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Metformin Improves BCL-2 Gene Expression, Testosterone, Platelet Indices, and Histopathology of Testis in Varicocele–Induced Rats

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ABSTRACT

The present study attempted to investigate the effects of metformin on B-cell lymphoma 2 (BCL-2) gene expression, testosterone, platelet indices, and the histopathology of testis in varicocele–induced rats. Twenty-four male adult Wistar rats, aged seven weeks, were selected and divided into four groups. Group I: Sham, II: VCL (varicocele–induced rats), III: MET, and IV: VCL-MET (varicocele–induced rats treated with metformin). Koksal surgical method was used to induce varicocele. One week later, the rats in the MET and VCL-MET groups received metformin (100 mg/kg), and the rats in the sham and VCL groups received normal saline orally for four weeks. Then, all rats were anesthetized, body weight was measured, and the left testis was isolated for BCL-2 gene expression. Also, their blood samples were collected to measure platelet indices and evaluate testosterone concentration. The most significant secondary weight loss relative to the initial body weight, lowest platelet count (PLT: 49.6 × 10_3/µl), highest plateletcrit level (PCT: 0.44 %), mean platelet volume (MPV: 6.0 fL), platelet distribution width (PDW: 18.15%), and the lowest BCL-2 gene expression was observed in the VCL group. The lowest MPV/PLT (0.008), and the highest testosterone level (3.21 ng/ml) were observed in the VCl-Met group. Spermatid cells are seen in the VCL-Met group, spermatocyte cells in the VCL group, and spermatozoa cells in the sham and Met groups. Metformin apparently improved testosterone levels and testicular histopathology by increasing BCL-2 gene expression. As an anti-inflammatory, it also mitigated the changes in platelet indices caused by experimental varicocele.

Introduction

Varicocele, the abnormal dilation of the veins of the scrotum, which leads to an increase in the heat of the testicles, is one of the most prominent causes of infertility in men. Research indicates that varicocele affects 35% of men with primary infertility and 80% of men with secondary infertility.[1] The precise mechanism by which varicocele leads to infertility is poorly understood.[2] In recent years, the role of oxidative stress (OS) as a mediating factor in most of these mechanisms has received much attention. Reactive oxygen species (ROS) can be mentioned among the oxidizing agents.

In various studies, high levels of ROS have been reported in the semen of varicocele patients.[3] In general, ROS refers to oxygen-derived free radicals and non-free radicals, such as singlet oxygen, superoxide anion, hydrogen peroxide, hydroxyl radical, and ozone, which have high. Unpaired electrons lead to chemical reactivity. Men with varicocele appeared to have higher levels of aberrant sperm morphology, lipid peroxidation, DNA fragmentation, and proton sparseness than those in fertile individuals.[4] The integrity of sperm DNA is crucial for delivering healthy progeny, and males with varicocele have lower fertility

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due to abnormalities in nuclear and mitochondrial sperm DNA caused by an imbalance between excessive ROS production and antioxidant defense.[5] Rare varicocele causes include venous thrombosis, renal artery abnormalities, and pampiniform plexus thrombosis. The connection between varicocele and poor spermatogenesis has been described by several theories: Heat, OS, and androgen deprivation are likely involved in the multifactorial cause. The testes typically have a temperature of 2 ^{o}C lower than the body's core temperature. Varicocele can raise this temperature, which in turn can lower sperm quality and increase sperm death.[6] After varicocele repair, testicular heat decreases. Increased venous pressure can cause testicular hypoxia, leading to the production of ROS and OS. These factors can reduce sperm quality by damaging DNA and causing other harm.[7] Reduced intra-testicular testosterone levels and detrimental effects on Leydig cell function could be further contributing causes. In comparison to men without varicocele, men with varicocele have smaller testis on the same side of the varicose veins, lower testosterone levels, and less sperm count. There appears to be a connection between hormonal dysfunction and varicoceles since they can lead to reduced testosterone synthesis and impaired sperm production. Experiments and clinical investigations have provided evidence for varicocele-related hypogonadism and the impact of therapy on testosterone.[8]

