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Effects of Silymarin on immunohistochemical Bax and 8-Ohdg expression, biochemical markers and sperm parameters in an experimental varicocele model in rats

Efectos de la Silimarina sobre la expresión inmunohistoquímica de los marcadores Bax y 8-OHDG sobre los parámetros espermáticos en un modelo experimental de varicocele en ratas

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ABSTRACT

In this study, the effects of silymarin on immunohistochemical Bcl-2-associated X protein (Bax) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) expression, biochemical markers and sperm parameters were investigated with an experimentally induced varicocele model in rats. The study was conducted on 36 Wistar albino rats. The distribution of rats within the group was made in an equal number. Rats in the control group were administered physiological saline daily via oral gavage. In the sham group, an incision was made on the midline and the renal vein (located on the left) was made visible. A probe was placed on this vein. The probe was coiled with the vein but not ligated. In the silymarin group, silymarin was administered by oral gavage at a dose of 75 mg/kg 3 times a week for 8 weeks. Ligation was performed on rats in the varicocele group, unlike the sham group. Varicocele was created in the varicocele+silymarin groups (50 mg/kg, 75 mg/kg). Silymarin application was started 8 weeks after varicocele induction and was applied 3 days a week for 8 weeks. After the analysis, it was seen that sperm parameters were negatively affected in the varicocele group. Additionally, severe caspase 3, 8-OHdG and Bax expressions were detected. Silymarin administration reduced the intensity of expression and had positive effects on spermatology. These positive effects were even more pronounced with the 75 mg dose. Based on the results obtained, silymarin may have the potential to reduce both clinical and pathological symptoms in varicocele cases.

Key words: Heat Shock Protein (HSP); silymarin; varicocele; transforming growth factor Alpha (TGF-α); vascular endothelial growth factor-A (VEGF-A)

RESUMEN

En este estudio se investigó los efectos de la silimarina sobre la expresión inmunohistoquímica de la proteína X asociada a Bcl-2 (Bax) y la 8-hidroxi-2'-desoxiguanosina (8-OHdG), los marcadores bioquímicos y los parámetros espermáticos con un modelo de varicocele inducido experimentalmente en ratas. El estudio se realizó en 36 ratas albinas Wistar. La distribución de ratas dentro del grupo se realizó en igual número. A las ratas del grupo de control se les administró diariamente solución salina fisiológica por sonda oral. En el grupo simulado, se practicó una incisión en la línea media y se hizo visible la vena renal (situada a la izquierda). Se colocó una sonda en esta vena. La sonda se enrolló con la vena pero no se ligó. En el grupo de la silimarina, ésta se administró por sonda oral a una dosis de 75 mg/kg 3 veces por semana durante 8 semanas. La ligadura se realizó en las ratas del grupo varicocele, a diferencia del grupo simulado. Se creó varicocele en los grupos varicocele+silimarina (50 mg/kg, 75 mg/kg). La aplicación de silimarina se inició 8 semanas después de la inducción del varicocele y se aplicó 3 días a la semana durante 8 semanas. Tras el análisis, se observó que los parámetros espermáticos se veían afectados negativamente en el grupo con varicocele. Además, se detectaron expresiones graves de caspasa 3, 8-OHdG y Bax. La administración de silimarina redujo la intensidad de la expresión y tuvo efectos positivos en la espermatología. Estos efectos positivos fueron aún más pronunciados con la dosis de 75 mg. Según los resultados obtenidos, la silimarina puede tener el potencial de reducir tanto los síntomas clínicos como los patológicos en los casos de varicocele.

Palabras clave: Proteína de choque térmico (HSP); silimarina; varicocele; factor de crecimiento transformante alfa (TGF-α); factor de crecimiento endotelial vascular A (VEGF-A)

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INTRODUCTION

Varicocele, defined as pathological dilatation of the veins in the spermatic cord, is considered one of the factors that cause reproductive problems in men. While it is seen in 15-22% of the adult male population, the rate of varicocele among infertile men reaches 30-40% [1 , 2]. Many conditions occur in varicocele that negatively affect fertility. These include many negative situations such as decreased spermatozoa concentration and viability and increased malondialdehyde (MDA) level [3 , 4]. Additionally, pathological levels of reactive oxygen species develop in varicocele. Reactive oxygen species (ROS) at pathological levels can cause many undesirable consequences such as defective sperm function, deterioration of sperm morphology, damage to sperm DNA and inadequate sperm-oocyte fusion [5 , 6].

