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

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**Research Article**

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

**Posted Date:** March 24th, 2023

**DOI:** https://doi.org/10.21203/rs.3.rs-2713835/v1

# Abstract

In this study, it was aimed to investigate the effects of jervine, which was isolated from *Veratrum album*, on testicular tissue obtained from induced varicocele rats by using histological, immunohistochemical, biochemical and molecular techniques.

In this experimental study, 40 male Sprague Dawley rats of 8-week-old with an average weight of 180–200 g were used. Rats were randomly divided into 6 groups; varicocele, healthy + jervine (10 mg/kg), varicocele + jervine (5 mg/kg) and varicocele + jervine (10 mg/kg), control, sham. After the left testes were dissected and excised, tissues were divided into three parts for histological, biochemical and Real-Time PCR examinations.

It was observed that seminiferous tubules were normal in the control, sham and healthy + jervine (10 mg/kg) groups but it was found that seminiferous tubules were destroyed in the varicocele and varicocele + jervine groups. *TNF-α* immunoreactivity was stronger in the varicocele group than that of others. It was found that SOD activity was decreased and CAT activity was raised in varicocele group matched to the control group. *TNF* and *p53* gene expression were downregulated due to the effects of jervine.

In summary, we conclude that jervine promotes sperm morphology and contributes to preventing varicocele-induced damage by reducing *TNF-α* and *p53* levels in experimental varicocele rats.


# Introduction

Varicocele means dilatation of the pampiniform plexus. It is generally considered as the most common cause of a male infertility but its treatment still remains controversial. The incidence of varicocele in the general male population is approximately 20%. However, varicocele is found in 40 percent of infertility cases (Pryor & Howards, 1987). Varicocelectomy is the preferred method in the treatment of varicocele. Although recoveries were observed in semen analysis (Cosentino, Chey, Takihara, & Cockett, 1984), testicular size (Lyon, Marshall, & Scott, 1982) and testicular histology (Johnsen & Agger, 1978; Lewis & Harrison, 1980). after varicocelectomy, the effect of varicocele on spermatogenesis is not known exactly. Likewise, the changes after varicocelectomy cannot be set out in full. The negative effect of varicocele on infertility can be reduced thanks to the assisted reproductive techniques and varicocelectomy (Newton, Schinfeld, & Schiff, 1980). Jervine is a steroidal alkaloid derived from plants of the genus veratrum, and it was discovered in 1991. It is also known as cyclopamine analogue which is an important steroidal alkaloid. Cyclopamine inhibits the hedgehog signalling pathway that plays a role in the proliferation of cancer cells. Therefore, jervine obtained from *V. album* is used by many studie (Lee, Wu, Pasca di Magliano, Peters, Wang, Hong, et al., 2007; Tang, Li, Shen, Jin, Yan, Liu, et al., 2008). In addition, studies mention antioxidant and anti-inflammatory effects of jervine (Dumlu, Aydin, Odabasoglu, Berktas, Kutlu, Erol, et al., 2019). Leukocytes and endothelial cells produce pro-inflammatory and anti-inflammatory cytokines to regulate the inflammation (Abbas, Lichtman, & Pillai, 2021; Babu, Davies, & Holgate, 2004).

One of these cytokines, *TNF-α*, is synthesized by some cells such as macrophages, fibroblasts, endothelial cells, monocytes and adipocytes. Some oxidant radicals are toxic to living organisms. Antioxidant enzymes such as superoxide dismutase and catalase protect the cells from harmful effect of these radicals (Freeman & Crapo, 1982). The *p53* protein has functions of DNA repair, regulation of gene expression, regulation of chromosome separation and DNA replication (Harris, 1996). In this study, it is aimed that examine the effect of jervine on the testiticular tissue taken from varicocele-induced rat model by using histological, immunohistochemical, biochemical and molecular methods.