The BCL-2 protein family consists of members who play a crucial role in regulating cell death (apoptosis), promoting and inhibiting it. In normal cells, these regulators are carefully balanced. However, any disruption to this balance can lead to one of two outcomes: cells being driven towards irreversible death or cells escaping apoptosis, resulting in the development of a cancerous clone. In the last twenty years, scientists have identified and categorized members of the BCL-2 family based on their domains and functions. These proteins are grouped into three subgroups: the anti-apoptotic/pro-survival proteins (BCL-2 and BCL-XL), the pro-apoptotic proteins (BAX and Bak), and the pro-apoptotic BH3-only proteins (BAD and BID). They are characterized by short conserved sequence regions known as BCL-2 homology (BH) motifs.[9] Seminal BAX is increased, and seminal BCL-2 is decreased in varicocele patients. In the pathogenesis of vascular diseases, platelets have a vital role. Originating from mature megakaryocytes in the bone marrow, these tiny fragments of cytoplasm have the vital task of regulating hemostasis and safeguarding vascular health. In clinical practice, the platelet indices typically used to assess platelet function in the human body include MPV, PDW, and PLT. MPV is the average volume of a single platelet in the peripheral blood. PDW is an index that measures the variation in platelet volume, represented by the coefficient of variation of the measured individual platelet volume. PLT refers to the number of platelets in a blood volume.[10] Previous studies have reported an association between MPV and vascular damage of inflammatory origin, such as stroke, angina, cerebrovascular, and kidney damage. The amount of PCT, MPV, PDW, and MPV/PLT increase, while PLT decreases in varicocele patients.[11] Metformin (N, N-dimethylbiguanide) is the primary drug to treat type 2 diabetes. It decreases the mass of adipose tissue and enhances tissue sensitivity to insulin. It also prevents inflammation and OS in tissues, reduces hyperglycemia, and normalizes the metabolism of fats and carbohydrates. It could decrease OS and apoptosis in renal tubular epithelial cells and testis of patients with varicocele. Additionally, metformin treatment has been found to minimize damage to spermatogenesis in rats with varicocele.[12] Clinical damages caused by ischemia-reperfusion due to dysfunction in the primary organ function or dysfunction in the function of other organs lead to an increase in mortality in patients, and considering that ischemia-reperfusion phenomenon occurs in most surgical methods, antioxidant drugs or free radical scavengers are used to reduce the clinical effects of such injuries. For the first time, this study has studied the therapeutic effect of metformin on BCL-2 gene expression, serum testosterone, and platelet indices in rats with varicocele.

Materials and Methods

Case Selection

For the experiments, 24 male adult Wistar rats (age: 7 weeks, weight: 200-300 gr) were obtained from the breeding and maintenance section of the Semnan University Laboratory Animal Center, a reputable source for laboratory animals. They were then housed in specially designed cages (light: 12 hours, temperature: 19-25 °C, humidity: 45- 55%). The rats were accustomed to their environment, starting one week before the experiment and consuming pellets. They were able to access water freely. The study protocol was conducted using ethical principles approved by the International Committees for the Protection of Laboratory Animals. The rats of the present study are divided into four groups of 6, including Group I (Sham): Rats that underwent laparotomy surgery without varicocele induction and received normal saline orally for up to 4 weeks. Group II (VCL): This group includes rats in which varicocele is induced and receives normal saline orally for up to 4 weeks. Group III (Met): This group contains rats that underwent laparotomy surgery without varicocele induction and received metformin at the rate of 100 mg/kg orally for four weeks. Group IV (VCL-Met):

includes rats in which varicocele is induced and receive metformin at 100 mg/kg orally for four weeks.[13]