Varicocele negatively affects reproductive physiology if left untreated or if treatment is delayed. The most appropriate treatment in varicocele cases is varicocelectomy. However, there are some unclear situations regarding varicocelectomy. In addition to the lack of pre- and postnatal data, there is still controversy regarding the methodology and the criteria applied. Additionally, there is information that infertility continues significantly after varicocelectomy [3 , 7 , 8 , 9].

Since the negative impact on reproductive function also affects psychology negatively, finding new alternative agents to surgical intervention is of great importance. For these reasons and because they present lower risks than surgery, many studies investigated the benefits of treatment with antioxidants. Silymarin is a flavonoid complex obtained from the seeds of milk thistle (Silybum marianum). Silymarin was reported to have many biological activities such as hepatoprotective [10], anti-cancer [11], and anti-inflammatory effects [12]. It acts as a powerful antioxidant by reacting with ROS and also potentiates the effects of physiological antioxidants such as glutathione and superoxide dismutase [13 , 14].

A review of the literature reveals the existence of studies examining silymarin and varicocele. However, it has been determined that the effects of silymarin on varicocele have not been comprehensively investigated within the framework of the methodology and markers utilized in this study. Therefore, an experimental varicocele model was established, and the effects of silymarin on biochemical markers (HSP-90, HSP-70, Inhibin B, VEGF-A, TGF-α, Testosterone), histopathological findings, immunohistochemical markers (Caspase 3), and immunofluorescent markers (8-OHdG, Bax), as well as its influence on spermatological parameters (Spermatozoa concentration, Motility, Abnormal spermatozoa rate), were evaluated.

MATERIALS AND METHODS

Animals and creation of experimental groups

The study was conducted using 36 male rats (*Rattus norvegicus*) (Wistar albino, 300-370 g). A total of 6 groups were arranged and groups included 6 rats. Rats were kept in individual cages in a suitable living space (21 ± 1 °C temperature, 55 ± 15% humidity and 12 hours of daylight). In the study, extreme care was taken about hygiene and surgical interventions were performed under xylazine/ketamine anesthesia. Blood was taken from all rats at the end of the study, including the control group, and orchiectomy was performed. The study received the necessary approval from the Van Yuzuncu Yil University Animal Experiments Ethics Committee with the number "2020/07-09". In addition, the study was carried out by taking into account the instructions of the relevant board.

Control group: Physiological saline was administered daily by oral gavage throughout the study period.

Sham group: After an incision was made on the midline, the renal vein on the left side was made visible. A probe was placed on this vein. This vein and the probe were wrapped around it with the help of a ligature without being tied. The midline incision was closed. After 60 days (d), blood was taken under anesthesia and orchiectomy was performed.

Silymarin group (75 mg/kg): Silymarin was administered by gavage 3 times a week for 8 weeks (75 mg/kg).

Varicocele group: After an incision was made on the midline, the renal vein on the left side was made visible. A probe was placed on this vein. This vein and the probe were connected with the help of a ligature. The midline incision was then closed. After 60 d, blood was taken under anesthesia and orchiectomy was performed.

Varicocele+silymarin group (50 mg/kg): Varicocele was induced. Silymarin administration was started 8 weeks after varicocele induction and applied 3 d a week for 8 weeks.

Varicocele+silymarin group (75 mg/kg): Varicocele was created. Silymarin administration was started 8 weeks after varicocele induction and applied 3 d a week for 8 weeks.

Varicocele induction

An incision was made at the top of the midline of the abdomen. After the renal vein was made visible, a probe was placed on it and tied with a ligature. The probe was removed. The vessel was allowed to expand within the boundaries of the ligature. The abdominal wall and anterior abdominal muscles were sutured separately and the incision area was closed. As a result of this narrowing process, intravascular pressure increased and this pressure was transferred to the spermatic vein, varicocele was formed. Overdoing the narrowing process may lead to kidney necrosis. If the narrowing process is inadequate, it causes insufficient intrarenal vein pressure. For this reason, great care was taken in the narrowing process [15].