## Materials And Methods

# Plant Material

It was taken from the Animal Experiments Local Ethics Committee of Kafkas University with the decision number 2020/030 .*V. album* was collected in Black Sea region. Root parts of plant were ground after drying in the shade without exposing them to the sun.

## Extraction And Isolation

Jervine was purified from *V. album* roots as in the previous study (Aydin, Cakir, Kazaz, Bayrak, Bayir, & Taşkesenligil, 2014). The plant sample (900 g) was macerated five times at room temperature with acetone (2.5 L x 24 hours) and filtered. The filtrates were combined and the solvents were removed in the rotary evaporator and 12.76 g of acetone extract (1.4%) were obtained. The acetone extract (12.76 g) was fractionated by silica gel column chromatography (CC) (47x4 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.

## Experimental Varicocele Induction In Rats

In this experimental study, 40 adult Sprague Dawley male rats (180–200 g) were used. The animals kept in a room under standard living conditions (20 ± 2°C; %50 ± 10 relative humidity; 12 h/12 h light/dark cycle) and were housed with four rats per cage. The subjects were divided into 6 groups as group 1 (control), group 2 (sham), group 3 (healthy + jervine 10 mg/kg), group 4 (varicocele), group 5 (varicocele + jervine 5 mg/kg), group 6 (varicocele + jervine 10 mg/kg). Each of the first two groups had 6 rats and each of the remaining groups had 7 rats. Apart from group 1 and 3, abdomen of all rats were opened surgically after 10 mg/kg xylazine and 90 mg/kg ketamine were administered intraperitoneally (ip). To create experimental varicocele animal model, the left renal vein of rats in group 4, 5 and 6 was partially ligated. In group 2, abdomen of rats was closed without creating an experimental varicocele model. At the end of the 4th week of this study, the rats were sacrificed by the method of blood collection from the heart

under anaesthesia (ketamine 90 mg/kg, xylazin 10 mg/kg) (Barqawi, Caruso, & Meacham, 2004; Fazlioglu, Yilmaz, Mete, Kurtulus, Parlakkilic, Güctas, et al., 2008). After the left testes removed, tissues were divided into three pieces for histological, biochemical and molecular examinations. Tissues for histological examination were preserved in 10% formalin. Those of for biochemical and molecular analyses were stored at -80°C. In the surgical application, 15 min after anaesthesia the anterior abdominal skin was shaved, and cleaned with an antiseptic. Laparatomy was performed with an incision of approximately 4 cm in the midline. After accessed to the peritoneal cavity, the juncture of the left spermatic vein and the left renal vein was made observable (Fig. 3). After removed the adipose tissue around this area and then the left renal vein was narrowed using 0.85 mm thick metal wire. Left testicular tissue pieces separated for histological examinations were fixed in 10% formol for 72 hours.

## Histopathologic Analysis

Serial section of 5 µm thickness from each tissue using microtome (Leica RM2125RTS). Hematoxylin and Eosin (H&E), Periodic Acid Shiff (PAS) and Masson trichrome stains were applied for histological examinations (Demir, 2001). The longest diameters of 4 seminiferous tubules from each tissue in control and sham groups and 3 seminiferous tubules from the other groups were measured using Cellsense Software program at 20x magnification on a light microscope (Olympus BX43). Twenty seminiferous tubule diameters were randomly selected from the same area and from each group for comparison among groups by 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). The degree of positivity for immunoreactivity in 5 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

For biochemical analysis, 0.1 g of tissue and 9 ml of homogenate buffer were mixed. It was for 1 min in the homogenizer by adding 10 µl of Triton X-100. The mixture was centrifuged at 18000 g for 15 min at 4°C. Obtained supernatant was used as enzyme solution (Sun, Oberley, & Li, 1988).