Experimental Design

Koksal surgical method was used to induce varicocele.[14] All treatments started one week after varicocele induction and continued for four weeks. All rats were anesthetized using xylazine and ketamine after the end of the treatment period. First, they were weighed, and then the left testes were isolated for BCL-2 gene expression and histopathologic assessment. Also, their blood samples were collected in tubes containing citrate anticoagulant to measure platelet indices by an automatic cell counter (Nihon Kohden) and simple tubes without anticoagulant to obtain serum to evaluate testosterone concentration.[15]

Before induction of varicocele and laparotomy, body weight (BW1) was measured. On the last day of the experiment (after the experiments and the end of the treatment period), BW2 was measured. Blood samples collected in citrate-anticoagulant tubes were used to assess platelet indices, including PLT, PCT, PDW, and MPV, as well as the MPV to PLT ratio. A blood sample was collected from the hearts of rats using a 5 cc syringe. The samples were then immediately centrifuged in tubes containing EDTA anticoagulant to measure platelet indices and in plain tubes without anticoagulant at 3000 rpm for 10 minutes. The separated serum was stored in a -80 °C freezer for analysis of serum testosterone levels. Serum testosterone concentration was measured using available kits (Demeditec Diagnostics kit, Germany).

After isolation, the rats' testes were first lysed and homogenized with utmost care, strictly following the manufacturer's instructions. The extracted RNA was then used for cDNA synthesis through reverse reaction using fermentase. cDNA was obtained using a master mix, and specific primers were used to measure mRNA levels of BCL-2 anti-apoptotic protein using RT-PCR.

BCL-2 forward: "5'-TGCAGAGATGTCCAGTCAG-3'",
BCL-2 reverse: "5'-GAACTCAAAGAAGGCCACAATC-3'"

The GAPDH gene is considered a housekeeping gene and an internal control.

GAPDH forward: "5'-GCAGCTCCTTCGTTGCCGGT-3'";
GAPDH reverse: "5'- CCCGCCCATGGTGTCCGTTC-3'."

The ratio of the target gene to the housekeeping gene is estimated using the $2^{-\Delta\Delta ct}$ method.

The testicular tissues of the rats were fixed in a 10% formalin solution. A tissue processor was used to process the tissues, which involved dehydration, cleaning, and impregnation. Once the tissues were prepared, they were embedded in melted paraffin and sectioned into five-micron-thick pieces using a microtome. To examine the testicular tissues under a microscope, hematoxylin and eosin staining were used to color the cytoplasm purple and the tissue nuclei blue. The histopathologic changes were then evaluated to determine the severity of testicular damage using the Johnson grading system, which ranges from grade 1 to 10. Each tubular section was assigned a score from 1 to 10 based on the presence of cells (Table 1).

Statistical Analysis

Statistical analysis of data was done using SPSS statistical software version 23 (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± standard deviation. Data with normal distribution between groups were compared by one-way ANOVA statistical test (and Tukey's *post hoc* test), and data with non-normal distribution were compared by Kruskal-Wallis statistical test (and Mann-Whitney U *post hoc* test). $p < 0.05$ are considered statistically significant differences. The figures were drawn using Prism software.

Results

Effect on Body Weight (BW)

The results of the primary (BW1) and secondary (BW2) body weights showed that body weight decreased after surgery in all groups. The most weight loss was observed in the VCL group (242.2 gr), and the lowest

Table 1. Johnson grading system.

