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Original Article

Effects of Metformin on Experimental Varicocele in Rats

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Abstract

The current study aimed to determine the effect of metformin (MET) on histopathologic evaluation and antioxidant enzyme activity in experimental varicocele-induced rats. A total of 60 rats were randomly divided into six experimental groups. Group 1 (control) received no medication and underwent no surgery. In group 2 (sham), the rats received no medication and the abdominal cavity was opened; however, there was no varicocele induction. In group 3 (varicocele), the abdominal cavity was opened and the rats underwent varicocele induction and received no medication. In group 4, the abdominal cavity was opened and the animals received 25 mg/kg of MET for 42 days and were varicocele-induced. Groups 5 and 6 were similar to group 4 except that the animals received 50 and 100 mg/kg of MET, respectively. At the end of the 21st and 42nd days, the rats were euthanized and the left testis was removed for histological analysis and measurement of superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GPx), and total antioxidant status levels. According to the results, a dose-dependent difference was observed in testis damage grade in the MET treated groups, compared to that reported for the varicocele group ($P<0.05$). No difference was observed between 25 and 50 mg/kg of MET ($P>0.05$). Tissue MDA levels significantly increased in varicocele rats ($P<0.05$); however, MET (25, 50, and 100 mg/kg) in a dose-dependent manner decreased varicocele-induced MDA ($P<0.05$). Experimental varicocele significantly decreased SOD activity, compared to that reported for the control group ($P<0.05$). The administration of MET (25, 50, and 100 mg/kg) significantly increased tissue SOD activity in varicocele rats ($P<0.05$). The MET (25, 50, and 100 mg/kg) in a dose-dependent manner increased GPx activity in varicocele rats ($P<0.05$). There was no difference in MDA, SOD, and GPx levels between 25 and 50 mg/kg MET groups ($P>0.05$). The aforementioned findings suggested that MET treatment had beneficial effects on varicocele.

Keywords: Antioxidant, Histologic evaluation, Metformin, Varicocele, Rat

Effets de la Metformine sur la Varicocèle Expérimentale chez les Rats

Résumé: L'étude actuelle visait à déterminer l'effet de la metformine (MET) sur l'évaluation histopathologique et l'activité enzymatique antioxydante chez les rats expérimentaux induits par la varicocèle. Un total de 60 rats ont été répartis au hasard en six groupes expérimentaux. Le groupe 1 (témoin) n'a reçu aucun médicament et n'a subi aucune intervention chirurgicale. Dans le groupe 2 (simulacre), les rats n'ont reçu aucun médicament et la cavité abdominale a été ouverte; cependant, il n'y a pas eu d'induction de varicocèle. Dans le groupe 3 (varicocèle), la cavité abdominale a été ouverte et les rats ont subi une induction de varicocèle et n'ont reçu aucun médicament. Dans le groupe 4, la cavité abdominale a été ouverte et les animaux ont reçu 25 mg/kg de MET pendant 42 jours et ont été induits par la varicocèle. Les groupes 5 et 6 étaient similaires au groupe 4 sauf que les animaux ont reçu respectivement 50 et 100 mg/kg de MET. À la fin des 21^{e} et 42^{e} jours, les rats ont été euthanasiés et le testicule gauche a été prélevé pour analyse histologique et mesure de la superoxyde dismutase (SOD), du Malondialdéhyde (MDA), de la glutathion peroxydase (GPx) et des niveaux de statut antioxydant total. Selon les résultats, une différence dose-dépendante a été observée dans le degré de lésion testiculaire dans les groupes traités MET, par rapport à celle rapportée pour le groupe varicocèle ($P<0.05$). Aucune différence n'a

