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Examination of effect of jervine purified from the *Veratrum album* on changes in testicular tissue of varicocele-induced rats

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Abstract: **Background**: Varicocele is a dilatation of the pampiniform plexus. It is generally considered the most common cause of male infertility. **Objectives**: This study aimed to investigate the effects of jervine on testicular tissue obtained from rats with induced varicocele. **Methods**: In this experimental study, 40 male Sprague Dawley rats were allocated randomly to 6 separate groups: varicocele, healthy + jervine (10 mg/kg), varicocele + jervine (5 mg/kg), varicocele + jervine (10 mg/kg), control and sham. After the left testes were dissected and excised, the tissue was divided into three parts for histological, biochemical and real-time PCR examinations. **Results**: We found a difference between the groups for the sperm anomaly rate. We also observed that seminiferous tubules were damaged in the varicocele and varicocele + jervine groups. TNF-α immunoreactivity was stronger in the varicocele group than in the others. We determined that superoxide dismutase activity was lower and catalase activity was higher in the varicocele group compared to the control group. Jervine can downregulat the *p53* gene when used in healthy rats. **Conclusion**: These findings suggest that Jervine may promote sperm morphology and contribute to the prevention of varicocele-induced damage by decreasing *TNF-α* and *p53* levels in experimental varicocele rats.

Keywords: *Jervine*; *p53*; Testes; *TNF-α*; Varicocele

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INTRODUCTION

An abnormal expansion and dilatation of the scrotal venous pampiniform plexus, which drains blood from each testicle, is known as a varicocele. Varicoceles are typically painless, but they are clinically significant since they are the most frequently found cause of reduced sperm count, poor motility, aberrant sperm morphology and abnormal semen analysis (Yetkin and Ozturk, 2018; Paick and Choi, 2019; Fallara *et al*., 2023). The most popular option for treating varicocele is thought to be varicocelectomy. Varicocelectomy has been demonstrated to decrease DNA fragmentation, enhance chromatin integrity and increase semen quality. Sperm concentration, motility and morphology were all en hanced by varicocelectomy, which also decreased DNA fragmentation. Furthermore, research demonstrated that abnormal DNA fragmentation index levels should be regarded as an indicator for varicocelectomy (Birowo *et al*., 2020). The scrotal pampiniform plexus exhibits varicose tortuosity, swelling and elongation in varicocele. A clinical varicocele typically is developed in 25-35% of infertile men (Nieschlag *et al*., 2023). Semen measures like sperm concentration, total sperm motility, progressive motile sperm count and sperm morphology have also shown notable changes following varicocelectomy. Varicocelectomy is advised according to the European Association of Urology 2023 guidelines for individuals with clinical varicocele and studies have shown that surgery enhances the likelihood of spontaneous pregnancies. Semen measures such as sperm quantity, progressively motile sperm count, total sperm motility and shape of sperm have also shown notable changes following varicocelectomy (Baazeem *et al*., 2011; Agarwal *et al*., 2023). However, medical treatments have been investigated as an alternative to varicocelectomy (Takacs *et al*., 2025). Jervine is a steroidal alkaloid derived from plants of the genus *Veratrum and* it was discovered in 1981. It was first named jervinone (Cordell, 1981). (Fig. 1).