## Rna Extraction And Reverse-transcription Polymerase Chain Reaction Analyses (Rt-PCR)

Total RNA were purified from testicular tissue using ECO-TECH total RNA Kit following the instructions described by the manufacturer. RNA was eluted using 50 µl nuclease free water (NFW) and stored at −80°C. The concentration 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. The 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 reverse-transcription polymerase chain reaction (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*). Data were collected using a 7500 Fast Real-Time PCR (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 groups, Tukey test of One-way ANOVA (IBM SPPS Statistic Program) was used to compare body weight, left testes weight, right testes weight, seminiferous tubule diameter, sperm length and biochemical analysis results. To determine diversity betwixt groups for the number of sperm head and tail anomalies, Chi-square test was used. Student's t-test was used to match the Real-Time PCR analysis results between control group and other groups. The *p* value for statistical differences among groups was accepted as < 0.05.

# Results

## Body Weight, Testis Weight And Sperm Results

Statistically important diversity was not found among the groups for body weight, right testis weight and left testis weight ($p > 0,05$). According to the results of statistical analyses, there was not statistical significant difference among groups in terms of sperm length, sperm head length and sperm tail length ($p > 0,05$). When the seminiferous tubule diameters were compared between the groups, it was seen that the mean of the seminiferous tubule diameter of the varicocele group was statistically significantly shorter than the mean of the seminiferous tubule diameter of all the other groups ($p < 0.05$). It was seen that the seminiferous tubule diameter length of the varicocele + jervine (5 mg/kg) group was between those of varicocele group and the control group. In our study, there was a powerful diversity between groups for the sperm anomaly rate ($p < 0.05$). Normal sperm rate of the control group was greater than that of the varicocele and varicocele + jervine (5 mg/kg) groups ($p < 0.05$). It was observed that the rate of sperm head anomaly in the control group was lower than the varicocele and varicocele + jervine (5 mg/kg) groups ($p < 0.05$). There was similarity among the groups for sperm tail anomaly ($p > 0.05$).

## Histopathologic Results

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

compared among groups, it was found that there was statically similarity among these three groups. It was determined that there was a statistical difference between these groups and the varicocele and varicocele + jervine groups in terms of *TNF-α* immunoreactivity. Varicocele, Varicocel + jervine (5 mg/kg), Varicocele + jervine (10 mg/kg): Histological examination on testicular tissue stained with H&E showed that seminiferous tubule structure was altered in varicocele group. In the varicocele group, it was determined that there was alteration in the loose connective tissue surrounding the seminiferous tubules where Leydig cells and blood vessels were found in interstitial region. Histological examination on testicular tissue stained with PAS indicated that there was irregularity in peritubular area and thickening of some part of basement membrane. PAS staining in the peritubular area, germs cells and basement membrane of varicocele + jervine (10 mg/kg) were similar to the control group. PAS reaction in question structures of varicocele + jervine (5 mg/kg) was stronger than those of varicocele group and weaker than those of control group. On the other hand, in Masson's trichrome staining, it was observed that the collagen increased in the blood vessel wall, tunica albuginea and seminiferous tubule wall in varicocele group compared to the other groups. *TNF-α* immunoreactivity in testicular tissue was found stronger in varicocele group than others. It was seen especially in spermatogonium and primary spermatocytes. *TNF-α* immunoreactivity was weak in Sertoli cells, and negative in Leydig and myoid cells. In varicocele group, it was seen that *TNF-α* immunoreactivity in germinal epithelial cells gradually weakened towards the lumen of seminiferous tubule. *TNF-α* immuneoreactivity in the same structures in varicocele + jervine (5 mg/kg) group was weaker than varicocele group and stronger than varicocele + jervine (10 mg/kg) group. *TNF-α* immunoreactivity results in Sertoli, Leydig and myoid cells in the varicocele + jervine groups were similar to the varicocele group. No staining was observed in the negative control.(Fig. 1)