Cell	Grade
Spermatozoa	8, 9, 10
Spermatid	6, 7
Spermatocyte	4, 5
Spermatogony	3
Sertoli	2
No cells were seen	1

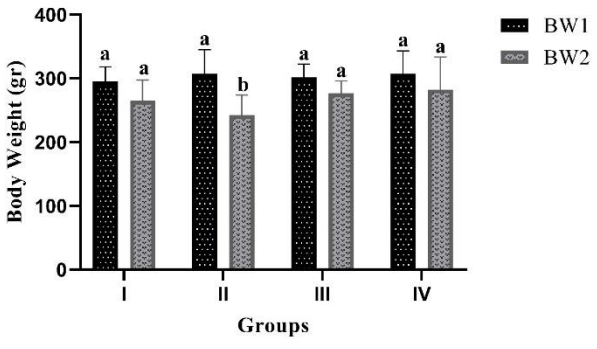

Figure 1. Comparison of body weight (gram) between studied groups. BW1: Primary body weight, BW2: Secondary body weight. Group I (Sham): Rats underwent laparotomy surgery without varicocele induction and received normal saline. Group II (VCL): Rats that varicocele is induced and receive normal saline. Group III (Met): Rats that underwent laparotomy surgery without varicocele induction received metformin. Group IV (VCL-Met): Rats that varicocele is induced and receive metformin. The same letters are not significantly different from each other.

amount was observed in the VCL-Met group (282.6 gr). A statistically significant difference ($p < 0.05$) was observed between the VCL group and other groups (Figure 1).

Effect on Platelet Indices

The impact of varicocele on platelet indices is demonstrated in Fig. 3. The VCL group exhibited a significantly lower PLT (49.6 × 10_3/µl) than the other groups ($p < 0.05$). The VCL group also had the highest PCT level (0.44 %). Furthermore, a significant difference was observed between the VCL and other groups ($p < 0.05$). MPV values were significantly increased in the VCL group (6.0 fl, $p < 0.05$). The VCL group demonstrated the highest percentage of PDW (18.15%). There was a significant difference between the VCL group and the other groups ($p < 0.05$). The VCL-Met group had the lowest amount of MPV/PLT (0.008) and the highest amount related to the VCL group (0.017). There was a significant statistical difference between the VCL and other groups. ($p < 0.05$) (Figure 2).

Effect on Testosterone Level

Figure 3 evaluates testosterone levels in the study groups. It is worth noting that the VCL-Met group exhibited the highest testosterone level (3.21 ng/ml), indicating the potential of metformin in treating varicocele. Conversely, the VCL group had the lowest level at 0.56 ng/ml. A significant statistical difference was found between the VCL and other groups ($p < 0.05$).

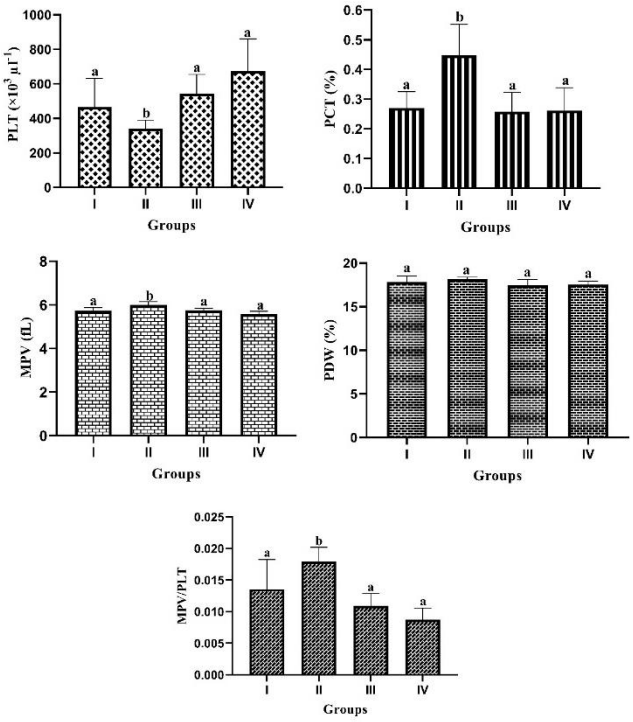

Figure 2. Comparison of platelet indices between studied groups. PLT: platelet count, PCT: plateletcrit, MPV: mean platelet volume, PDW: platelet distribution width. Group I (Sham): Rats that underwent laparotomy surgery without varicocele induction and received normal saline. Group II (VCL): Rats that varicocele is induced and receive normal saline. Group III (Met): Rats that underwent laparotomy surgery without varicocele induction received metformin. Group IV (VCL-Met): Rats that varicocele is induced and receive metformin. The same letters are not significantly different from each other.