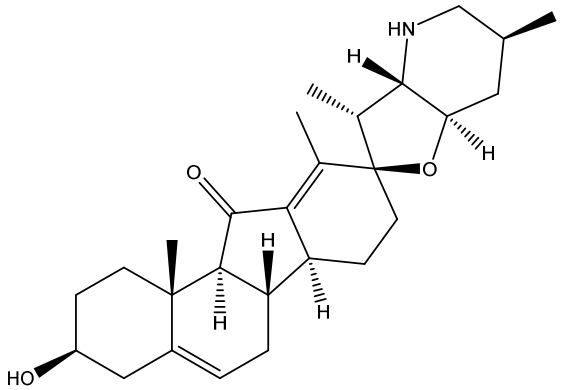

Fig. 1: Structure of Jervine

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It is also known as a cyclopamine analogue and is an important steroidal alkaloid that inhibits the hedgehog signaling pathway, which plays a role in the proliferation of cancer cells. Therefore, jervine, obtained from *Veratrum album,* has been used in previous studies (Lee *et al*., 2007; Tang *et al*., 2008). In addition, studies have reported the antioxidant and anti-inflammatory effects of jervine (Dumlu *et al*., 2019) Leukocytes and endothelial cells produce pro-inflammatory and anti-inflammatory cytokines to regulate inflammation (Abbas *et al*., 2021; Babu *et al*., 2004). One of these cytokines, *TNF-α*, is synthesized by cells such as macrophages, fibroblasts, endothelial cells, monocytes and adipocytes. Certain oxidant radicals are toxic to living organisms. Most investigations have shown an aberrant rise in pro-inflammatory cytokines, such as interleukin (IL)-1 and tumor necrosis factor alpha (*TNF-α*), in seminal plasma, testicular tissue and even peripheral blood, as well as anti-sperm antibodies, in animal models and varicocele patients (Fang *et al*., 2021). Studies have shown that inhibition of inflammation may attenuate varicocele-mediated pathogenesis (Mazhari *et al*., 2018).

Antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) protect cells from the harmful effects of these radicals (Freeman and Crapo, 1982). The *p53* protein has functions in DNA repair, gene expression regulation, chromosome separation, regulation of gene expression, regulation of chromosome segregation and DNA replication (Harris, 1996). In the present study, the aim was to examine the effect of jervine on testicular tissue obtained from a varicocele-induced rat model using histological, immunohistochemical, biochemical and molecular methods.

MATERIALS AND METHODS

Plant material

Veratrum album was collected from the Zigana Mountains of Trabzon (Türkiye) in July 2020. The root parts of the plant were ground after drying in the shade before being put out in the sun. In order to obtain this active substance, it was identified by Gül Esma Akdogan, a member of the Department of Biology at Kafkas University. The plant sample was preserved at Ağrı İbrahim Çeçen University Central Research and Application Laboratory.

Extraction and isolation

Jervine was purified from *Veratrum album* roots as described previously. The plant sample (900 g) was macerated five times at room temperature with acetone (2.5 l × 24 h) and filtered. The filtrates were combined and the solvents were removed in a rotary evaporator and 12.76 g of acetone extract (1.4%) was obtained. The acetone extract (12.76 g) was fractionated by silica gel column chromatography (CC) (47 × 4 cm, 150 g, 70–230 mesh); ethyl acetate:methanol 1:0, 9:1, 8:2, 6:4, 4:6). The fractions (40 ml each) were checked by thin layer chromatography (TLC) and fractions that gave the same stain on TLC were combined. The major compound was isolated in 69–81 fractions (1.1 g). It was determined that the chemical structure of the compound was jervine by 1D ^{1}H and ^{13}C NMR spectroscopic methods. The NMR spectra were published in our previous study (Aydin *et al*., 2014). It was determined by high performance liquid chromatography (HPLC) that the jervine was 97% pure. ^{1}H NMR ($CDCl_3$): 5.35 (*d*, *J*=4.8, H6; 3.44–3.55 (m, H3); 3.29 (*dt*, *J*=10.3, 4.0, H23); 3.06 (*dd*, *J*=12.4, 4.0, H_a26; 2.65 (*t*, *J*=9.2, H22); 2.31 (*t*, *J*=12.5, H_b26); 2.15 (s, H18); 0.98 (s, H19); 0.94 (*d*, *J*=6.0, H21); 0.93 (*d*, *J*=6.2, H27). ^{13}C NMR ($CDCl_3$): 207.0 (C11); 145.9 (C12); 142.6 (C5); 137.4 (C13); 121.1 (C6); 85.8 (C17); 76.6 (C23); 71.8 (C3); 66.8 (C22); 62.8 (C9); 54.8 (C26); 45.1 (C20); 41.7 (C4); 40.6 (C14); 39.1 (C24); 38.2 (C8); 37.3 (C10); 37.0 (C1); 31.7 (C25); 31.4 (C16); 31.2 (C2); 30.9 (C7); 24.6 (C15); 18.9 (C27); 18.7 (C19); 12.3 (C18); 10.9 (C21).

Experimental varicocele induction in rats

In this experimental study, 40 adult male Sprague Dawley rats (180–200 g) were used. The animals were kept in a room under standard living conditions (20 ± 2 °C; between 40% and 60% relative humidity; 12 h/12 h light/dark cycle) and were housed with four rats per cage. They were divided into separate 6 groups as follows:

Varicocele

7 rats; the abdomen of each rat was opened surgically, the left renal vein was partially ligated and then the abdomen was closed.

Varicocele + jervine 5 mg/kg

7 rats; the abdomen of each rat was opened surgically, the left renal vein was partially ligated, then the abdomen was closed and jervine 5 mg/kg was administered by oral gavage. The jervine solution was prepared using distilled water.

