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Effect of sea buckthorn (Hippophae rhamnoides) on kidney and testicular damage, sperm quality and expression of Irisin and Asprosin in Streptozotocin-Induced diabetic rats

Efecto del espino amarillo (Hippophae Rhamnoides) sobre el daño renal y testicular, la calidad del esperma y la expresión de Irisina y Asprosina en ratas diabéticas inducidas por Estreptozotocina

Merve Pekince-Özöner[1], Sema Timurkaan[2], Fatih Mehmet Gür[3], Berrin Tarakçi-Gençer[2], Hatice Eröksüz[4], İbrahim Halil Güngör[5].*

[1]*Department of Histology and Embryology, Faculty of Veterinary Medicine, Siirt University, Siirt, Turkey.*
[2]*Department of Histology and Embryology, Faculty of Veterinary Medicine, Fırat University, Elâzığ, Turkey.*
[3]*Department of Histology and Embryology, Faculty of Medicine, Niğde Ömer Halisdemir University, Nigde, Turkey.*
[4]*Department of Pathology, Faculty of Veterinary Medicine, Fırat University, Elazığ, Turkey.*
[5]*Department of Reproduction and Artificial Insemination, Faculty of Veterinary Medicine, Firat University, Elazig, Turkey.*
**Correspondence author:* merve.pekince@siirt.edu.tr, *mervepknc2323@gmail.com*.

ABSTRACT

In recent years, many antioxidants have been used against hyperglycemia and oxidative damage in diabetes. The purpose of this study is to investigate the protective effects of Sea buckthorn (*Hippophae rhamnoides*) against adverse effects of diabetes on testicular and renal tissues. A total of 39 male Sprague-Dawley rats were assigned to 5 groups as follows: control, citrate, SBT, diabetes, and diabetes+SBT. Diabetes induction was made by streptozotocin (50 mg/kg intraperitonally) to diabetes and diabetes+SBT group. SBT oil was administered to SBT and diabetes+SBT group (50mg/kg/48h by oral gavage). At the end of study, testicular and kidney samples for histochemical and immunohistochemical examinations, serum samples for biochemical examinations and sperm samples for spermatogenic examinations were collected. The results of the analysis showed that SBT reduced body weight loss and lowered blood glucose levels by reducing the harmful effects of diabetes-induced oxidative stress. When serum TAS and TOS levels were evaluated, it was determined that TAS level was the highest in the SBT group and TAS level increased in the diabetes + SBT group compared to diabetes and the other groups. While TOS level increased in the diabetes group, it decreased in the diabetes+SBT group. SBT also increased sperm density and motility and reduced total abnormality (head-tail) in diabetic rats. In SBT-treated diabetic rats, histopathological changes during the course of diabetes significantly reduced. In addition, decreased irisin expression in renal tissue and decreased irisin and asprosin expression in testicular tissue in the diabetes group were significantly normalized in the diabetes+SBT group. In this study, it was found that the application of SBT oil significantly prevented hyperglycemia and oxidative stress in diabetes, and protected testicular and renal tissues from the functional and histopathological changes in these organs caused by hyperglycemia and oxidative stress in diabetic animals. These results showed that SBT oil was an effective nutritional supplement that can be used to protect against the adverse effects of diabetes.

Key words: Asprosin; diabetes; Irisin; oxidative Stress; sea buckthorn; sperm quality

RESUMEN

En los últimos años, se han utilizado muchos antioxidantes contra la hiperglucemia y el daño oxidativo en la diabetes. El objetivo de este estudio es investigar los efectos protectores del espino amarillo (*Hippophae rhamnoides*) contra los efectos adversos de la diabetes en los tejidos testicular y renal. Se asignaron 39 ratas macho Sprague-Dawley a 5 grupos: control, citrato, SBT, diabetes y diabetes+SBT. Se indujo diabetes mediante estreptozotocina (50 mg/kg por vía intraperitoneal) a los grupos diabetes y diabetes+SBT. Se administró aceite SBT al grupo SBT y diabetes+SBT (50mg/kg/48h por sonda oral). Al final del estudio, se recogieron muestras testiculares y renales para exámenes histoquímicos e inmunohistoquímicos, muestras de suero para exámenes bioquímicos y muestras de esperma para exámenes espermatogénicos. Los resultados de los análisis mostraron que el SBT reducía la pérdida de peso corporal y disminuía los niveles de glucosa en sangre al reducir los efectos nocivos del estrés oxidativo inducido por la diabetes. Cuando se evaluaron los niveles séricos de TAS y TOS, se determinó que el nivel de TAS era el más alto en el grupo SBT y el nivel de TAS aumentó en el grupo diabetes + SBT en comparación con la diabetes y los otros grupos. Mientras que el nivel de TOS aumentó en el grupo de diabetes, disminuyó en el grupo de diabetes + SBT. El SBT también aumentó la densidad y la motilidad espermáticas y redujo la anormalidad total (cabeza-cola) en las ratas diabéticas. En las ratas diabéticas tratadas con SBT, los cambios histopatológicos tras la diabetes se redujeron significativamente. Además, la disminución de la expresión de irisina en el tejido renal y la disminución de la expresión de irisina y asprosina en el tejido testicular en el grupo diabético se normalizaron significativamente en el grupo diabetes+SBT.En este estudio, se descubrió que la aplicación de aceite SBT prevenía significativamente la hiperglucemia y el estrés oxidativo en la diabetes, y protegía los tejidos testicular y renal de los cambios funcionales e histopatológicos en estos órganos causados por la hiperglucemia y el estrés oxidativo en animales diabéticos.Estos resultados demostraron que el aceite de SBT era un suplemento nutricional eficaz que puede utilizarse para proteger contra los efectos adversos de la diabetes.