Effect on BCL-2 Gene Expression

The effect of varicocele on BCL-2 gene expression is shown in Fig. 5. Notably, the VCL group had a significant decrease compared to the other groups ($p < 0.05$), emphasizing the effect of varicocele on BCL-2 gene expression. However, the statistical difference between the other groups was not significant (Figure 4).

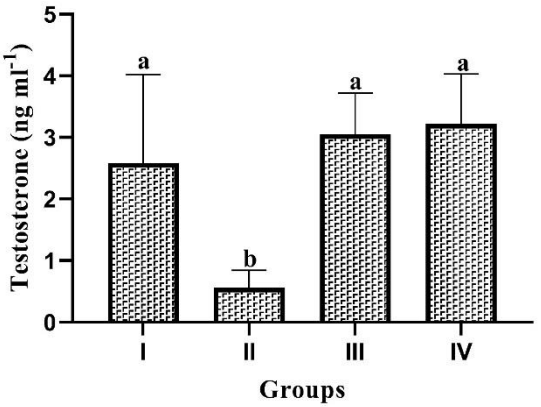

Figure 3. Comparison of testosterone level between studied groups. Group I (Sham): Rats that underwent laparotomy surgery without varicocele induction and received normal saline. Group II (VCL): Rats that varicocele is induced and receive normal saline. Group III (Met): Rats that underwent laparotomy surgery without varicocele induction received metformin. Group IV (VCL-Met): Rats that varicocele is induced and receive metformin. The same letters are not significantly different from each other.

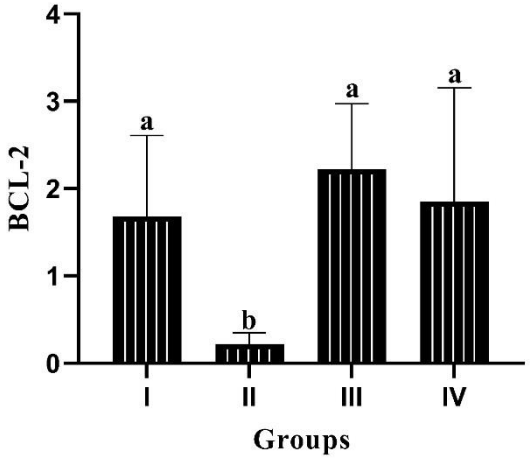

Figure 4. Comparison of BCL-2 gene expression between studied groups. BCL-2: B-cell lymphoma 2. Group I (Sham): Rats that underwent laparotomy surgery without varicocele induction and received normal saline. Group II (VCL): Rats that varicocele is induced and receive normal saline. Group III (Met): Rats that underwent laparotomy surgery without varicocele induction received metformin. Group IV (VCL-Met): Rats that varicocele is induced and receive metformin. The same letters are not significantly different from each other.

Johnson Grading Analysis

Figure 5 show the comparison of the pathology of the studied groups based on the Johnson grading system. Spermatid cells are seen in the VCL-MET group (6 to 7 score), spermatocyte cells in the VCL group (4 < score < 5), and spermatozoa cells in the sham and Met group (8 to 10 score).