## Biochemical Results

Biochemically, varicocele significantly increased CAT activity ($p < 0,05$). It was determined that the level CAT activity in varicocele + jervine (5 mg/kg) group became closer to that of control group ($p < 0,05$), and CAT of varicocele + jervine (10 mg/kg) group was statistically similar to that of the control group ($p > 0,05$). These findings show that jervine has an inhibitory effect on CAT activity. It was seen that there was a decline in SOD activity in the varicocele group contrast to the control group ($p < 0,05$). Varicocele + jervine groups had statistically similar SOD level to the control group ($p > 0,05$). This result show that jervine has an activating effect on SOD activity. (Table 1, Table 2 Table 3)

# Real-Time PCR (RT-PCR) Results

# Expression level changes of three genes were quantified using real time quantitative PCR (RT-qPCR) and are shown in Table 3-4. Expression level of beta actin was not different in varicocele-induced testes and other groups. *According to*

**_RT-PCR analysis, TNF-α expression level_ significantly up-regulated _in the varicocele group matched to the control group_ (p<0,05). _TNF-α expression levels decreased varicocele + jervine_ (5 mg/kg) group (p<0,05) and varicocele + jervine (10 mg/kg) group _matched_ to the control group (p<0,05). _TNF_ downregulation in our study may be due to the effects of jervinee.This study show that i_in the varicocele group_ has high p53 expression level. There was decrease in the varicocele + jervine (5 mg/kg) group _(p<0,05)_, in healthy + jervine (10 mg/kg) group _(p<0,05)_. (Figure 2,)**

## Discussion

Changes in body weights and testes weights of animals are important criterion in analysing drug toxicity and efficacy. Some studies experimental varicocele study in rats, they reported that there was statistically similarity betwixt the control group and the varicocele group in terms of body weight. In another varicocele rat experimental study, it was reported that there was no statistically important distinction betwixt the groups in terms of body weight and testis weights (De Stefani, Silingardi, Micali, Mofferdin, Sighinolfi, Celia, et al., 2005; Erfani Majd, Sadeghi, Tavalaee, Tabandeh, & Nasr-Esfahani, 2019; Hosseini, Shaygannia, Rahmani, Eskandari, Golsefid, Tavalaee, et al., 2020; Missassi, Dos Santos Borges, de Lima Rosa, Villela, da Cunha Martins, Barbosa, et al., 2017). In our study, although we did not find important distinction betwixt the groups for body weight and testis weight, we found that the mean body weight of the varicocele groups was partially lower than that of the other healthy groups but this was not reflected in the statistics. Zhu et al reported that varicocele disrupts the structure of the seminiferous tubules, there are fewer germ cells in the seminiferous tubules, and the distribution of these cells is irregular (Zhu, Rao, Yang, Ning, Yu, Ruan, et al., 2017). Zhao et al determined that the varicocele rat model, the seminiferous epithelium was severely damaged, the germ cells were irregularly distributed and numerically decreased. In the histological examinations we found that there were deteriorations in the germinal epithelium (Zhao, Liu, Wang, Wang, Zhang, Jin, et al., 2019). In the control, sham and healthy ± jervine groups, the spermatozoa in the seminiferous tubule and its lumen had normal appearance, there were deteriorations in the seminiferous tubule structure in the varicocele and varicocele ± jervine groups. We also saw that shedding germ cells into the lumen, and spermatozoa decreased in the lumen. Zheng et al reported that varicocele impairs Leydig cell function (Zheng, Zhang, Zhou, Cheng, Rao, & Yao, 2008). Dobashi et al determined that got thickening of the basement membrane of the seminiferous tubule compared to that of control group (Dobashi, Fujisawa, Yamazaki, Okada, & Kamidono, 2002). In a different varicocele study, it was reported that 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 (Köse, 2007). In our study, it was observed that Leydig cells partially decreased in the varicocele group and gave weak PAS reaction. As a result of PAS staining performed on testicular tissue in the varicocele group, we determined that there was a thickening of the basement membrane, and a decrease in the number of Sertoli cells and the amount of collagen. We observed that Sertoli cell distribution was closer to normal in jervine groups. Mohammadi et al informed that the percentage of sperm with abnormal morphological structure in the varicocele group was significantly higher than the control-sham groups (Mohammadi, Hassani-Bafrani, Tavalaee, Dattilo, & Nasr-Esfahani, 2018) Gur et al described a statistically powerful diversity in the rate of having abnormal sperm morphology between the varicocele group and the control group (Gur, Timurkaan, Taskin, Guven, Gur, Senturk, et al., 2021). In the same study, it was revealed that anomalies in all groups were mostly tail anomalies and head anomalies were reported to be quite rare. In our study, we found an important relationship between the anomaly and the groups ($p < 0.05$). Normal sperm rate of control was greater than that of the varicocele, varicocele + jervine (5 mg/kg) groups ($p < 0.05$). It was observed that the rate of sperm head anomaly in the control group was lower than the varicocele, varicocele + jervine (5 mg/kg) groups ($p < 0.05$). There was no diversity betwixt the groups for sperm tail anomaly ($p > 0.05$).