Palabras clave: Asprosina; diabetes; Irisina; estrés oxidativo; espino amarillo; calidad del esperma.

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INTRODUCTION

Diabetes mellitus is a metabolic disorder which results in impaired glucose homeostasis after the failure of the synthesis of the hormone insulin and/or defects in the secretion of the hormone insulin. Impaired glucose balance leads to macrovascular (peripheral vascular disease, ischemic stroke and coronary heart disease) and microvascular (nephropathy, neuropathy, retinopathy and erectile dysfunction) disturbances in the body [1]. Previous studies have also reported that hyperglycemia together with diabetes causes damage to the urinary system due to disorders in germ cells and disorders in kidney functions and kidney histology due to disorders in the pituitary-hypothalamic system [2 , 3].

Today, the pathogenesis of diabetes is still not fully determined. However, the studies have indicated that the impaired glucose balance during the course of diabetes causes oxidative stress by increasing the amount of free radicals [4]. In recent years, there have been many studies investigating the effectiveness of antioxidant substances against free radicals that occur during the course of diabetes [5]. Sea Buckthorn (*Hippophae rhamnoides* L. Sea Buckthorn, SBT) is a shrub which can grow in different climates and belongs to the family Elaeagnaceae, and the color of its fruits varies from yellow to orange [6]. The SBT plant contains high concentration of unsaturated fatty acids, and also high levels of omega-7, omega-9, omega-6, and omega-3, which are abundant in its seeds [6 , 7]. Numerous studies have shown that this plant has antidiabetic, antiulcerogenic, antioxidant, cardioprotective and antiatherogenic effects [5 , 8 , 9].

Irisin, first discovered by Boström *et al.* [10], a hormone-like polypeptide called as fibronectin type III domain 5 in skeletal muscle. Irisin is a myokine that increases energy expenditure in the body by converting white adipose tissue into brown adipose tissue. It has been reported that irisin may have positive effects on glucose homeostasis and insulin sensitivity [10 , 11 , 12].

Asprosin, discovered by Romere *et al.* [13] in studies conducted in individuals diagnosed with neonatal Progeroid Syndrome, is a glucogenic adipokine synthesized from white adipose tissue after fasting. Asprosin causes a rapid release of glucose from liver cells via the G protein-cAMP-PKA pathway, leading to elevated blood glucose levels. It has been revealed that serum asprosin levels of individuals with mutations in hunger- related genes have decreased. In cases where asprosin is not supplied to the blood, a decrease in the amount of glucose and insulin is observed. It has been reported that the level of asprosin released into the circulation increases in fasting and decreases after feeding (high glucose state) [13 , 14].

The aim of the present study was to investigate the ameliorative effects of Sea Buckthorn on the biochemical, spermatogenic and histopathological changes occurring in renal and testicular tissues after STZ-induced diabetes. In addition, it was aimed to determine the immunoreactivity of irisin and asprosin, whose relationship with energy and glucose metabolism in testicular and renal tissues of diabetes and diabetes+SBT rats.

MATERIALS AND METHODS

The present study was conducted in accordance with Ethics Committee Decision taken at the meeting of Fırat University Animal Experiments Local Ethics Committee dated and numbered 2022/8.

In the study, totaly 39, seven-week-old male Sprague Dawley rats (*Rattus norvegicus)* weighing 200-250 g were used (Necklife FLY 500, Turkey). A total of 5 groups were randomly selected from these rats obtained as control, citrate, SBT, diabetes and diabetes+SBT groups. During the experiment, all animals were housed in standard cages in an environment at 22-25 °C and with 12 hours (h) of light/12 h of darkness and fed *ad libitum* with commercial rat feed

Control group (n:7) was not subjected to any treatment for 8 weeks, the experimental period.

Citrate buffer (SST) group (n:7) Single dose of 0.1 M SST was administered intraperitoneally (i.p.).

SBT Group (n:7) SBT oil (70mg/kg/48 h dose) was given by oral gavage for two months. The nutritional supplement Sea Buckthorn Oil Capsules (Pharma Nord, Erfurt, Germany) was used as a source of SBT Oil. TABLE I shows the nutritional content of these capsules.

Diabetes group (n:9) STZ 50 mg/kg dissolved in 0.1 M SST (pH:4.5) was administered to each rat i.p. as a single dose. Blood glucose level was measured 72 h after the injection from the tail vein. Rats with blood glucose levels above 250 mg/dL were considered as diabetic and included in the study. STZ- treated rats were given 5% dextrose for 12 h after the injection to prevent sudden hypoglycemic shock.

Diabetes+ SBT group (n:9) STZ 50 mg/kg was dissolved in 0.1 M SST (pH:4.5) and administered i.p. as a single dose to each rat. Rats with blood glucose levels above 250 mg/dL 72 h after injection were considered diabetic. SBT oil was administered to diabetic rats via oral gavage catheter at a dose of 70 mg/kg every 48 h for two months.