Varicocele + jervine 10 mg/kg

7 rats; the abdomen of each rat was opened surgically, the left renal vein was partially ligated, then the abdomen was closed and jervine 10 mg/kg was administrated by oral gavage.

Healthy + jervine 10 mg/kg

7 rats; the abdomen was not opened surgically but jervine 10 mg/kg was administrated by oral gavage.

Control

6 rats; no application was conducted in the control group.

Sham

6 rats; the abdomen of each rat was opened surgically and closed without ligation of the left renal vein.

The abdomens of all rats were opened surgically after 10 mg/kg xylazine and 90 mg/kg ketamine were administered intraperitoneally (ip). The jervine was administered every day after the day of inducing varicocele during the study. Jervine was used in doses of 5 mg/kg and 10 mg/kg in our study (Yakan *et al*., 2019). At the end of week 4 of the study, the rats were sacrificed by blood collection from the heart under anesthesia (ketamine 90 mg/kg, xylazine 10 mg/kg) (Barqawi *et al*., 2004, Fazlioglu *et al*., 2008). After the left testes were removed, the tissue was divided into three pieces for histological, biochemical and molecular examinations. The tissue for histological examination was preserved in 10% formalin. That for biochemical and molecular analyses was stored at -80°C. During the surgical procedure, 15 min after anesthesia the anterior abdominal skin was shaved and cleaned with an antiseptic. Laparotomy was performed with an incision of approximately 4 cm in the midline. After access was gained to the peritoneal cavity, the juncture of the left spermatic vein and the left renal vein was made observable. After removal of the adipose tissue around this area, the left renal vein was narrowed using 0.85-mm-thick metal wire. The left testicular tissue pieces separated for histological examinations were fixed in 10% formalin for 72 h.

Histopathologic analysis

Serial sections of 5 µm thickness from each tissue were obtained using a microtome (Leica RM2125RTS). Hematoxylin and eosin (H&E), periodic acid Schiff (PAS) and Masson's trichrome stains were applied for histological examinations (Demir, 2001). The longest diameters of 4 seminiferous tubules from each tissue in the control and sham groups and three seminiferous tubules from the other groups were measured using Cellsense Software at 20× magnification with a light microscope (Olympus BX43). Twenty seminiferous tubule diameters were selected by simple random method from the same area and from each group for comparison among the groups using statistical analysis. The immunoreactivity was graded from 0 to +3 according to the degree of positivity (0 = no reaction; 1 = minimal reaction; 2 = moderate reaction; 3 = strong reaction) (Moghimian, Soltani, Abtahi & Shokoohi, 2017). The degree of positivity for immunoreactivity in five areas from each subject was scored by the same person. Data from 30 random areas from each group were used for statistical analysis.

Biochemical analysis

SOD activity

The SOD activity assay is based on the formation of superoxide radicals produced by xanthine and xanthine oxidase, which react with Nitroblue Tetrazolium to form a formazan dye (Sun *et al*., 1988). This formazan dye gives maximum absorbance at a wavelength of 560 nm and the decrease in absorbance gives SOD activity. To determine the SOD activity, the testes tissue was homogenized in phosphate buffer (0.1 mM, pH 3) using a homogenizer. The homogenates was centrifuged at 18,000 × g for 60 min. The supernatants (each 500 µl) were added to 2450 µl of a mixture containing sodium pyrophosphate buffer (0.052 mM, pH 7.0), xanthine (0.3 mM), EDTA (0.6 mM), phenazine methosulfate (186 µM), Na_2CO_3 (0.4 M), xanthine oxidase (50 µl), bovine serum albumin (1.2 g/l) and NBT (150 µM). The enzyme reaction was initiated by adding 0.2 ml of NADH (780 µM) and stopped precisely after 1 min by adding 100 µl of $CuCl_2$ solution (0.8 mM). The amount of formazan was measured spectroscopically at 560 nm and the results were expressed as mM/min/mg tissue. All experiments were performed in triplicate.