In a varicocele study, it was stated that TNF-α immunoreactivity in testicular tissue was stronger in the varicocele group than that of in the control group (Hassani-Bafrani, Najaran, Razi, & Rashtbari, 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 the other groups. Moderate TNF-α immunoreactivity was seen in the varicocele + jervine groups, while weak TNF-α immunoreactivity was seen in the control and sham 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, Choi, You, Shin, Cui, Lee, et al., 2019). Interleukin-1, IL-6, and TNF concentrations have been found to be significantly higher in the semen of infertile patients (Moretti, Collodel, Mazzi, Campagna, Iacoponi, & Figura, 2014). In the present study, the expression of *TNF-α and p53* were assessed in varicocele induced rat testis tissue and testicular tissue treated with jervine. We only observed a significant up-regulation (72 times) for *TNF-α* in varicocele induced testis tissue. ($p < 0.05$). TNF expression levels were close to 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 ($p < 0.05$) in the study comparing the main molecular markers of the hypoxia (HIF1- and P53) and inflammation (TLR-2, TLR-4, and TNF-) pathways between infertile men with varicocele and fertile individuals. TNF level was also lower (Ghandehari-Alavijeh, Tavalaee, Zohrabi, Foroozan-Broojeni, Abbasi, & Nasr-Esfahani, 2019). In an experimental varicocele study, it was found that there was no statistical difference 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, Wen, Dong, Zhao, & Shi, 2015). In our study, *p53* expression levels increased about 3,5-fold in the varicocele group ($p > 0.05$), and jervine caused a downregulation in p53 level in both healthy and varicocele groups. It decreased in the varicocele + Jervine (5 mg/kg) group ($p < 0.05$), in the

healthy + jervine (10 mg/kg) group ($p < 0.05$) compared to the control group. Due to this decrease in the healthy + jervine (10 mg/kg) group, it was thought that jervine had strong inhibitory effect on *p53*. Karna et al. determined that SOD and CAT enzyme activities decreased statistically significantly in the varicocele group. In our study, the result of SOD activity in the testis of varicocele group was similar to Sadeghi et al finding (Sadeghi, Erfani-Majd, Tavalaee, Tabandeh, Drevet, & Nasr-Esfahani, 2020). However, we observed that CAT activity was higher in the varicocele group matched to the control. We found that varicocele significantly ($p < 0.05$) decreased SOD activity. SOD activity leve was increased in the varicocele ± jervine groups ($p < 0.05$) and even reaching higher than that of the control group. According to our results, it was determined that varicocele significantly increased the CAT enzyme activity ($p < 0,05$). 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 jervine (at a dose of 5 mg/kg) administered, approached CAT enzyme activity to that of the control group; and jervine (10 mg/kg) administered, declined CAT activity and made it similar to that of the control group.

# Conclusion

We suggested jervine as a candidate medicine because of its curative effect on sperm abnormality in varicocele, its anti-inflammatory and antioxidant regulatory effects on testicular tissue. However, due to its inhibitory effect on p53, we thought that jervine is an active substance that should be investigated in detail in terms of side effects.