The body weights of the rats were recorded at the beginning and end of the experimental procedures. At the end of the eight-week experimental period, the rats were euthanized and blood, sperm, testes and kidney samples were taken. Testes and kidney samples were immersed in 10% buffered neutral formalin solution and fixed for 24 h for future histopathological examinations. For biochemical analysiś, serum samples were stored at -20°C (Vestfrost Vf 4820 Nf, Turkey). Semen samples were sent to the laboratory for analysis.

TABLE I. Sea Buckthorn Oil Capsules Nutrional Information

NUTRIONAL INFORMATION	Per 2 capsules (1000 mg)
Sea bucthorn oil contains:	1000 mg
Saturated fatty acids	210 mg
Monounsaturated fatty acids	480 mg
Of which	
Omega 7 (palmiloic asit)	240 mg
Omega 9 (oleic asit)	180 mg
Omega 7 (cis vassenik asit)	60 mg
Polyunsaturated fatty acids	300 mg
Of which	
Omega 6 (linoleic asit)	170 mg
Omega 3 (alfa linoik asit)	130 mg
Vitamin E (as alpha tocopherol)	4,0 mg
Vitamin A (from beta carotene)	400 ug

Biochemical analysis

At the beginning and end of the experimental procedures, blood-glucose values were measured with a glucometer (Rina Check AP 10, Germany) from the blood taken from the tail veins of the rats. After the other blood samples were placed in serum extraction tubes and centrifuged (3400 g, 10 min, Hettich EBA 200, Germany), TAS (Reel Assay Total Oxidant Status Test Kit, Gaziantep, Turkey) and TOS (Reel Assay Total Oxidant Status Test Kit, Gaziantep, Turkey) levels in these samples were measured using Erel's method [15].

Spermatological analysis

To examine sperm density in the study, the right cauda epididymis was separated from the testes and placed in a special glass petri dish with 1 mL of 0.9% NaCl. It was then cut into small pieces and incubated at room temperature for four h. The supernatant, which contained the spermatozoa, was combined with a 1:200 solution of eosin. Approximately 10 µL of this mixture was transferred to the thoma slide. The mixture was then spread over both fields and left for five minutes. After this period, the sperm density was evaluated under a 200x magnification (Celestron 44348 Penta View LCD Digital Microscope, USA).

To assess sperm motility, one drop of semen were collected from the left cauda epididymis, mixed it with a Tris buffer solution on a slide and analyzed it under the microscope at 37 °C (Celestron 44348 Penta View LCD Digital Microscope, USA). Motility rate was calculated as a percentage at 400 magnification in three different fields.

To determine the percentage of morphologically abnormal spermatozoa, 20 µL of the Tris buffer-spermatozoa mixture used for motility assessment was taken and dripped onto a microscope slide heated to 37°C. A thin smear of this mixture was applied to the slide and allowed to dry at room temperature. The slides were then examined under a light microscope at 400× magnification (Celestron 44348 Penta View LCD Digital Microscope, USA). A total of 200 spermatozoa were examined on each slide, and the head, tail, and total abnormality rates of the spermatozoa were expressed as percentages [16].

Histopathological analysis

Tissue processing and Haematoxylin and Eosin (H&E) staining. Fixed testicular and kidney tissues were subjected to routine tissue processing procedures. Subsequently, the tissue samples were embedded in paraffin blocks. Sections of 5 µm thickness were excised from the paraffin blocks using a rotary microtome (Thermo Scientific, USA), and the slides were stained with H&E, Periodic Acid Schiff (PAS) and Crossman methods to determine morphological and histopathological changes for histopathological analysis. Right and left testicular tissues stained with H&E were examined for histopathological changes such as interstitial edema, congestion and subcapsular hemorrhage and scored as undamaged, -; slightly damaged, +; moderately damaged, ++ and very damaged, ++++. The degree of maturation of germ cells was determined by evaluating at least 20 seminiferous tubules under a light microscope with a 40x objective (Olympus BX50, Japan) according to the Johnsen scoring [17]. After measuring the mean seminiferous tubule diameter (MSTD) and mean seminiferous epithelial thickness (MSET) in the seminiferous contorti tubules closest to the round in different regions using the Olympus Cell Sens Standard program (Version 1.1.17), the data obtained were statistically evaluated [18].

Haematoxylin and Eosin stained renal tissues were examined for histopathological changes such as glomerular atrophy, glomerular lobulation, tubular damage, vacuolization and the findings were rated as follows: No damage, 0; less than 10% damaged, 1; 25% damaged, 2; 26-50% damaged, 3; more than 75% damaged, 4. In addition, PAS stain was applied to determine the basement membrane thickness in both testicular and renal tissues. These tissues were photographed after staining and evaluated.

Immunohistochemical analysis

The immuno-peroxidase method was used for immunohistochemical analysis according to kit (Ultravision Detection System, Thermo Fisher Scientific, USA). Briefly, 5-µm thick sections of renal tissues were deparaffinized in xylol and passed through a series of alcohols. Antigen retrieval procedure was performed by boiling the tissue sections in 0.01 M citrate buffer (pH: 6) at 90-95°C for 30 min. Then, tissue sections were treated with H2O2 solution to inhibit endogenous peroxidase activity for 15 min and then treated with Ultra V Block solution for 10 min and incubated overnight at +4°C in slide tray (eBioscience StainTray, Thermo Scientific, USA) with primary antibodies (Irisin, 1/100 dilution, rabbit polyclonal, 201r-0335, Sun Red, China; Asprosin 1/200 dilution, rabbit polyclonal, 201r-5721, Sun Red, China). The tissue sections were incubated with secondary antibody following streptavidin peroxidase for 30 min in a in slide tray (eBioscience StainTray, Thermo Scientific, USA) at 37°C. Hematoxylin was used as the counterstain.