été observée entre 25 et 50 mg/kg de MET (P>0.05). Les taux de MDA tissulaire ont augmenté de manière significative chez les rats varicocèles (P<0.05); cependant, MET (25, 50 et 100 mg/kg) d'une manière dose-dépendante a diminué la MDA induite par la varicocèle (P<0.05). La varicocèle expérimentale a diminué de manière significative l'activité de la SOD, comparée à celle rapportée pour le groupe témoin (P<0.05). L'administration de MET (25, 50 et 100 mg/kg) a augmenté de manière significative l'activité de la SOD tissulaire chez les rats varicocèles (P<0.05). La MET (25, 50 et 100 mg/kg) d'une manière dose-dépendante a augmenté l'activité de la GPx chez les rats varicocèles (P<0.05). Il n'y avait aucune différence dans les niveaux du MDA, de la SOD et de la GPx entre les groupes MET de 25 à 50 mg/kg (P>0.05). Les résultats susmentionnés ont suggéré que le traitement avec la MET avait des effets bénéfiques sur la varicocèle.
Mots-clés: Antioxydant, Evaluation Histologique, Metformine, Varicocèle, Rat

1. Introduction

Varicocele is an abnormal vascular dilatation of the pampiniform plexus. Clinically, varicocele is more commonly observed on the left side, although there is wide variation in the reported prevalence of bilateral varicocele. Most anatomic studies have been conducted on the internal spermatic vein and varicocele formation; however, there are some data to suggest that dilated external spermatic veins can also contribute to primary or recurrent varicocele (Masson and Brannigan, 2014). The pathophysiology of testicular damage in varicocele is not completely perceived; nevertheless, gross testicular alterations associated with varicocele are well documented. Based on the literature, it is suggested that varicocele causes a progressive decline in fertility and can continue to induce the impairment of spermatogenesis despite prior fertility (Celik-Ozenci et al., 2006; Masson and Brannigan, 2014).

Metformin (N, N-dimethylbiguanide; MET) is an orally administered biguanide that is commonly prescribed for the treatment of type 2 diabetes (Soraya et al., 2012). In addition to its glucose-lowering effect, MET positively affects vascular endothelial function and atherosclerosis. The MET inhibits pro-inflammatory response and apoptosis in human vascular wall cells (Schramm et al., 2011). The association of varicocele with male infertility derives from the first century AD when Celsius reported that there is a link between dilated scrotal veins and testicular atrophy (Masson and Brannigan, 2014).

Despite the fact that several investigations have been carried out on the role of MET against cell death (Oishi et al., 2014), there has been scarce information on the role of MET in male infertility. According to the evidence, it was reported that as MET possesses a non-genomic action, it could be an interesting molecule for the treatment of sperm which can improve fertility (Bertoldo et al., 2014). It was also reported that MET improves semen characteristics in men with metabolic syndrome (Morgante et al., 2011). Spermatozoa are particularly vulnerable to the oxidation of their lipid plasma membranes due to the composition of fatty acids in the membrane and the relative inability to combat oxidative stress. It was shown that MET has the ability to decrease reactive oxygen species (ROS) (Bertoldo et al., 2014).

The mechanism of MET action has been primarily studied in the context of diabetes and is poorly perceived. In the context of atherosclerosis, MET inhibits the activation of nuclear factor kappa-light-chain-enhancer of activated B cells and decreases C-reactive protein levels; moreover, it inhibits the inflammatory response (Hirsch et al., 2013). Although many infertile individuals have a varicocele, its relationship with male infertility still remains unexplained (Zhang et al., 2006). Based on a literature review, there has been no report on the effect of MET on experimental unilateral varicocele-induced rats. Therefore, the current study aimed to determine the effect of MET on histopathologic evaluation and

antioxidant enzyme activity in experimental varicocele-induced rats.

2. Material and Methods

2.1. Study Animals

To survey the protective role of MET in spermatozoa characteristics in experimental unilateral varicocele-induced rats, a total of 60 male Wistar rats (weight: 230-250 g) were allocated into six treatment groups. The rats were individually housed under standard laboratory conditions according to European community suggestions for laboratory animals at a temperature of 21±2°C, relative humidity of 55-60%, and 12-hour light period. All the animals had free access to chow pellets and fresh water. All experimental procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals to Investigate Experimental Pain in Animals (Zimmermann, 1983). Animal handling and experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) and the current laws of the Iranian government.