# Declarations


### Acknowledgment

This article was financed by the Scientific BAP of Kafkas University (Project No:2019-TS-54). This results described in this paper were part of student thesis (Serdar YİĞİT's PhD thesis 2021) at Kafkas University, Medical Science Institute


**Ethical Approval:** It was taken from the Animal Experiments Local Ethics Committee of Kafkas University with the decision number 2020/030

**Competing interests:** The authors declare no competing interests

**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


**Funding:** This article was financed by the  Scientific BAP of Kafkas University (Project No:2019-TS-54).


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

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# Tables

**Table 1:** The effect of jervin molecule isolated from V. album on Catalase (CAT) enzyme activity in rat testes with varicocele. a,b,c,d,e There is a statistical diversity between groups which have superscripts (p<0,05).

| Groups | N | CAT activity (mmol/min/mg tissue) |
|---|---|---|
| Varicocele | 7 | $115,2\pm0,7^{e}$ |
| Varicocele +jervin (5 mg/kg) | 7 | $95,7\pm0,6^{c}$ |
| Varicocele +jervin (10 mg/kg) | 7 | $93,0\pm0,4^{b}$ |
| Healthy + jervin (10 mg/kg) | 7 | $90,7\pm0,4^{a}$ |
| Control | 6 | $92,0\pm0,6^{a,b}$ |
| Sham | 6 | $97,8\pm0,5^{d}$ |

**Table 2**: The effect of jervin molecule isolated from *V. album* on Superoxid Dismutase (SOD) enzyme activity in rat testes with varicocele. $^{a,b,c,d,e}$ There is a statistical diversity between groups which have

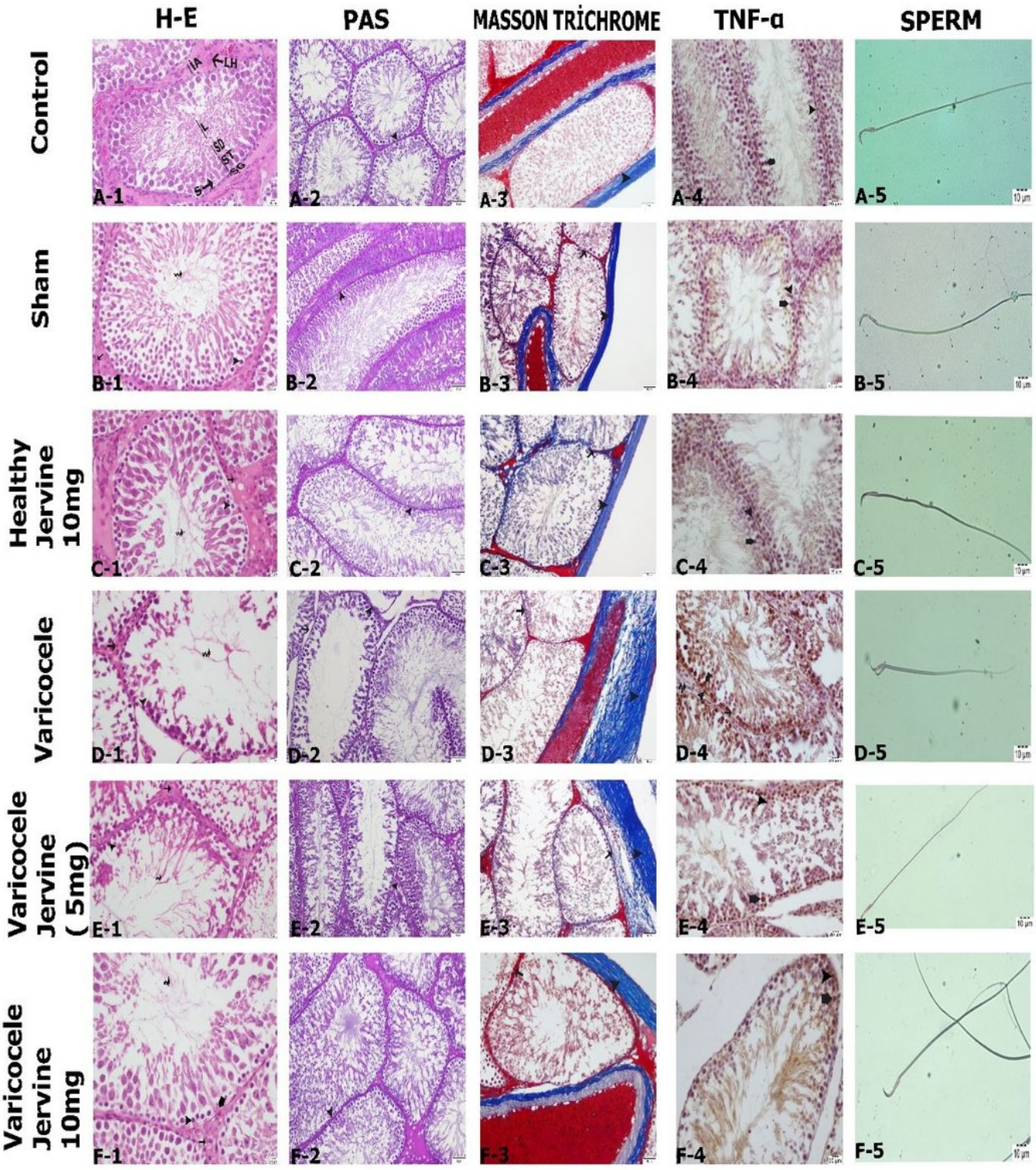
superscripts (p<0,05).