CAT activity

CAT activity in the testes tissue of the rats was determined as described by Aebi (Aebi, 1984). To this end, the testes tissue (each piece 0.5 g) of the rats was homogenized in 4.5 ml of phosphate buffer (50 mM, pH 7.8) and the homogenates were centrifuged at 18,000 × g at 4 °C for 60 min. The supernatant was then used for the assay. After the supernatants were incubated on ice for 30 min, the reaction was started by the addition of aliquots (20 µl) of supernatant to 980 µl of a preparation containing 50 µl of 1 mol/l Tris-HCl buffer (5 mmol/l EDTA, pH 8.0) and 900 µl of 10 mmol/l H_2O_2 and then adding 30 µl of H_2O to bring the final volume up to 1 ml. The rate of decomposition of H_2O_2 (9 mmol/l) was measured by changes in absorbance at 240 nm. Catalase activity was described as the amount of enzyme required to decompose 1 nmol of H_2O_2 per min at 25 °C and pH 7.8 and the results were expressed as mmol/min/mg tissue. All experiments were performed in six replicates.

RNA extraction and reverse-transcription polymerase chain reaction (RT-PCR) analysis

Total RNA was purified from the testicular tissue using the ECO-TECH total RNA kit following the instructions provided by the manufacturer. RNA was eluted using 50 µl of nuclease free water and stored at -80 °C. The concentrations of the RNA samples were assessed using Thermo Scientific™ NanoDrop™ One/OneC Microvolume UV-Vis (Thermo Fisher Scientific, Waltham, MA, USA). The total RNA was used for RT-qPCR analysis. A High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA) was used to reverse-transcribe RNA into cDNA in accordance with the manufacturer's instructions for the RT-PCR. RT-PCR analyses were performed using the resulting total cDNA on a TaqMan® custom plate (Applied Biosystems) designed with 2 genes (*TNF-α* and *p53*). The data were collected using a 7500 Fast Real-Time PCR system (Applied Biosystems). Each gene's expression level was normalized to the level of the reference gene (*β-Actin*) and the relative expression of the genes was computed using the $2^{-\Delta\Delta Ct}$ technique.

Statistical analysis

Among the groups, the post hoc test of one-way ANOVA (IBM SPSS) was used to compare body weight, left testis weight, right testis weight, seminiferous tubule diameter, sperm length and biochemical analysis results. To determine differences between the groups for the number of sperm head and tail anomalies, the chi-squared test was used. The normality of the RT-PCR data for each group was confirmed using the Shapiro–Wilk test. Given the normal distribution of the data, differences in gene expression levels were evaluated using one-way ANOVA. ANOVA followed by Tukey's post hoc test was used for pairwise group comparisons to account for multiple comparisons. The *p* value for significant differences among the groups was set at <0.05. The analyses were performed in Origin Pro 2024 software.

RESULTS

Body weight, testis weight and sperm results

Statistically significant difference was not found among the groups for body weight, right testis weight, or left testis weight. According to the results of the statistical analyses, there was no significant difference between the groups in terms of sperm length, sperm head length, or sperm tail length. When the seminiferous tubule diameters were compared between the groups, it was seen that the mean seminiferous tubule diameter of the varicocele group was significantly shorter than those of all the other groups. The seminiferous tubule length of the varicocele + jervine (5 mg/kg) group was between those of the varicocele group and the control group. In our study, there was a difference between the groups in the sperm anomaly rate. The normal sperm rate of the control group was greater than that of the varicocele and varicocele + jervine (5 mg/kg) groups. Moreover, the rate of sperm head anomaly in the control group was lower than that in the varicocele and varicocele + jervine (5 mg/kg) groups. There was similarity between the groups for sperm tail anomalies.

Histopathologic results

Control, sham and healthy + jervine (10 mg/kg) groups: Histological examination of the testicular tissue stained with H&E showed that the seminiferous tubule structure was similar and normal in these three groups. Leydig cells in the interstitial region were seen singly or in clusters per tubule. Normal basement membrane, Sertoli cells and germinal epithelial cells were observed. In Masson's trichrome staining, the collagen on the walls of blood vessels, tunica albuginea and seminiferous tubule walls were stained and were normal in appearance. Weak *TNF-α* immunoreactivity was determined in the spermatogonia and primary spermatocytes. It was also seen in the Sertoli, Leydig and myoid cells. When *TNF-α* immunoreactivity was compared among the groups, significant similarity was observed between them. It was determined that there was a significant difference between these groups and the varicocele and varicocele + jervine groups in terms of *TNF-α* immunoreactivity.