Immunohistochemical staining intensity was graded in the range of 0-3. 0, negative; 1 light; 2, medium; and 3 were rated as intense. Also, negative fields are 0; <25% stained areas as 0.1; 26–50% stained areas 0.4; Areas 51–75 stained 0.6; Areas stained between 76–100% were rated as 0.9. After obtaining these data, the histoscore was determined using the area x density formula [19 , 20].

Statistical analysis

The data were determined as mean ± standard deviation and statistical analyses were performed using SPSS 22 software (IBM Corp., Armonk, NY, USA). Kruskal Wallis and Posthoc Dunn test, were used for the evaluation between groups. The value of P<0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Diabetes has many systemic effects on the body. Findings from several studies have shown observable changes in body composition in experimental diabetic rats, including decreased testicular weight [21]. In contrast, a different study by Scarano *et al.* [22] reported that the while body weight of diabetic rats decreased significantly, there was no difference in testicular weight and as a result relative testicular weight increased. In the present study, although the relative testicular weights of the rats in the diabetes group increased compared to the control, citrate, and SBT groups, this increase was not statistically significant (TABLE II). This can be explained by the preservation of testicular weight in conjunction with a decrease in body weight among diabetic animals.

In nephropathy caused by diabetes, kidney weight increases due to cell proliferation and hypertrophy. In studies, it has been observed that nephropathy occurs in diabetic animals due to tubulointerstitial fibrosis and increased expression of angiogenic factors [23 , 24]. In the study, relative kidney weights were similar in the control, citrate, and SBT groups. Although there was an increase in the diabetes+SBT and diabetes groups compared to other groups, the relative kidney weights in the diabetes+SBT group were closer to control and citrate groups. However, these changes were not statistically significant (P>0.05) (TABLE II). This may be explained by the preservation of kidney weight with a decrease in body weight when calculating relative kidney weight in diabetic animals. The fact that the kidney weight of SBT supplemented diabetic animals was close to the control group suggests that SBT may prevent the increase in kidney weight caused by hyperglycaemia due to its antioxidant content. However, it was hypothesised that the reason for the lack of statistical difference may be attributable to the individual differences observed in the animals.

There are numerous studies showing that diabetes causes a decrease in body weight as a result of lipid and protein degradation [2 , 3]. However, in contrast to this decrease, it has been shown that administering different doses of SBT to diabetic animals causes an increase in body weight [25]. In this study, the post-experimental body weights of the Control, citrate, SBT and diabetes+SBT groups were similar (P>0.05). It was found that the body weights of diabetic rats decreased significantly compared to the other groups (P<0.05) (TABLE II). When the data obtained and the literature presented above were evaluated together, it was concluded that SBT may be effective against body weight loss due to diabetes as a result of reducing cellular oxidative stress and preventing lipid and protein degradation.

TABLE II. Results of Body Weight, Relative Kidney Weight, Relative Testicular Weight and Results of Spermatogenic Examination

GROUPS	Control	Citrate	SBT	Diabetes	Diabetes+ SBT
Body Weight	322±22^{a}	313±77^{ac}	345±35^{a}	234±18^{bc}	276±58^{a}
Relative Kidney Weight	0,83±0,059^{a}	0,96±0,35^{a}	0,82±0,063^{a}	1,3±0,093^{a}	1±0,16^{a}
Relative Testicular Weight	0,92±0,11^{a}	0,94±0,3^{a}	0,90±069^{a}	1±0,11^{a}	0,90±0,24^{a}
The amount of sperm motility	63±16^{a}	53±12^{a}	58±8,1^{a}	43±24^{a}	51±19^{a}
Amount of Sperm Density	84±12^{a}	88±23^{a}	90±27^{a}	71±9,7^{a}	84±14^{a}
Sperm Abnormality	4,8±1,8^{a}	4,1±2,4^{ac}	1,9±0,58^{bc}	5,1±1,8^{a}	5,1±2,3^{a}

All values are in the form of mean ± standard deviation. Different letters in the same line show that the statistically different from each other.

In a previous study in which STZ-induced diabetes model was established, it was reported that administration of SBT to rats decreased the blood-glucose value [5 , 9 , 26]. In another study, it was reported that SBT administered to rats fed a high-fat diet decreased blood glucose levels [27]. In the present study, it was observed that blood sugar levels between the groups were similar in control, citrate and SBT groups (P>0.05), while blood sugar levels in the diabetes + SBT group were significantly reduced compared to the diabetes group (P<0.05) (TABLE IV). In this study, consistent with other literature [5 , 9 , 26], it was observed that blood glucose levels decreased in diabetic rats administered SBT oil.

Sea buckthorn oil has been found to protect against oxidative stress caused by oxidizing lipids [25]. In the present study, it was determined that the serum TAS level was the highest in the SBT group, and that the TAS level increased in the diabetes + SBT group compared to the diabetes and other groups (P<0.05). The highest TOS value was observed in the diabetes group. In addition, while it was similar in the control, citrate and SBT groups, the TOS level decreased in the diabetes + SBT group compared to the diabetes group altough there was no significant difference (P>0.05) (TABLE III). The data obtained suggested that sea buckthorn may have a protective effect against oxidative stress caused by diabetes.