Semen collection and spermatological evaluation

While the rats were under anesthesia, one testis was removed immediately after blood collection and before the body cooled. The cauda epididymis of the excised testis was initially used to assess sperm motility under a light microscope (ECLIPSE E 400 Nikon JAPAN), set at 37°C. During the evaluation of motility, the dense rat semen were diluted in saline at 37°C. The percentage of sperm motility was evaluated at 200x magnification using a heated-stage light microscope as described by Sonmez *et al.* [16].

Sperm density and abnormal sperm rates were determined from the suspension mixture obtained by slicing the same cauda epididymis section (after motility assessment) in 2 ml of saline. Sperm density was determined using a slightly modified method

described by Sonmez *et al.* [16] The semen sample was drawn into an Eppendorf tube using a 10 µL automatic pipette, and 990 µL of eosin solution was added. Approximately 10 µL of the diluted sperm suspension was transferred to the counting chambers of a Thoma slide (Germany) and left for 5 min. The sperm count was performed under a phase-contrast microscope at 200x magnification, and the sperm density was calculated using the appropriate formula. The mean of three consecutive evaluations was used as the final motility score.

To assess abnormal sperm cells, the method reported by Turk *et al.* [17] was applied. Briefly, a drop of the semen sample was mixed with an equal amount of eosin-nigrosin stain (1.67% eosin, 10% nigrosin, and 0.1 M sodium citrate), and a thin smear was prepared. A total of 300 sperm cells were examined under a light microscope (Olympus BX 51, JAPAN) at 400x magnification.

Histopathological examination of testicles

Testicular tissue samples were first detected (10% formaldehyde). Then, the sections prepared in paraffin blocks were stained with hematoxylin-eosin (HE) and microscopic evaluation was performed (Olympus BX 51, JAPAN). Sections were evaluated as none (-), mild (+), moderate (++) and severe (+++) according to their histopathological findings

Immunohistochemical examination

For immunohistochemical examination, tissue sections placed on adhesive (poly-L-Lysin) slides were deparaffinized and dehydrated. Then, endogenous peroxidase was inactivated by keeping in 3% H2O2 for 10 minutes. Then, the tissues were boiled in 1% antigen retrieval (citrate buffer (pH+6.1) 100X) solution and left to cool at room temperature. To prevent nonspecific background staining in the tissues, the sections were incubated with protein block for 5 minutes. Then, primary antibody (Caspase 3 Cat No: sc-56053, Dilution Ratio: 1/100, US) was dropped onto the tissues and incubated according to the instructions for use. 3-3' Diaminobenzidine (DAB) chromogen was used as the chromogen in the tissues. Stained sections were examined with a light microscope (Zeiss AXIO GERMANY).

Double immunofluorescence staining method

The 4-µm sections were taken on adhesive slides, deparaffinized, dehydrated, and washed with PBS. Endogenous peroxidase was inactivated in 3% H2O2 for 10 min. Then, the samples were boiled in 1% antigen retrieval (citrate buffer (pH + 6.1) 100X) solution and cooled to room temperature. Sections were incubated with protein block for 5 min to abolish nonspecific background staining. Then, the primary antibody (8 OHdG cat no: sc-66036, dilution ratio: 1/100, US) was added according to the manufacturer's instructions. This was followed by the immunofluorescence 2nd antibody marker (FITC cat no. ab6785, diluent ratio 1/500, UK) and dark incubation for 45 min. Then, the primary antibody (Bax cat no. sc-7480, dilution ratio 1/100, US) was dripped onto the sections and incubated according to the manufacturer's instructions. A secondary immunofluorescence antibody was used as a secondary marker (Texas Red cat no. ab6719, diluent ratio 1/500, UK) and kept in the dark for 45 min. Then, DAPI with mounting medium (cat no. D1306, dilution ratio 1/200, UK) was dripped onto the preparations and kept in the dark for 5 min. Afterward, the stained sections were covered with a coverslip and examined under a fluorescence microscope (Zeiss AXIO, Germany).

Biochemical analyses

The rats were euthanized using xylazine 10 mg·kg-1 IP (2% Rompun® Bayer) and Ketamine (HCl) (10% Alfamine® Atafen) 75 mg·kg-1 IP injectable anesthetics, and the animals were sacrificed by high volume blood collection under anesthesia and testicular tissue was taken. The blood taken was centrifuged (Nuve, NF 800R, Turkey) at 4000 G for 10 min and the serum was separated. Serum samples were stored (Dt, Fydl– 268, Turkey) at -40 °C until the day of study. Phosphate buffer (pH: 7.2–7.4) was added to the testicular tissues at 10 times their weight. It was homogenized with the aid of a homogenizer. Care was taken to ensure the cold chain at all stages of the experiment. The homogenates were centrifuged at 2000–3000 rpm, +4°C for 20 minutes and the supernatant was separated.