Varicocele, varicocele + jervine (5 mg/kg) and varicocele + jervine (10 mg/kg) groups

Histological examination of the testicular tissue stained with H&E showed that the seminiferous tubule structure was altered in the varicocele group. In the varicocele group, there was alteration in the loose connective tissue surrounding the seminiferous tubules where Leydig cells and blood vessels were found in the interstitial region. Histological examination of the testicular tissue stained with PAS indicated that there was irregularity in the peritubular area and thickening of some part of the basement membrane. PAS staining in the peritubular area, germs cells and basement membrane in the varicocele + jervine (10 mg/kg) group were similar to those in the control group. PAS reactions in the structures in question of the varicocele + jervine (5 mg/kg) group were stronger than those of the varicocele group and weaker than those of the control group. On the other hand, in Masson's trichrome staining, it was observed that the collagen level was higher in the blood vessel wall, tunica albuginea and seminiferous tubule wall in the varicocele group compared to the other groups.

TNF-α immunoreactivity in the testicular tissue was stronger in the varicocele group than in the others. It was seen especially in the spermatogonium and primary spermatocytes. *TNF-α* immunoreactivity was weak in the Sertoli cells and negative in the Leydig and myoid cells. In the varicocele group, *TNF-α* immunoreactivity in the germinal epithelial cells gradually weakened toward the lumen of the seminiferous tubule. *TNF-α* immunoreactivity in the same structures in the varicocele + jervine (5 mg/kg) group was weaker than in the varicocele group and stronger than in the varicocele + jervine (10 mg/kg) group. *TNF-α* immunoreactivity results in the Sertoli, Leydig and myoid cells in the varicocele + jervine groups were similar to those in the varicocele group. No staining was observed in the negative control (fig. 2).

Biochemical results

Biochemically, varicocele significantly increased CAT activity. It was determined that the level of CAT activity in the varicocele + jervine (5 mg/kg) group became closer to that of the control group and the CAT activity of the varicocele + jervine (10 mg/kg) group was significantly similar to that of the control group. These findings show that jervine has an inhibitory effect on CAT activity. There was a decline in SOD activity in the varicocele group compared to the control group. The varicocele + jervine groups had SOD levels significantly similar to that of the control group (Table 1). This result shows that jervine has an activating effect on SOD.