TABLE III. Results of Biochemical Blood-glucose, Serum TAS value, Serum TOS value

GROUPS	**Control**	**Citrate**	**SBT**	**Diabetes**	**Diabetes+ SBT**
Blood-glucose value	82±23^{a}	69±11^{a}	67±12^{a}	430±66^{b}	326±69^{c}
Serum TAS value	0,93±0,063^{ac}	0,87±0,11^{ac}	1.0±0,13^{bc}	0,85±0,044^{a}	0,90±0,091^{ac}
Serum TOS value	8,7±1,4^{a}	8,6±1,2^{a}	8,6±0,72^{a}	10±0,82^{a}	9,5±2,3^{a}

All values are in the form of mean ± standard deviation. Different letters in the same line show that the statistically different from each other

Oxidative stress in course of diabetes has been documented to reduce sperm count, sperm motility and increase abnormal sperm rate [28 , 29]. Navarro-Casado *et al.* [28] reported diabetes decreased sperm density and increased sperm total abnormality (head-tail) but did not affect sperm motility. In another study, it was determined that sperm count, sperm viability and motility rates, which decreased during the course of diabetes, increased as a result of quercetin administration [29]. In this study, percentage motility, density and sperm total abnormality (head-tail) were similar in the control and citrate groups.There was a decrease in motility in the diabetes group. When sperm motility and density values were compared with the diabetes group, an increase was observed in the diabetes + SBT group altough there was no significant difference (P>0.05). A slight increase in sperm density was observed in the SBT group. The rate of sperm anomaly (in the head and tail regions) was similar in the control, citrate, diabetes and diabetes + SBT groups and significantly decreased in the SBT group compared to the other groups except citrate group (P<0.05) (TABLE II). This results are consistent with literature [28 , 29]. In summary in this study, although not statistically significant, sperm density and motility decreased. Sperm anomalies was more evident in diabetic rats. On the other hand, sperm motility and density increased and sperm anomalies decreased in rats treated with SBT. This result suggests that SBT oil can be used to improve sperm quality in normal and diabetic animals.

Previous studies reported diabetes lead to a decrease in MSTD and MSET parameters, an increase in interstitial connective tissue and seminiferous tubule basement membrane thickness, and histopathological changes such as atrophy and degeneration in tunica albuginea and seminiferous tubules in testicular tissue [30 , 31]. In the present study, Hematoxylin-Eosin, Crossman trichrome and PAS staining results of rats in control, citrate and SBT groups showed normal histological structure in testicular tissue (FIG. 1). Histopathological changes such as decreased spermatogenic activity, atrophy in some seminiferous tubules, interstitial edema, hyperemia in blood vessels and increased basement membrane thickness were found in the testicular tissues of rats in the diabetes group. The diabetes + SBT group exhibited enhanced morphologic abnormalities in comparison to the diabetes group. In the testicular tissues of the rats in this group, the capillaries in the interstitial region were full and hyperemic (FIG. 1).

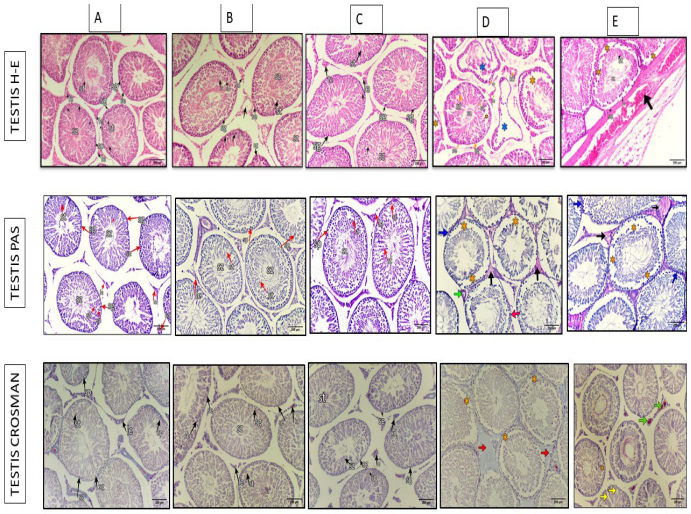

FIGURE 1. H-E, Crossman Triple and PAS staining of testicular tissue. A, control group. B, citrate buffer group. C, SBT group. D, diabetes group. E, diabetes+SBT group. Spermatogonium (sg), spermatocyte (sp), spermatid (st), spermatozoa (sz), blood vessel (kd), Leydig cell (le), sertoli cell (se), spermatogenic separating from basement membrane cells (orange star), interstitial edema (red arrow), hyperemia in capillaries (green arrow), vacuolization in tubules (yellow arrow), degenerated tubules (blue star), interstitial edema (black arrow).