In serum and testicular tissue, HSP-90 (Catalog No: SG-20499, SinoGeneClon Biotech, China), HSP-70 (Catalog No: SG-20498, SinoGeneClon Biotech, China), inhibin B (Catalog No: SG-20735, SinoGeneClon Biotech, China), transforming growth factor-alpha (TGF-α) (Catalog No: SG-20059, SinoGeneClon Biotech, China), and VEGF-A (Catalog No: SG-21042, SinoGeneClon Biotech, China) levels were detected with species-specific ELISA kits. In addition, serum testosterone level was measured with the Abott Architect ci16200 (Germany) device using a commercial kit.

Statistical analysis

For statistical analysis of sperm parameters, one-way Analysis of Variance (ANOVA) was used to compare group means and post-hoc Tukey test was used to determine the difference between groups following analysis of variance. In order to determine the intensity of positive staining on images obtained as a result of immunohistochemical and immunofluorescence staining, 5 random areas were selected from each image and evaluated with the ZEISS Zen Imaging Software program. Data were statistically described as mean and standard deviation (mean±SD) for % area. One-way ANOVA followed by Tukey's test was performed to compare positive immunoreactive cells and immunopositive stained areas with healthy controls. One-way ANOVA was used to determine the difference between groups in terms of biochemical parameters, and the Duncan test, one of the multiple comparison tests, was used to determine which group caused the differences.

RESULTS AND DISCUSSION

Although varicocelectomy is known to be a suitable method to treat infertility in varicocele, there is a trend towards some non-surgical techniques because infertility continues from time to time after varicocelectomy. In the literature review, the effectiveness of many chemical agents was investigated in this field [18 , 19].

The values obtained regarding sperm findings are presented in TABLE I. A non-significant difference was detected between the control group, sham group and silymarin group in terms of sperm parameters (spermatozoa concentration, motility, abnormal spermatozoa rate) (P>0.05). However, in the other 3 groups (varicocele group, varicocele+50, varicocele+75), the differences detected in terms of all three sperm parameters were significant (P<0.05). In the current study, silymarin increased spermatozoa concentration and motility, while significantly reducing the abnormal spermatozoa rate and

leading to significant improvements in sperm parameters. Similar significant changes in sperm parameters (sperm vitality, motility and count) were reported in a different varicocele studie evaluating the effect of sodium selenite on testicular damage induced by experimental varicocele in male Wistar rats [20].

TABLE I .
Sperm parameters in different treatment groups in an experimental varicocele model in rats

Groups	Spermatozoa concentration ($\times 10^6$)	Motility (%)	Abnormal spermatozoa rate (%)
Control	126.16 ± 0.98a	78.33 ± 0.51a	11.16 ± 0.40d
Sham	125.16 ± 0.40a	77.50 ± 0.54a	11.50 ± 0.54d
SMN75	126.50 ± 0.83a	78.50 ± 0.54a	10.50 ± 0.54d
VC	72.16 ± 0.75d	30.83 ± 0.40d	43.50 ± 0.54a
VC+SMN50	88.16 ± 0.98c	45.83 ± 0.98c	32.16 ± 0.40b
VC+SMN75	96.50 ± 0.54b	53.16 ± 0.98b	27.83 ± 1.16c

a b c d; Different letters in the same column indicate a statistically significant difference (P<0.05). SMN75: Silymarin (75mg/kg). VC: Varicocele. VC+SMN50: Varococele + Silymarin (50 mg/kg). VC+SMN75: Silymarin (75 mg/kg).

In histopathological examination, the testicular tissues of the control group, sham group and silymarin 75 group had normal histological appearance (FIG. 1). In the evaluation performed in the varicocele group, severe degeneration and necrosis of spermatocytes, thinning of the tubule wall, edema in the intertubular region and hyperemia in the vessels were detected (FIG. 1). The findings detected in the histopathological examination of the varicocele group were moderate in the varicocele+silymarin 50 group (FIG. 1). However, the hyperemia of vessels was severe. The findings detected in the varicocele group were at a mild level in the varicocele+silymarin 75 group (FIG. 1). However, a statistically significant difference (P<0.05) was detected when the varicocele+silymarin 75 group was compared with the varicocele group. It is possible to see the histopathological findings in TABLE II.