| Groups | N | SOD activity (mmol/min/mg tissue) |
|---|---|---|
| Varicocele | 7 | 124,0±0.4[a] |
| Varicocele +jervin (5 mg/kg) | 7 | 128,5±0.4[b] |
| Varicocele +jervin (10 mg/kg) | 7 | 133,0±0.7[d] |
| Healthy + jervin (10 mg/kg) | 7 | 136,3±0.5[e] |
| Control | 6 | 127,3±0.3[b] |
| Sham | 6 | 130,7±0.6[c] |

**Table 3**: Relative expression of the two genes analyzed in the testis

| Gene | | Varicocele testis | Varicocele +5mg jervin | Varicocele +10 mg jervin | Healthy +10 mg jervin | Control | Sham |
|---|---|---|---|---|---|---|---|
| | **Relative gene expression** | | | | | | |
| *TNF-α* | | | | | | | |
| *p53* | | | | | | | |
| | **Ratio** | 72,50 | 0,04 | 0,21 | 0,04 | 1,00 | 0,45 |
| | | 3,63 | 0,30 | 0,81 | 0,07 | 1,00 | 1,08 |
| | **SD** | 29,60 | 0,02 | 0,21 | 0,03 | 0,42 | 0,40 |
| | | 0,85 | 0,18 | 0,67 | 0,04 | 0,47 | 0,86 |
| | **P value** | 0,00 | 0,00 | 0,01 | 0,00 | 1,00 | 0,12 |
| | | 0,00 | 0,03 | 0,81 | 0,01 | 1,00 | 0,70 |

# Figures




## Figure 1

Histological staining. A-1 Control ,B-1 Sham, C-1 Healthy + jervin 10 mg/kg, D-1 Varicocele ,E-1 Varicocele + jervin 5 mg/kg, F-1 Varicocele + jervin 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 immunoreaktivity, arrowhead: spermatogonium, arrow: spermatocyt. A-5, B-5, C-5, D-5, E-5, F-5 sperm images.