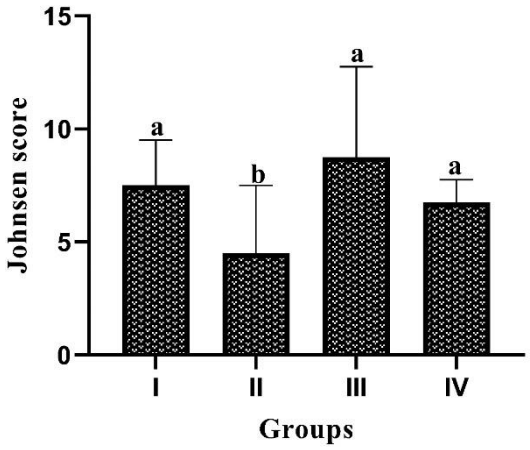

Figure 5. Comparison of the pathology of the studied groups based on the Johnson grading system. Group I (Sham): Rats that underwent laparotomy surgery without varicocele induction, and receive normal saline. Group II (VCL): Rats that varicocele is induced and receive normal saline. Group III (Met): Rats that underwent laparotomy surgery without varicocele induction and received metformin. Group IV (VCL-Met): Rats that varicocele is induced and receive metformin. The same letters are not significantly different from each other.

Discussion

Varicocele is the dilation of the pampiniform venous plexus that drains blood from the testis, causing venous blood reflux. While it can be asymptomatic and discovered incidentally, it is a common issue among patients seeking medical help for infertility problems or experiencing chronic scrotal pain or discomfort.[16] Several studies have identified various mechanisms involved in the pathophysiology of varicocele disease. Researchers have focused on the main mechanisms leading to infertility in varicocele diseases, such as hypoxia, OS, hyperthermia, and apoptosis. Medical management, including antioxidants, may help reduce OS and cell death in varicocele-induced infertility.[17] So, the present study attempted to investigate the effects of metformin, a powerful antioxidant, on BCL-2 gene expression, testosterone, platelet indices, and the histopathology of testis in varicocele–induced rats. The results showed that BW2 decreased after the surgery, more in the untreated group and less in the groups treated with metformin. In other words, metformin could compensate for the weight loss resulting from the surgery. It was reported that metformin alone and in combination with vitamin E improved body weight.[18] In the present study, metformin compensated for the decrease in testicular weight caused by varicocele, and group IV had the highest testicular weight. Metformin was reported to have improved semen parameters, leading to weight loss, increased testis weight, and reduced testis cell apoptosis.[13] The adverse effects of varicocele on spermatogenesis, increased ROS, testosterone deficiency, and the accumulation of toxic stimuli related to the apoptosis process have been reported. In a study on the effect of varicocele on testosterone, it was reported that varicocele causes a decrease in testosterone levels and damages the function of Leydig cells by increasing apoptosis and suppressing the expression of Star protein. Experimental varicocele decreased testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). Metformin administration (50 and 100 mg/kg) significantly increased testosterone, LH, and FSH.[19] On the other hand, when administered during pregnancy, metformin at 10 times the therapeutic concentration significantly reduced testosterone secretion and the number of Sertoli cells in rats.[12] It was observed that 6-week-old chickens treated with metformin for three weeks showed a reduction in testis weight and testosterone level. Exposure to environmental carcinogens or endocrine disruptors is significantly linked to male infertility, affecting reproductive hormones, spermatogenesis, glycolytic pathways, and sperm capacitation and leading to OS. metformin at 50 and 100 mg/kg has been shown to improve testicular damage in rats with varicocele and reduce the level of the tissue antioxidant malondialdehyde.[20] Metformin's weak effect is due to its ability to combat lipid peroxidation in the testis membrane. Treatment significantly increased total sperm count, reduced OS and lipid peroxidation, and restored expected levels of pituitary-gonadal hormones.[21] Metformin's recovery and protective phase involve increasing the activity of 5'-AMP-activated protein kinase, restoring normal hormone levels to monitor male fertility, and regulating testosterone function through LH and FSH hormones. Exposure to arsenic trioxide leads to decreased testosterone and FSH activity. It inhibits the hypothalamic-pituitary axis and causes changes in LH and FSH concentration in the plasma. It can disrupt Leydig cell function and reduce testosterone production.[22] In another study, a significant increase in testis weight was reported in a diabetic model treated with metformin, consistent with the present study's findings.[23] The positive effects of metformin on hormonal levels are related to its antioxidant activity in varicocele patients.[19] The results of this study are consistent with the present study's findings. In a study on the effect of varicocele on platelet indices in adult and adolescent rats, it was reported that varicocele caused a decrease in PLT, an