2.2. Experimental Creation of Varicocele

All surgical procedures were performed under anesthesia by an intraperitoneal (IP) injection of 60 mg/kg ketamine hydrochloride 10% and 10 mg/kg xylazine hydrochloride 2%. The upper left abdominal quadrant was approached through a midline laparotomy incision. Herein, the renal and adrenal veins and left spermatic vein inserted into the left renal vein. With a midline incision, the left renal vein was exposed. Moreover, after the fine dissection of the proximal left renal vein, the left renal vein was tied using a silk suture (4-0) (Turner, 2001; Sahin et al., 2005). At the point of medial to the insertion of the adrenal and spermatic vein into the renal, a metal probe (with a diameter ranging from 0.4-0.85mm based on the size of the renal vein) was placed. The ligature was made around the probe, and then the probe was removed and the vein allowed expanding within the boundary of the ligature. This procedure leads to a decrease in renal vein diameter to one-half. The midline incision of the abdominal wall and the anterior abdominal muscles were separately repaired (Celik-Ozenci et al., 2006).

2.3. Study Design

A total of 60 rats were randomly divided into six experimental groups (n=10). Group 1 (control) received no medication and underwent no surgery. In group 2 (sham), the rats received no medication and the abdominal cavity was opened; however, there was no varicocele induction. In group 3 (varicocele), the abdominal cavity was opened and the rats underwent varicocele induction and received no medication. In group 4, the abdominal cavity was opened and the animals received 25 mg/kg of MET for 42 days and were varicocele-induced. Groups 5 and 6 were similar to group 4 except that the rats received 50 and 100 mg/kg of MET, respectively. At the end of the 21st and 42nd days, the rats were euthanized with an overdose injection of pentobarbital (300 mg/kg, IP), peritoneum opened, and the left testis was removed for further investigations.

2.4. Histologic Evaluation

The tissue was fixed in Bouin's solution (7.5 mL saturated picric acid, 2.65 mL glacial acetic acid, and 2.5 mL 7% formaldehyde), post-fixed in 70% alcohol, and embedded in paraffin blocks. A tissue section (5 µm) was obtained, deparaffinized, and stained with hematoxylin and eosin (H&E). The testicular tissue was evaluated in random order using standard light microscopy by an observer who was unaware as to which group the rat belonged to. Then, the testis tissue samples from the experimental rats were fixed at Bouin's solution for complete fixation and processed for paraffin sectioning. A tissue section about 5 µm thickness was taken and stained with H&E. The testis sections were numerically graded to assess the degree of histological changes associated with seminiferous tubule injury as previously described by Johnsen (1970) as follows:

- 10: complete spermatogenesis and perfect tubules

- 9: many spermatozoa present but disorganized spermatogenesis
- 8: only a few spermatozoa present
- 7: no spermatozoa but many spermatids present
- 6: only a few spermatids present
- 5: no spermatozoa or spermatids present but many spermatocytes present
- 4: only a few spermatocytes present
- 3: only spermatogonia present
- 2: no germ cell present
- 1: neither germ cells nor Sertoli cells present

2.5. Antioxidant Activity

Malondialdehyde (MDA) is a standard to determine free radical damage. The detecting kit was purchased from Randox (Randox Laboratories Ltd., Crumlin, Antrim, United Kingdom). The MDA was formed as an end product of lipid peroxidation and treated with thiobarbituric acid to produce a colored product that was measured at 532 nm (Paglia and Valentine, 1967). The commercial kit was obtained from Randox (Randox Laboratories Ltd., Crumlin, Antrim, United Kingdom). According to this method, glutathione peroxidase (GPx) catalyzes the oxidation of glutathione. Furthermore, in the presence of glutathione reductase and nicotinamide adenine dinucleotide phosphate (NADPH), oxide glutathione converts to the reduced form by the changes in the oxidation of NADPH to oxidized nicotinamide adenine dinucleotide phosphate ($NADP^+$) in absorbance at 340 nm (Paglia and Valentine, 1967).