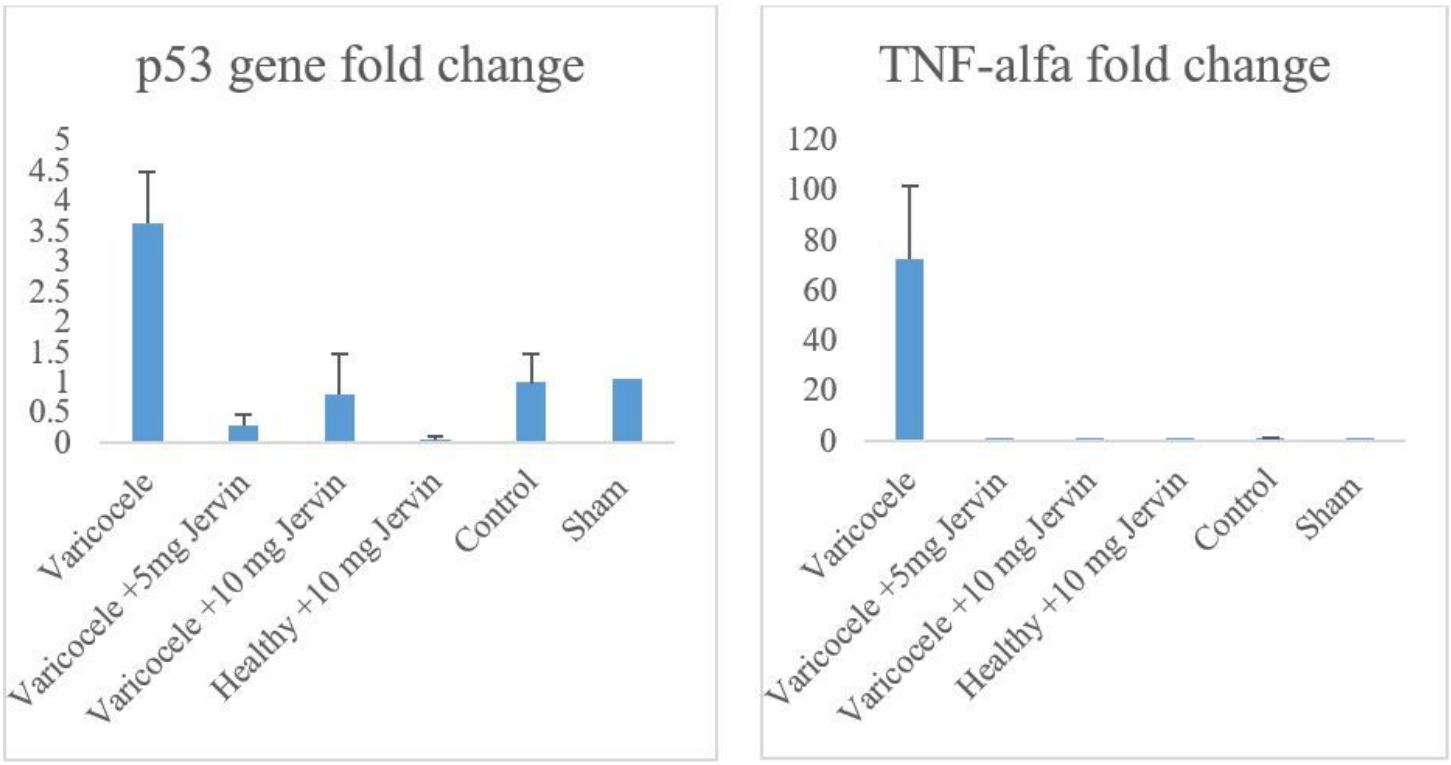



**Figure 2**

*TNF-α and p53 gene expression levels in testis tissue. TNF-α* gene expression levels were significant in varicocele, varicocele*+ jervin* (5 mg/kg), varicocele + jervin (10 mg/kg), and healthy+jervin (10 mg/kg) group,($p<0.05$). *p53* gene expression levels were significant in varicocele, varicocele *+ jervin* (5 mg/kg) , and healthy+jervin (10 mg/kg) group, except control ($p<0.05$).

Image not available with this version

**Figure 3**

Image not available with this version