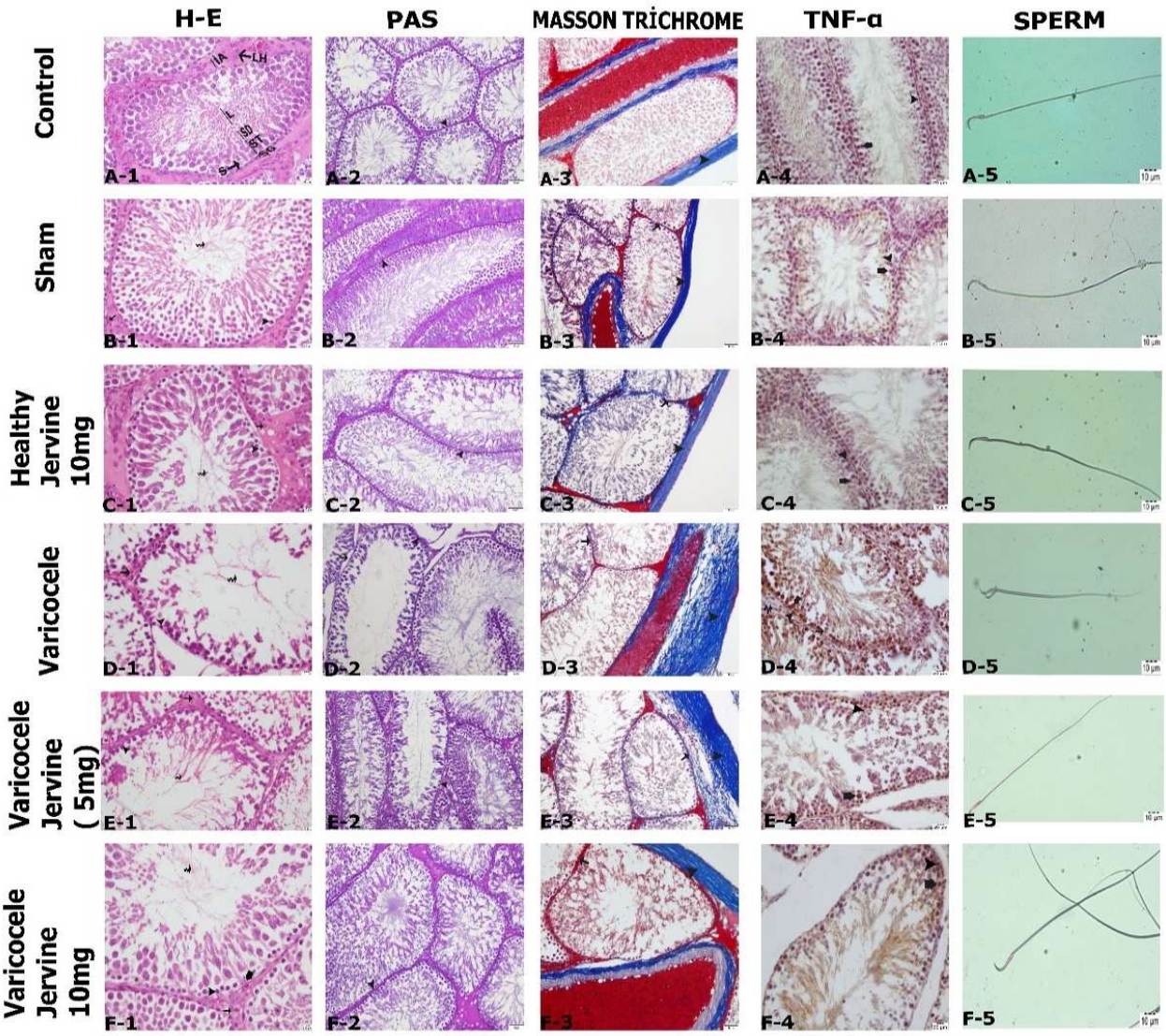

Fig. 2: Histological staining.
A-1 Control, B-1 Sham, C-1 Healthy + Jervine 10 mg/kg, D-1 Varicocele, E-1 Varicocele + Jervine 5 mg/kg, F-1 Varicocele + Jervine 10 mg/kg: A-1 LH: Leydig cell, IA: Interstitial area L: Lumen, SG: Spermatogonium, SD: Spermatid, ST: Spermatocyt. A-1, B-1, C-1, D-1, E-1, F-1 Curved arrow: Lumen, arrowhead: Spermatogonium, arrow: Interstitial area. A-2, B-2, C-2, D-2, E-2, F-2 PAS Staining, arrow: Basement membrane. A-3, B-3, C-4, D-4, E-4, F-4 Masson trichrome staining, Arrow: connective tissue. A-4, B-4, C-4, D-4, E-4, F-4 *TNF-α* immunoreactivity, arrowhead: Spermatogonium, arrow: Spermatocyt. A-5, B-5, C-5, D-5, E-5, F-5 Sperm images.

Table 1: Comparison of CAT and SOD activity in testis.

Groups	N	CAT activity (mmol/min/mg tissue±SD)	SOD activity (mmol/min/mg tissue±SD)
Varicocele	7	115.2±0,7^{e}	124.0±0.4^{a}
Varicocele +jervine (5 mg/kg)	7	95.7±0,6^{c}	128.5±0.4^{b}
Varicocele +jervine (10 mg/kg)	7	93.0±0,4^{b}	133.0±0.7^{d}
Healthy +jervine(10 mg/kg)	7	90.7±0,4^{a}	136.3±0.5^{e}
Control	6	92.0±0,6^{a,b}	127.3±0.3^{b}
Sham	6	97.8±0,5^{d}	130.7±0.6^{c}

CAT activity decreased to the level of the healthy (control) group by the doses of jervine administered with varicocele; Dismutase (SOD) enzyme activity (mmol/min/mg tissue±SD) activity increased to the level of the healthy (control) group and even increased above the level of the healthy (control) group with increasing dose of jervine.^{a,b,c,d,e} There is significant difference between the groups that have superscripts ($p<0.05$). SD: standard deviation.