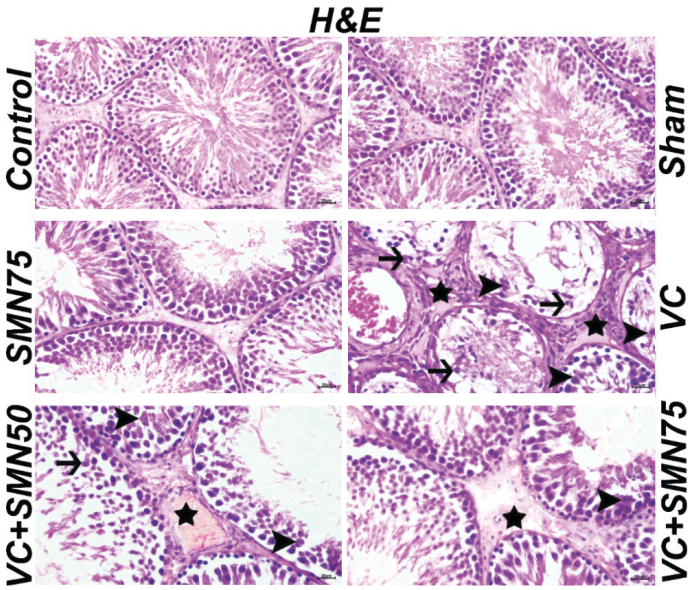

FIGURE 1. Testicular tissue in different treatment groups in an experimental varicocele model in rats. Degeneration of spermatocytes in the tubular wall (arrowheads), necrosis (arrows), thinning of the tubular wall, hyperemia in the vessels, edema in the intertubular spaces (stars), H&E, Bar: 20 µm. SMN75: Silymarin (75mg/kg). VC: Varicocele. VC+SMN50: Varococele + Silymarin (50 mg/kg). VC+SMN75: Silymarin (75 mg/kg).

TABLE II .
Histopathological findings and scoring for testicular tissues with experimental varicocele of the left spermatic vein

Parameters	Control	Sham	SMN 75	VC	VC+ SMN 50	VC+ SMN 75
Degeneration in spermatocytes	-	-	-	+++	++	+
Necrosis in spermatocytes	-	-	-	+++	++	-
Hyperemia in vessels	-	-	-	+++	+++	+
Thinning of the tubular wall	-	-	-	+++	++	+
Edema in the intertubular region	-	-	-	+++	++	+

SMN75: Silymarin (75mg/kg). VC: Varicocele. VC+SMN50: Varococele + Silymarin (50 mg/kg). VC+SMN75: Silymarin (75 mg/kg)

While caspase 3 expression was at a severe level in the varicocele group, it was at a moderate level in the varicocele+silymarin 50 group and at a mild level in the varicocele+silymarin 75 group (FIG. 2). A statistically significant difference (P<0.05) was detected when the varicocele+silymarin 75 group was compared with the varicocele group. Data for immunohistochemical findings are presented in TABLE III. Oxidative stress resulting from increased Reactive oxygen species in varicocele was reported to increase apoptosis [21]. Activated caspase 3 can induce spermatogenic cell apoptosis in varicocele [22]. In this regard, caspase 3 expression was evaluated immunohistochemically in the current study. The results obtained regarding caspase 3 in current study confirm previous reports that caspase 3 expression was reported to be at severe levels in the varicocele group [23 , 24].