TABLE IV. Scoring of histopathological changes in testicular tissue and kidney tissue

Histopathological changes		Control	Citrate	SBT	Diabetes	Diabetes+ SBT
TESTIS	Interstitial Edema	0,71±0,76^{a}	0,86±0,69^{a}	0,57±0,53^{a}	1,2±0,83^{a}	1,1±0,93^{a}
	Congestion	0,86±0,69^{ac}	0,29±0,49^{bc}	0,86±0,38^{ac}	1,2 ±0,67^{a}	0,44±0,53^{bc}
	Subcapsular Hemorrhage	0±0^{a}	0±0^{a}	0±0^{a}	0,11±0,33^{a}	0±0^{a}
	Johnsen Score	9,1±0,69^{a}	9,3±0,96^{a}	9,6±0,79^{a}	7,8±1,3^{b}	8,1±0,86^{b}
	Spermatogenic Activity	2,8±0,41^{a}	2,6±0,79^{ac}	2,1±0,6^{b}	2,3±0,76^{b}	2,8±0,41^{bc}
	MSET	98±9,3^{ac}	90±4,4^{bc}	93±4,8^{ac}	85±4,3^{b}	91±2,4^{bc}
	MSTD	285±9,3^{ac}	271±13^{b}	290±10^{a}	270±15^{ab}	270±5,8^{bcd}
KIDNEY	Glomerular Atrophy	0±0^{a}	0±0^{a}	0±0^{a}	0,67±0,71^{b}	0,22±0,44^{a}
	Glomerular Lobulation	0±0^{a}	0,14±0,38^{a}	0,14±0,38^{a}	0,56±0,53^{b}	0,11±0,33^{a}
	Tubular Damage	1,3±0,52^{a}	0,71±0,49^{a}	1,4±0,53^{a}	3,7±0,87^{b}	2,8±1,2^{b}
	Vacuolization	1,4±1,2^{a}	1,1±0,83^{a}	0,14±0,38^{a}	0±0^{b}	0,14±0,38^{b}
	Expansion in Bowman's capsule	1,6±0,53^{a}	1,2±0,83^{a}	0,9±0,9^{ac}	0,43±0,53^{bc}	0,71±0,49^{a}

All values are in the form of mean ± standard deviation. Different letters in the same line show that the statistically different from each other

It was established that the Johnsen scores of the rats in the control, citrate, and SBT groups were closely aligned, while the Johnsen score of the rats in the diabetes group exhibited a substantial decline in comparison to these groups ($P < 0.05$). Despite an increase in the Johnsen score of the rats in the diabetes + SBT group compared to the diabetes group, this increase was not statistically significant ($P > 0.05$) TABLE IV).

When MSET values were compared between groups, it was found that MSET in the diabetes group decreased statistically significantly compared to the control, citrate, and SBT groups ($P<0.05$), whereas, MSET in the diabetes + SBT group increased and approached the control group ($P>0.05$) (TABLE IV). When evaluating the MSTD results, it was found that MSTD values decreased in the citrate, diabetes, and diabetes + SBT groups compared to the control and SBT groups, while there was no significant difference between the diabetes and diabetes + SBT groups ($P>0.05$) (TABLE IV). In accordance with the findings, it was noted that the diameter and thickness of the seminiferous tubules decreased in diabetes. These results indicates that diabetes had a harmful effect on the histological structure of seminiferous tubules. In this study, results of the diabetes + SBT group exhibited a greater similarity to those of the control group suggests that SBT reduced testicular damage caused by diabetes.

In diabetic kidney tissues, it causes damage to many histopathological changes including atrophy of molar glomeruli, expansion of Bowman's capsule and thickening of basement membrane, shedding and vacuolization of tubular epithelium, atrophy of tubules and proteinosis filtrate [32 , 33]. A literature review revealed that a study on the effects of SBT on the kidney showed that SBT ameliorated renal damage [32]. In histochemical staining of sections of kidney tissue, it was observed that the histological structure of the cortex and medulla regions of the kidneys of control, citrate and SBT groups was normal. In H&E stained kidney tissues of the diabetic group, histopathological changes such as atrophy of some glomeruli, expansion of Bowman's capsule, intense bleeding in some areas, vacuolization of tubules (Armani-Ebstein lesions), atrophy and proteinosis filtrate were observed. Although tubular degeneration, vacuolization, hemorrhage, mononuclear cell infiltration and clear structures were observed in the kidney tissues of the Diabetes + SBT group, pathological changes were reduced compared to the diabetes group (FIG. 2).

TABLE IV shows the scoring of histopathological changes in the kidney tissue. Examination of Crossman triple stained sections showed a slight increase in collagen and fibrosis in the interstitial space in the diabetes group (FIG. 2). Examination of PAS stained sections showed shedding of the tubular epithelium and thickening of the basement membrane of the Bowman capsule in the diabetes group (FIG. 2). Shedding of the tubular epithelium was observed in the diabetes + SBT group, but other histopathological changes observed in the diabetes group were significantly decreased (FIG. 2). The findings of the present study are consistent with the findings of previous studies confirming the pathological effects of diabetes on the kidneys. In this study, SBT, which has antioxidant effects, decreased the histopathological changes mentioned above in the kidney tissues of the diabetic group.

Irisin is a hormone-like polypeptide which discovered by Boström *et al.* [10] in skeletal muscles. In their study, Eser *et al.* [34] observed that irisin level decreased significantly after STZ-induced diabetes [34]. In a study exploring the relationship between oxidative stress and irisin during the course of diabetes, it was found that irisin level decreased as oxidative stress increased [35]. Immunohistochemical expression of irisin has been shown in tissues such as brain, heart, stomach, pancreas, testis, epididymis, liver and testis [19 , 36]. Aydın *et al.* [19] reported that irisin-positive immunostaining was detected in spermatogenic and Leydig cells in human fetus, strong immunostaining was detected in Leydig cells and weak immunostaining was detected in spermatogenic cells in adult human testicular tissue. In the present study, the immunohistochemical staining pattern of irisin in the testicular tissues of the control, citrate and SBT group rats was similar and weak immunostaining was observed in Leydig cells. The lowest Irisin expression was observed in the diabetes group, and the highest in the diabetes + SBT group. Irisin immunoreactivity was statistically different between Diabetes and in favor of diabetes+SBT groups ($P<0.05$). This result may be due to antioxidant content of SBT to reduce oxidative stress [36]. No positivity was observed in the negative control preparations (FIG. 3). Histoscore values between the groups are given in TABLE V.