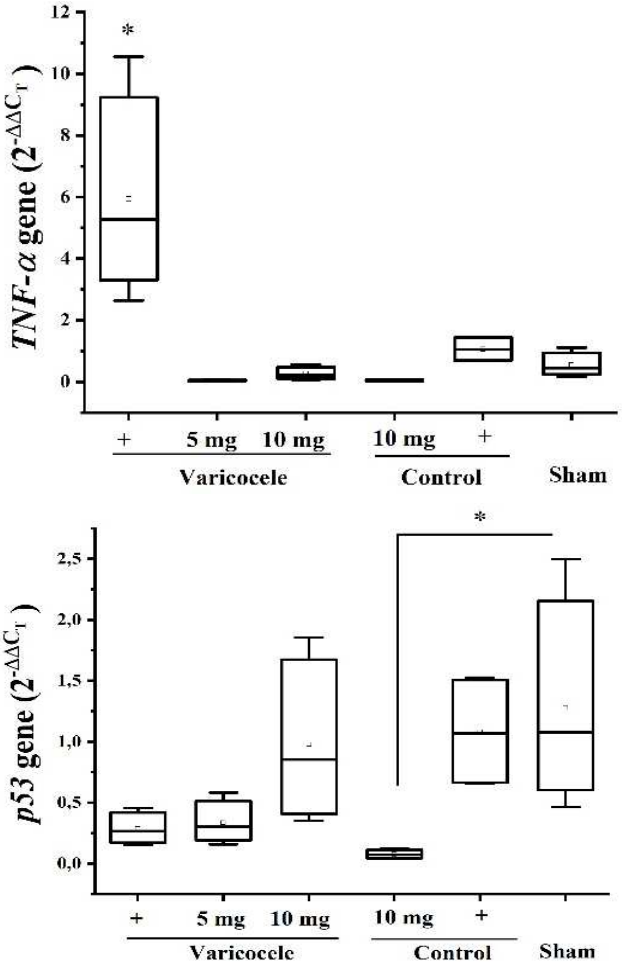

Fig. 3: Relative expression of the two genes analyzed in the testis. Varicocele, Varicocele + Jervine 5 mg/kg, Varicocele + Jervine 10 mg/kg, Control, Healthy + Jervine 10 mg/kg, Sham

Real-time PCR (RT-PCR) results

Expression level changes of two genes were quantified using real-time quantitative PCR (RT-qPCR) and are shown in fig. 3. According to the RT-PCR analysis, TNF-α expression level was significantly upregulated in the varicocele group compared to the other groups ($p<0.001$). TNF-α expression levels were decreased in the varicocele + jervine (5 mg/kg) group and varicocele + jervine (10 mg/kg) group *compared* to the control group. It was observed that high doses of jervine decreased *p53* expression when given to the healthy group. Fig. 3 TNF-α and p53 gene expression levels in testis tissue. TNF-α gene expression levels were significant in varicocele ($p<0.001$). *p53* gene expression levels were significant in the control + jervine (10 mg/kg) group. Error bars show SD (Standard deviation) $p<0.001$.

DISCUSSION

Changes in the body and testis weights of animals are important criteria when analyzing drug toxicity and efficacy. In some experimental varicocele studies in rats, it was reported that there was significant similarity between the control group and the varicocele group in terms of body weight. In another varicocele rat experimental study, no significant difference was found between the groups in terms of body or testis weights (Hosseini *et al*., 2020; De Stefani *et al*., 2005; Erfani Majd *et al*., 2019; Missassi *et al*., 2017). In our study, although we did not find a significant difference between the groups for body or testis weights, we found that the mean body weight of the varicocele groups was somewhat lower than that of the other healthy groups but this was not reflected in the statistics. Zhao *et al*. determined in the varicocele rat model that the seminiferous epithelium was severely damaged and the germ cells were irregularly distributed and numerically decrease. Zheng *et al*. (2008) reported that varicocele impairs Leydig cell function (Zheng *et al*., 2008). Dobashi *et al*. determined that there was thickening of the basement membrane of the seminiferous tubule compared to that of the control group (Dobashi *et al*., 2002). In a different varicocele study, a decrease in the number of Sertoli cells per seminiferous tubule, shedding in the germinal epithelium, thickening of the seminiferous tubule basement membrane and an increase in the amount of collagen were observed (Köse, 2007).

In our study, in the control, sham and healthy ± jervine groups, the spermatozoa in the seminiferous tubule and its lumen had a normal appearance, while there were deteriorations in the seminiferous tubule structure in the varicocele and varicocele ± jervine groups. We also saw germ cells shedding into the lumen and spermatozoa were decreased in the lumen. Leydig cells partially decreased in the varicocele group and gave a weak PAS reaction. As a result of PAS staining performed on testicular tissue in the varicocele group, we determined a thickening of the basement membrane and a decrease in the number of Sertoli cells and amount of collagen. We observed that Sertoli cell distribution was closer to normal in the jervine groups. Mohammadi *et al*. reported that the percentage of sperm with abnormal morphological structure in the varicocele group was significantly higher than that in the control/sham groups (Mohammadi *et al*., 2018). Gur *et al*. described significant difference in the rate of having abnormal sperm morphology between the varicocele group and the control group (Gur *et al*., 2021). In the same study, the anomalies in all groups were mostly tail anomalies and head anomalies were quite rare. In our study, it was observed that the rate of sperm head anomalies in the control group was lower than that in the varicocele and varicocele + jervine (5 mg/kg) groups. There was no difference between the groups in terms of the sperm tail anomaly.