increase in MPV, PCT, PDW, and MPV/PCT, which is consistent with the results of the present study.[11] Platelet volume indices were higher in varicocele patients compared to healthy subjects, as reported by another study. MPV is widely recognized as an indicator of platelet function. It has been reported that the rate of PDW was significantly higher in varicocele patients. PDW can help confirm varicocele diagnosis and for post-varicocelectomy follow-up.[24] Several studies have reported the presence of more giant platelets as a possible risk factor for the development of vascular disorders. Giant platelets may be a risk factor for diseases and conditions such as testis torsion, varicocele, stroke, myocardial infarction, angina, coronary artery atherosclerosis, malignancy, ulcerative colitis, familial Mediterranean fever, Alzheimer's disease, Behçet's disease. Platelets are crucial in secreting the critical mediators for coagulation, inflammation, thrombosis, and atherosclerosis.[25] Platelet indices, such as MPV, PDW, and PCT, are standard indicators of platelet function in disease pathophysiology. MPV is an essential marker of platelet size and activation. Platelet activation is vital to inflammation and may contribute to varicocele development. A higher MPV value indicates increased platelet activity. Activated platelets produce cytokines such as interleukin-17 (IL-6) and C-reactive protein (CRP). A study on the relationship between MPV, PLT, and PCT reported that high PLT and MPV are generally associated with vascular disease. It was reported that metformin reduces the expression of inflammatory cytokines such as Interferon-γ (IFN-γ), Interleukin-17 (IL-17), interleukin 1 beta (IL-1β), and tumor necrosis factor-alpha (TNF-α), impacting inflammation.[26,27] The meta-analysis study found that the MPV of varicocele patients was significantly higher compared to healthy people. When activated, platelets produce cytokines like interleukin-6 (IL-6) and CRP, which are closely related to inflammation. The activated platelets can promote varicocele. When this lesion is repaired, the change is also corrected. The link between varicocele and MPV may be persistent inflammation triggered by platelets. Metformin was reported to reduce the expression of inflammatory cytokines such as IFN-γ, IL-17, IL-1β, and TNF-α, impacting inflammation. In the current study, MPV increased in varicocele patients and decreased after treatment with metformin. Perhaps metformin, with its anti-inflammatory properties, has improved the inflammatory effects of varicocele. The results of a meta-analysis of existing studies to evaluate the role of platelets in the pathogenesis of varicocele have four main conclusions. This study confirmed that varicocele patients and healthy controls have significantly different MPV values. The meta-analysis found no significant differences in PDW values between varicocele patients and healthy control subjects. However, the PLT in varicocele patients was significantly lower than in healthy individuals. Varicocele surgery may lower the elevated MPV levels in varicocele patients before the procedure.[27] The results of a previous study conducted by our research team indicated that the average PCT in varicocele rats was significantly higher than in healthy rats, possibly due to the elevated MPV in varicocele rats. Either the number of PLT is involved in the pathogenesis of varicocele, or the number of PLT affects MPV.[4] The results of the current research are consistent with our previous study. The relationship between PLT and MPV is inverse, so as the ratio of MPV/PLT increases, PLT decreases and MPV increases. A decreased PLT could signify heightened coagulation and platelet activation in cardiovascular conditions with elevated glycoprotein VI and inflammatory markers.[28] MPV/PLT ratio increases in varicocele patients. All the platelet indices in varicocele rats increased significantly, unlike PLT. These indices are appropriate for diagnosing and monitoring varicocele. The current study's results align with this finding.[11] In varicocele rats, HIF1-α and Bax mRNA levels were higher, while BCL-2 levels in testis tissues were lower compared to healthy rats. The expression of HIF1-α and Bax increased in varicocele rats compared to the control group, while BCL-2 was reduced. In the present study, the BCL-2 level was decreased in varicocele patients compared to the healthy and metformin treatment groups.[28] The expression of HIF1-α and Bax increased in varicocele rats compared to the control group, while BCL-2 was reduced.[29] Previous reports indicate that infertile men with varicocele have high levels of OS in their semen, as shown by increased ROS and reduced total antioxidant capacity (TAC). It suggests a link between sperm dysfunction and OS in varicocele patients. Metformin is mainly transported into cells by organic cation transporters, and recent studies suggest it acts through the AMP-activated protein kinase (AMPK) pathway. It inhibits the activity of the respiratory electron transport chain in mitochondria, induces epigenetic modifications, and decreases OS and apoptotic activity.[30,31] Metformin seems to play an antioxidant role in induced varicocele through the mentioned mechanism. The varicocele leads to increased lipid peroxidation, generating excessive free radicals and reducing the activity of endogenous antioxidants. Varicocele can disrupt the balance between pro-oxidant and antioxidant elements in the affected testis. Other studies have shown that excessive ROS production in varicocele-affected testis may damage DNA and protein, leading to apoptosis. Extreme ROS production can initiate apoptosis by releasing cytochrome C from mitochondria to the cytoplasm, leading to apoptosome development and activation of pro-caspase 9. The intrinsic apoptosis mechanism