When the immunoreactivity of Irisin in kidney tissue was examined, positive cells with weak/medium staining intensity were found in the proximal and distal tubules in the cortex of the kidney tissue of the control, citrate and SBT groups. Although the expression decreased in the diabetes group compared to the control group, it was not statistically significant ($P>0.05$). Intense positive Irisin immunostaining was seen in the proximal tubules of the diabetes + SBT group rats and a statistical difference was detected compared to other groups ($P<0.05$). Negative control staining did not show positivity (FIG. 3). TABLE V presents the intergroup histoscore values of irisin in kidney tissue.

In this study, it was determined the intensity of irisin-positive staining in Leydig cells in both testicular and kidney tissues of diabetic rats was weaker than in other groups, while the staining intensity in the diabetes + SBT group was significantly increased compared to the diabetes group. It is hypothesised that positive enhancing effect of SBT on irisin expression is by its intense antioxidant content and therefore its effect of reducing oxidative stress.

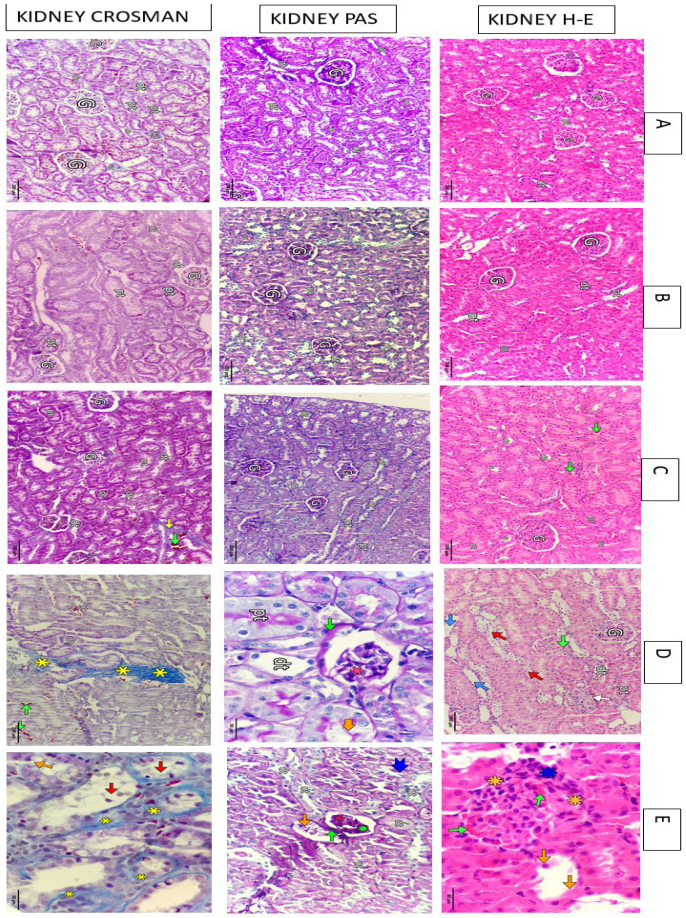

FIGURE 2. H-E, Crosman Triple and PAS staining of kidney tissue. A, control group. B, citrate buffer group. C, SBT group. D, diabetes group. E, diabetes+SBT group. Glomerulus (G), proximal tubule (pt), distal tubule (dt), macula densa (md), hyperemia (green arrow) tubular dilatation (blue arrow), tubular vacuolization (Armani Ebstein lesions) (red arrow), narrowing of Bowman's space (white arrow), mononuclear cell infiltration (blue star), exfoliation of tubular epithelium (orange arrow), proteinous filtrate (black arrow), connective tissue increase (yellow star), thickening of basement membrane (green arrow)