Superoxide dismutase (SOD) detecting kit was purchased from Randox (Randox Laboratories Ltd., Crumlin, Antrim, United Kingdom). The role of SOD is to accelerate the dismutation of the toxic superoxide radical (O_2), produced during the oxidative energy processes, to hydrogen peroxide and molecular oxygen. This method employs xanthine and xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride to form a red formazan dye detectable at 505 nm (Paoletti and Mocali, 1990). Nicotinamide adenine dinucleotide oxidation was measured at 340 nm and expressed as U/mg tissue. The total antioxidant status (TAS) detecting kit was obtained on the basis of suppression in color production which was measured at 600 nm and expressed as mmol/ml (Miller et al., 1993).

2.6. Statistical Analysis

Shapiro-Wilk tests were used for the normality of the obtained data. Then, the parametric data were analyzed by one-way analysis of variance using SPSS software (version 24.0) and expressed as mean±standard error of the mean. The differences between groups were analyzed using Duncan's Multiple Range Test. The histopathological scores were analyzed by the Kruskal Wallis test. A p-value of less than 0.05 was regarded as a significant difference between the groups.

3. Results

The effect of different doses of MET on the injury score for the assessment of the experimental testicular varicocele-induced rats on the 21st and 42nd days following the surgery are illustrated in Figures 1 and 2. As depicted in Figure 1, the varicocele group had the lowest testis damage grade, compared to other groups ($P<0.05$). The testis damage grade was higher in the varicocele group in comparison to that reported for the control group ($P>0.05$). The administration of MET in a dose-dependent manner improved testis damage, compared to that reported for the varicocele group ($P<0.05$). No difference was observed between 25 and 50 mg/kg of MET ($P>0.05$) (Figures 3-12).

Table 1 shows the effect of different doses of MET (i.e., 25, 50, and 100 mg/kg) on the tissue values of MDA, SOD, GPx, and TAS in the experimental testicular varicocele-induced rats on the 42nd day. According to the results, tissue MDA levels significantly increased in the varicocele rats ($P<0.05$); however, MET (25, 50, and 100 mg/kg) in a dose-dependent manner decreased varicocele-induced MDA ($P<0.05$). No difference was observed in MDA levels between 25 and 50 mg/kg MET groups ($P>0.05$).

Experimental varicocele significantly decreased SOD activity, compared to that reported for the control group

(P<0.05). The administration of MET (25, 50, and 100 mg/kg) significantly increased tissue SOD in the varicocele rats (P<0.05). There was no difference in SOD levels between 25 and 50 mg/kg MET groups (P>0.05). Different doses of MET (i.e., 25, 50, and 100 mg/kg) in a dose-dependent manner increased GPx activity in the varicocele rats (P<0.05). No difference was noticed in GPx levels between 25 and 50 mg/kg MET groups (P>0.05). The MET (25, 50, and 100 mg/kg) had no significant effect on TAS levels in the testicular varicocele-induced rats (P>0.05).