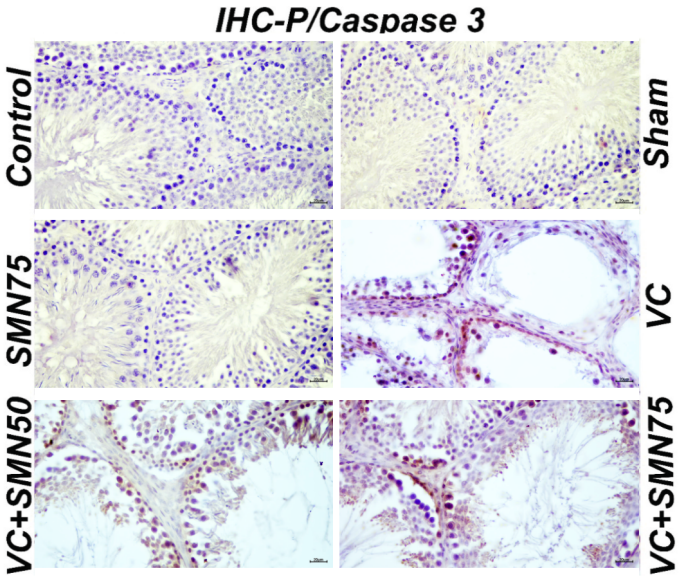

FIGURE 2. Testis tissue caspase 3 expression, IHC, Bar: 20 µm. SMN75: Silymarin (75mg/kg). VC: Varicocele. VC+SMN50: Varococele + Silymarin (50 mg/kg). VC+SMN75: Silymarin (75 mg/kg).

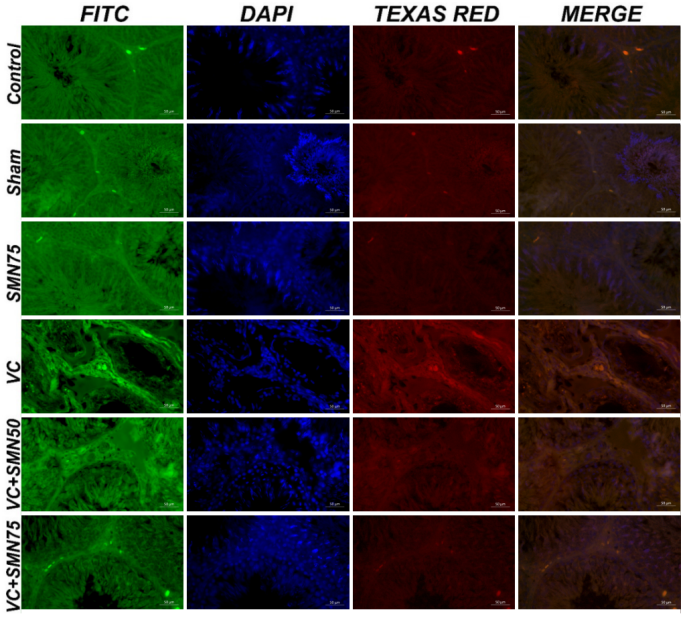

FIGURE 3. Testis tissue 8-OHdG expression (FITC) and Bax expression (Texas Red), IF, Bar: 50 µm. SMN75: Silymarin (75mg/kg). VC: Varicocele. VC+SMN50: Varococele + Silymarin (50 mg/kg). VC+SMN75: Silymarin (75 mg/kg).

When the testicular tissues stained with the immunofluorescence staining method were examined, 8-OHdG and Bax expressions were negative in the control group, sham group and silymarin 75 group (FIG. 3). While 8-OHdG and Bax expressions were at severe levels in the varicocele group, they were at moderate levels in the varicocele+silymarin 50 group and at mild levels in the varicocele+silymarin 75 group (FIG. 3). A statistically significant difference (P<0.05) was detected when the varicocele+silymarin 75 group was compared with the varicocele group. Data for immunohistochemical findings are presented in TABLE III. In current study, silymarin significantly reduced the expression levels of both 8-OHdG and Bax. The high levels of 8-OHdG and Bax expressions detected in the varicocele group are compatible with the results of previous studies [25].

TABLE III .
Data and statistical analysis of immunohistochemical and immunofluorescence findings for testicular tissues with experimental varicocele of the left spermatic vein.

Groups	Caspase 3	8-OHdG	Bax
Control	18.49 ± 0.56^{a}	24.61 ± 0.75^{a}	20.03 ± 0.91^{a}
Sham	19.16 ± 0.41^{a}	24.52 ± 0.39^{a}	21.18 ± 0.43^{a}
SMN75	19.03 ± 0.7^{a}	24.63 ± 0.26^{a}	20.84 ± 0.51^{a}
VC	64.95 ± 1.83^{b}	80.66 ± 1.54^{b}	71.5 ± 1.55^{b}
VC+SMN50	43.38 ± 1.26^{c}	53.16 ± 1.43^{c}	48.66 ± 1.4^{c}
VC+SMN75	31.54 ± 1.33^{d}	35.7 ± 0.87^{d}	32.15 ± 0.93^{d}

a b c d; Different letters in the same column indicate a statistically significant difference (p<0.05). SMN75: Silymarin (75mg/kg). VC: Varicocele. VC+SMN50: Varococele + Silymarin (50 mg/kg). VC+SMN75: Silymarin (75 mg/kg).