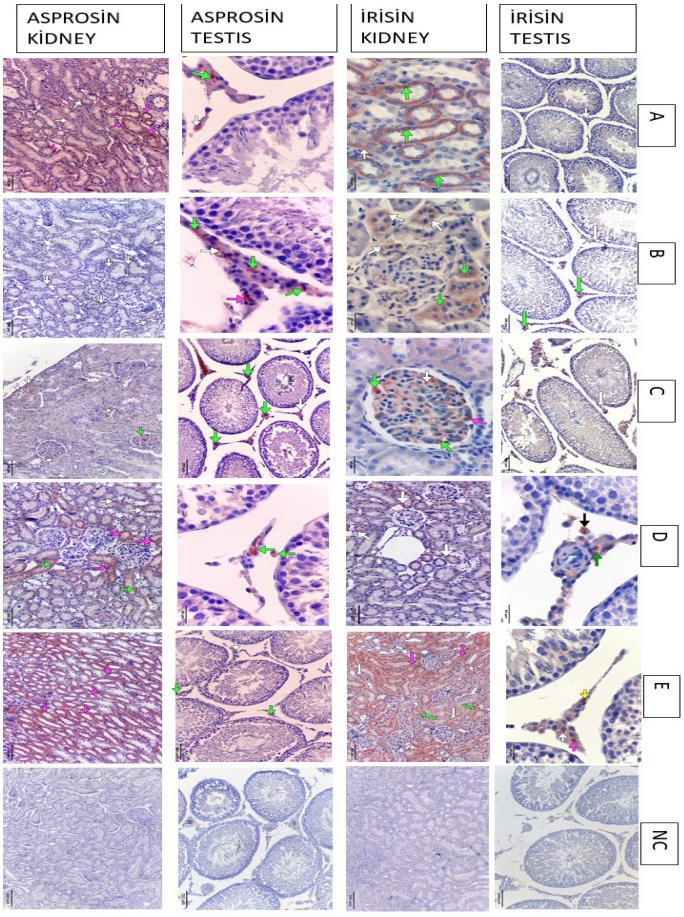

FIGURE 3. Irisin and Asprosin immunoreactivity in testicular and kidney tissue. A, control group. B, citrate buffer group. C, SBT group. D, diabetes group. E, diabetes+SBT group. Weak Leydig cell immunopositivity (white arrow), moderate Leydig cell immunopositivity (green arrow, pink arrow), strong Leydig cell immunopositivity (yellow arrow)

TABLE V. Evaluation of Irisin and Asprosin immunoreactivity in testis and kidney tissue

Immunohistochemical evaluation	Control	Citrate	SBT	Diabetes	Diabetes+ SBT
Irisin immunoreactivity of testicular tissue	0,5±0,20^{ac}	0,8±0,46^{ac}	0,6±0,69^{ac}	0,35±0,41^{a}	1,0±0,23^{bc}
Irisin immunoreactivity of kidney tissue	0,7±0,12^{a}	0,92±0,64^{a}	0,66±0,44^{ac}	0,58±0,26^{a}	1,5±0,63^{bc}
Asprosin immunoreactivity of testicular tissue	0,90±0,14^{a}	0,6±0,28^{a}	0,6±0,28^{a}	0,47±0,12^{a}	0,76±0,41^{a}
Asprosin immunoreactivity of kidney tissue	0,8±0^{a}	0,5±0,14^{a}	0,6±0,28^{a}	1,1±0,23^{a}	1,3±0,50^{a}

All values are in the form of mean ± standard deviation. Different letters in the same line show that the statistically different from each other.

Asprosin is a recently discovered adipokine secreted from white adipose tissue [13]. In a previous study, asprosin administration increased blood glucose concentration in healthy rats; however, it was reported that it did not cause any changes in diabetic rats [37]. One study showed immunohistochemical localisation of asprosin in distal tubule cells in the kidney and Leydig cells in the testes of diabetic rats. In the same study, when comparing control and diabetes in terms of expression, asprosin was found to be increased in the stomach and testes of diabetic rats, decreased in liver, kidney and heart tissues, and there was no significant change in brain tissue [38]. Another study found that serum asprosin levels were high in people with type 2 diabetes-induced nephropathy and that asprosin levels increased with the severity of nephropathy [39]. In a study determining the relationship between asprosin and aging-related spermatogenetic activity, it was determined that asprosin level decreased in parallel with the decrease in testosterone level. The data obtained in the same study suggest that a decrease in asprosin level, which is associated with insulin sensitivity and glucose transport, may cause regression in testicular development [40]. In a study by Keskin *et al*. [41], it was indicated that intracerebral administration of asprosin

increased hypothalamic GnRH, mRNA and protein levels in male rats, thus causing an increase in LH, FSH and testosterone levels. In addition, it was determined that sperm density and motility increased in the testes after asprosin administration. These results suggest that asprosin has regulatory roles in the reproductive system and testicular functions [41]. When asprosin immunoreactivity was examined in testicular tissue, the immunoreaction of testicular tissues of Control, Citrate and SBT groups was similar. The lowest asprosin immunoreactivity was observed in the diabetes group. The immunoreaction of asprosin was almost the same in control and diabetes + SBT groups. The tissues used as negative controls were asprosin negative (FIG. 3). No positivity was observed in the negative control preparations Histoscore values between the groups are given in TABLE V.

Kidney tissue asprosin expression was found to be similar in the control, citrate and SBT groups. The highest asprosin expression was seen in the diabetes + SBT group. It was detected that immunoreactivity increased in the diabetes and diabetes + SBT groups compared to the other groups, but it was not a significant increase ($P>0.05$). Negative control staining did not show positivity (FIG. 3). These results may be related to severity of nephropathy [39]. Histoscore values between the groups are given in TABLE V. In the present study, it was found that asprosin expression in the testicular tissues of diabetic rats decreased compared to the control group, while asprosin expression in the kidney tissues increased. The increase in asprosin expression in the kidney tissues of the diabetic group was probably due to nephropathy. It was thought that the decrease of Asprosin immunoreactivity in testicular tissue may be due to insulin sensitivity, glucose transport, and testicular development.

CONCLUSION

The study revealed that SBT oil administered to rats with experimental diabetes reduced diabetes-induced body weight loss, lowered blood glucose levels, and reduced the harmful effects of oxidative stress during the course of diabetes. SBT administration increased sperm density and motility, reduced abnormalities, significantly reduced histopathological damage in testicular and renal tissues during the course of diabetes, and had positive effects on irisin and asprosin expression related to glucose and energy metabolism in diabetic rats. In order to determine more precisely the therapeutic efficacy of SBT against the negative effects of diabetes on testicular and renal tissues, it is thought that it would be beneficial to plan studies in the future to investigate the effectiveness of SBT at different doses.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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