGY

Several studies have reported that the hepatocytes of STZ-induced diabetic mice showed cytoplasmic alterations similar to those observed in so-called oncocytic cells. The effects of diabetes on hepatic structure include hypertrophy and autophagic vacuoles in hepatocytes. It was suggested that, in type II diabetes, there were several pycnotic nuclei and dilated sinusoids. The hepatocytes' nuclei were generally enlarged and sometimes showed irregular contours and intranuclear inclusions.[20,21,25]

In this study, we observed similar morphological changes in STZ-NA treated diabetic rat liver. In untreated diabetic rats and L-NNA treated diabetic rats, the nuclei of hepatocytes were enlarged. Moreover hepatocytes with pycnotic nuclei and sinusoidal structures were seen in the liver cells of diabetic control and L-NNA diabetic groups. Additionally, in the SrB treated diabetic group, cells were seen empty and nuclei were seen separated from the cytoplasm. We suggest that these histological changes induced by diabetic complications.

The intensity of changes in SrB and L-NNA treated diabetic rats was less severe when compared to untreated diabetic rats.

CONCLUSION

As a result, the extracts of *Stevia rebaudiana* Bertoni leaves have some beneficial effects on diabetes-induced histological, oxidative and hyperglycemic changes. It was determined that L-NNA was less efficient in the treatment of type II diabetes when compared to SrB. Further studies on SrB concerning the treatment of diabetes seem warranted.

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