Serum biochemical parameters of all groups are presented in TABLE IV, and testicular tissue biochemical parameters are presented in TABLE V. There was no significant difference between the groups in terms of serum HSP-90 levels (P>0.05). Although the serum HSP-70 level in the varicocele group was lower than the control group, this decrease was not significant (P>0.05). Serum HSP-70 level was significantly lower in the varicocele+silymarin 50 group (P<0.05). Testicular tissue HSP-90 levels were similar in all groups. The highest HSP-90 level belonged to the varicocele group. Although the HSP-90 level in the varicocele groups treated with silymarin was lower than in the varicocele group, this decrease was not significant. (P>0.05). Varicocele group testicular tissue HSP-70 level was higher than the control and silymarin groups. Although testicular tissue HSP-70 levels in the varicocele groups treated with silymarin were lower than those in the varicocele group, this decrease was not significant (P>0.05). When the varicocele group was compared with the control group, serum HSP-70 and HSP-90 levels were found to decrease in the varicocele group. Silymarin (75 mg) administered to the varicocele group was able to cause an increase in serum HSP-70 level. The results obtained for serum HSP-70 in the present study were obtained by administering testosterone and VitE in a previous study [26]. In current study, HSP-70 and HSP-90 levels in testicular tissue increased in the varicocele group, and this increase was consistent with a previous study [27]. The upregulation of HSPs detected in the varicocele group may be a cellular response to scrotal hyperthermia occurring in varicocele.

Serum inhibin B level in the varicocele group was lower than the other two groups (control and sham groups) (P<0.05). Although serum inhibin B levels in the varicocele groups treated with silymarin were higher than the varicocele group, this increase was not significant (P>0.05). Testicular tissue inhibin B levels were similar in all groups. The lowest inhibin B level was detected in the varicocele group. Findings with inhibin B are similar to previous studies [25 , 28]. Both doses of silymarin

administered in the current study significantly regulated the inhibin B levels reduced by varicocele. A similar effect to silymarin on inhibin B was obtained with berberine in another study [28].

Serum VEGF-A level in the varicocele group was significantly higher than all other groups except the control (P<0.05). Serum VEGF-A levels in the varicocele+silymarin groups were found to be significantly lower than the varicocele group (P<0.05). Tissue hypoxia, which is likely to occur in varicoceles, increases VEGF-A expression and increased VEGF-A causes damage to the seminiferous tubules [29]. Serum and testicular VEGF-A levels evaluated in the current study increased in the varicocele group compared to the control group, consistent with previous studies [30]. This condition expression may have been elevated in the varicocele group as a compensatory mechanism to counteract the hypoxic state that can impair testicular function.

The group with the lowest serum TGF-α level was the varicocele group. Serum TGF-α level in the varicocele group was significantly lower than in only one group (varicocele+silymarin 50 group) (P<0.05). Varicocele+silymarin 50 group testicular tissue TGF-α level was found to be significantly higher than all other groups (P<0.05). Although the testicular tissue TGF-α level of the varicocele group was lower than the control group, this decrease was not significant (P>0.05). In the current study, both serum and tissue TGF-α levels were significantly lower in the varicocele group compared to the other groups. This detected situation supports the findings of previous studies [31]. Both doses of silymarin administered in the current study increased serum and tissue TGF-α levels.

Testosterone levels in the varicocele group were significantly lower than the other 3 groups (control, sham and silymarin groups) (P<0.05). Although the serum testosterone level in the varicocele groups treated with silymarin was higher than the varicocele group, it was not significant (P>0.05). The serum level of testosterone hormone, which is considered a clinical marker for detecting physiological activity in Leydig cells, was significantly decreased in the varicocele group compared to the control and sham groups, and this finding is similar to previous varicocele studies [25 , 28 , 32]. In the study found that silymarin increased testosterone levels, but this increase was not significant.