In a varicocele study, it was stated that *TNF-α* immunoreactivity in testicular tissue was stronger in the varicocele group than that in the control group (Hassani-Bafrani *et al*., 2019). In our study, it was observed that

TNF-α immunoreactivity in the testicular tissue in the varicocele group was quite diffuse and stronger than that in the other groups. According to certain studies, subfertility and cytokine levels may be related. *TNF-α,* a cytokine that promotes inflammation, was expressed at a higher level in the varicocele group compared to the control group, according to research by Karna *et al*. (Karna *et al*., 2019). *Interleukin-1*, *IL-6* and *TNF-α* concentrations have been found to be significantly higher in the semen of infertile patients (Moretti *et al*., 2014). In the present study, the expression of *TNF-α and p53* was assessed in varicocele-induced rat testis tissue and testicular tissue treated with jervine. We only observed a significant upregulation of *TNF-α* in varicocele-induced testis tissue. *TNF-α* expression levels were close to the control values in all jervine-treated groups. The mean expression of the hypoxia pathway markers (HIF1- and *p53*) was significantly higher in infertile men with varicocele than in fertile individuals in a study comparing the main molecular markers of the hypoxia (HIF1- and *p53*) and inflammation (*TLR-2*, *TLR-4* and *TNF-α*) pathways between infertile men with varicocele and fertile individuals. *TNF-α* level was also lower (Ghandehari-Alavijeh *et al*., 2019). In an experimental varicocele study, no significant difference was found in terms of *p53* expression in testicular tissue between the control and sham groups and *p53* expression was higher in the varicocele group compared to the other group (Liang *et al*., 2015).

In our study, *p53* expression levels decreased in the varicocele group and jervine caused a downregulation in *p53* level in the both healthy and varicocele groups. A significant difference was found in terms of *p53* expression in testicular tissue in the healthy + jervine (10 mg/kg) group compared to the sham group. Due to this decrease in the healthy + jervine (10 mg/kg) group, it was thought that jervine had a strong inhibitory effect on *p53* gene expression. Karna *et al*. determined that SOD and CAT enzyme activities decreased significantly in the varicocele group. In our study, the result for SOD activity in the testes of the varicocele group was similar to those reported by Sadeghi *et al*., Shokoohi *et al*. and Dolatkhah *et al*. (Sadeghi *et al*., 2020). However, we observed that CAT activity was higher in the varicocele group compared to the control group. We found that varicocele significantly decreased SOD activity. SOD activity levels were increased in the varicocele ± jervine groups and even reached higher levels than those of the control group. According to our results, varicocele significantly increased the CAT enzyme activity. It was observed that jervine at a dose of 5 mg/kg administered with varicocele brought the CAT enzyme activity closer to that of the control group. We observed that when jervine at a dose of 5 mg/kg was administered, CAT enzyme activity was similar to that of the control group; when it was administered at a dose of 10 mg/kg, CAT activity declined and it was similar to that of the control group.

In this study, there are limitations. The first one is that we could not determine the details of the molecular mechanism of jervine on gene expression and other molecules. The last one is that the downregulation of p53, which plays a vital role in cellular stress response and DNA repair, raises safety concerns.

CONCLUSION

In the present study, the results indicated that jervine may be a drug candidate due to its curative effect on sperm abnormalities in varicocele and its anti-inflammatory and antioxidant regulatory effects on testicular tissue. However, due to its inhibitory effect on *p53*, jervine was thought to be an active substance that should be investigated in detail in terms of its side effects. We regard jervine as an active substance but it should be studied in detail in terms of raising safety concerns, toxicological profilin and the mechanism of its effect on gene expression in future studies.

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Authors' contributions
Concept: SY, Design: SY, SAB, MY, Supervising: SY, Data collection and entry: SY, FDA, NE, TA, FNK, Analysis and interpretation: SY, SAB, Literature search: SY, SAB, Writing: SY, SAB, Critical review: SAB

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Data availability
It is confirmed that the data supporting the findings of the study are available within the article.

Ethical approval
It was obtained from the Animal Experiments Local Ethics Committee of Kafkas University with the decision number 2020/030

Conflict of interest
The authors declare that there is no conflict of interest regarding the publication of this document.

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