involves anti-apoptotic proteins (e.g., BCL-2, BCL-XL) that inhibit apoptosis and pro-apoptotic proteins (e.g., BAX, Bad, Bid) that trigger the apoptotic pathway.[32] Based on the research, the OS caused by ROS generation heightens BCL-2 family phosphorylation, leading to an imbalance of pro-apoptotic and anti-apoptotic proteins in favor of the pro-apoptotic agents.[33] In the VCL group, there was a significant decrease in BCL-2 expression compared to the VCL-Met group. Other studies suggest that varicocele-induced OS may decrease BCL-2 expression, leading to apoptosis, which aligns with this study. Studies have shown that varicocele induction in rats results in the seminiferous tubules being covered only by Sertoli cells. Varicocele can lead to hypospermatogenesis, which involves reducing germ cells in the seminiferous tubules. Tissue changes include maturation cessation, Sertoli cell presence without germ cells at each stage of spermatogenesis, and tubule hyalinization. The severity of microscopic changes in the testicular tissue was obtained using Johnson's grading below 8, and after treatment with metformin, this number reached more than 8. It has been reported that OS can damage the space between cells and germ cells and reduce the number of Sertoli cells.[34] The development of varicocele can impede sperm production and result in the degeneration of seminiferous tubules. Varicocele can lead to increased lipid accumulation in Sertoli cells by increasing the lipid content in their cytoplasm when they engulf residual or damaged cells. In the present study, the histopathological examination revealed the presence of spermatocytes in the VCL group. Metformin stimulates lactate production, crucial for germ cell development and Sertoli cells' anti-apoptotic effect. Metformin's anti-apoptotic effect in rat testis has been reported to be mediated through caspase-3. The study's findings support previous research indicating that metformin reduces testicular apoptosis in varicocele.[12] Metformin improves varicocele by weakening the apoptotic and pro-inflammatory processes in the testicle. Treatment by metformin increases sperm motility, the number of Leydig cells, Sertoli, and spermatogonia, reduces the number of small, atrophic, and distorted seminiferous tubules, and improves their morphology.[35] The present study examined the impact of metformin on the expression of the BCL-2 gene, testosterone hormone, histopathology of the testis, and platelet indices in rats' experimental varicocele model. Metformin apparently improved testosterone levels and testicular histopathology by increasing BCL-2 gene expression. As an anti-inflammatory, it also mitigated the changes in platelet indices caused by experimental varicocele.

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Conflict of Interest

The authors declare no conflict of interest.

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