TABLE IV .
Serum biochemical parameters with experimental varicocele of the left spermatic vein

Groups	HSP-90 (pg/ml)	HSP-70 (pg/ml)	Inhibin B (pg/ml)	VEGF-A (pg/ml)	TGF-α (pg/ml)	Testosteronee (ng/ml)
Control	512.48 ± 51.3^{a*}	423.98 ± 12.58 ^{a}	52.90 ± 4.38 ^{a}	20.09 ± 4.11 ^{a,b}	53.73 ± 3.78 ^{a,b}	4.79 ± 0.47 ^{a}
Sham	480.24 ± 33.22^{a}	424.89 ± 19.97 ^{a}	50.63 ± 4.14 ^{a}	16.73 ± 3.14 ^{b,c}	50.61 ± 4.85 ^{a,b}	4.72 ± 0.45 ^{a}
SMN75	453.37 ± 39.83^{a}	425.51 ± 15.90 ^{a}	49.09 ± 4.66 ^{a,b}	15.97 ± 3.77 ^{b,c}	52.16 ± 3.32 ^{a,b}	4.40 ± 0.73 ^{a}
VC	497.48 ± 39.87^{a}	409.24 ± 30.43 ^{a}	45.20 ± 2.41 ^{b}	23.06 ± 4.78 ^{a}	47.86 ± 3.98 ^{b}	3.43 ± 0.48 ^{b}
VC+SMN 50	482.02 ± 51.99^{a}	373.53 ± 18.52 ^{b}	49.40 ± 5.14 ^{a,b}	14.54 ± 1.67 ^{c}	54.68 ± 6.64 ^{a}	3.67 ± 0.62 ^{b}
VC+SMN 75	463.37 ± 54.77^{a}	413.47 ± 11.93 ^{a}	47.83 ± 2.92 ^{a,b}	15.81 ± 3.16 ^{b,c}	50.15 ± 3.02 ^{a,b}	3.62 ± 0.63 ^{b}

*; Different letters in the same column indicate a statistically significant difference (P<0.05). SMN75: Silymarin (75mg/kg). VC: Varicocele. VC+SMN50: Varococele + Silymarin (50 mg/kg). VC+SMN75: Silymarin (75 mg/kg).

TABLE V .
Biochemical parameters for testicular tissue with experimental varicocele of the left spermatic vein

Groups	HSP-90	HSP-70	Inhibin B	VEGF-A	TGF-α
Control	363.36 ± 36.81^{a}	306.21 ± 44.17^{b}	40.51 ± 5.78^{a}	11.76 ± 1.01^{c}	40.75 ± 3.78^{b}
Sham	354.09 ± 30.30^{a}	324.93 ± 19.91^{a,b}	40.35 ± 4.79^{a}	12.57 ± 1.69^{c}	37.81 ± 4.48^{b}
SMN75	361.56 ± 27.27^{a}	305.94 ± 24.13^{b}	38.68 ± 3.16^{a}	12.79 ± 1.05^{c}	41.39 ± 6.79^{b}
VC	414.72 ± 59.20^{a}	350.78 ± 22.64^{a}	35.63 ± 3.37^{a}	13.57 ± 1.98^{b,c}	34.82 ± 4.26^{b}
VC+SMN 50	386.38 ± 10.25^{a}	318.18 ± 24.54^{a,b}	41.41 ± 4.44^{a}	15.44 ± 1.81^{a,b}	48.37 ± 6.88^{a}
VC+SMN 75	404.11 ± 107.10 ^{a}	337.94 ± 48.80^{a,b}	41.38 ± 4.38^{a}	16.31 ± 3.51^{a}	40.99 ± 8.04^{b}

*; Different letters in the same column indicate a statistically significant difference (P<0.05). SMN75: Silymarin (75mg/kg). VC: Varicocele. VC+SMN50: Varococele + Silymarin (50 mg/kg). VC+SMN75: Silymarin (75 mg/kg).

CONCLUSIONS AND IMPLICATIONS

Varicocele negatively affects sperm parameters, creates some changes in biochemical parameters and exacerbates apoptosis by increasing the expression levels of caspase-3, Bax and 8-OHdG. Silymarin administration had positive effects, especially on sperm quality parameters, biochemical parameters and enhancement of apoptosis by reducing the expression levels of caspase-3, Bax and 8-OHdG. Both doses of silymarin had positive effects on TGF-α levels. Based on these results, we can affirm that silymarin can be used therapeutically in varicocele, as it reduces the level of deterioration resulting from testicular pathology.

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Conflict of interest

The authors declare no competing interests.

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