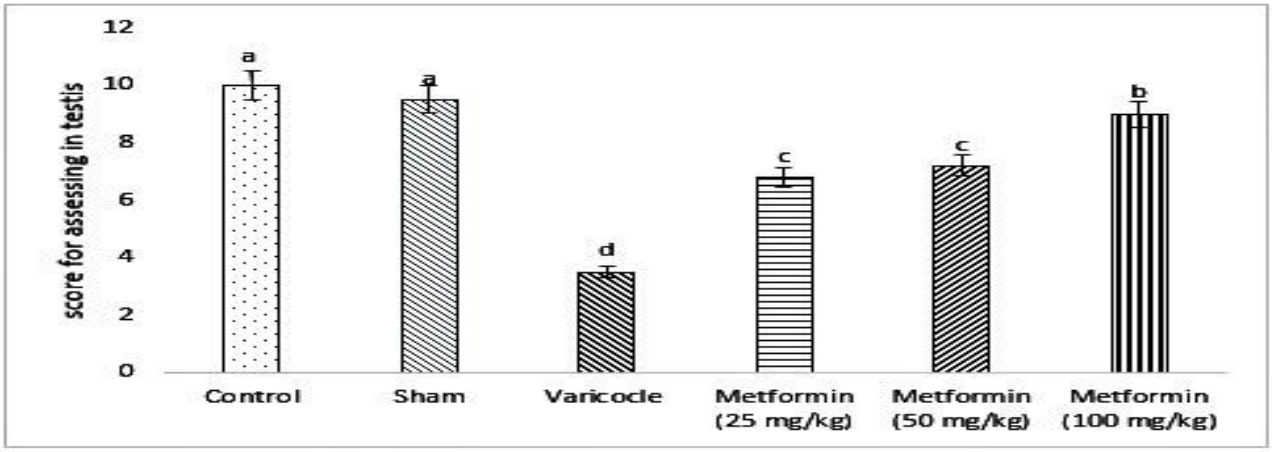

Figure 1. Histological score for score for assessing in testis" to "score for assessment of testis associated with seminiferous tubules injury after 21 days in experimental varicocele rats; control group: no medication and surgery; sham group: no medication, abdominal cavity opened, but no varicocele induction; varicocele group: abdominal cavity opened, varicocele induction, and no medication; metformin (25 mg/kg) group: abdominal cavity opened, varicocele induction, and 25mg/kg of metformin for 42 days; metformin (50 mg/kg) group: abdominal cavity opened, varicocele induction, and 50 mg/kg of metformin for 42 days; metformin (100 mg/kg) group: abdominal cavity opened, varicocele induction, and 100 mg/kg of metformin for 42 days; different letters (i.e., a-d) in each column indicating significant differences between treatments (P<0.05)

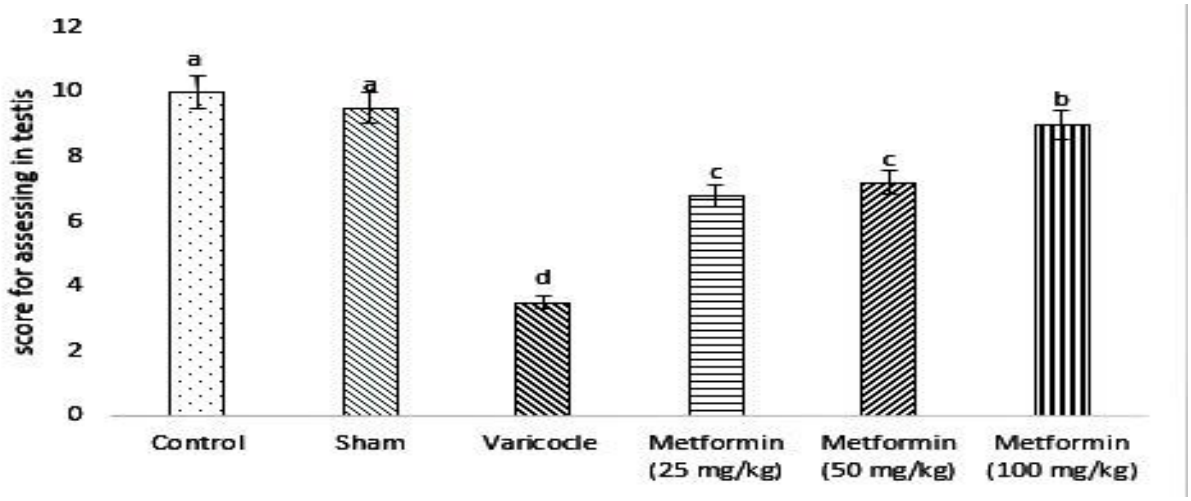

Figure 2. Histological score for the assessment of testis associated with seminiferous tubules injury after 42 days in experimental varicocele rat; control group: no medication and surgery; sham group: no medication, abdominal cavity opened, but no varicocele induction; varicocele group: abdominal cavity opened, varicocele induction, and no medication; metformin (25 mg/kg) group: abdominal cavity opened, varicocele induction, and 25mg/kg of metformin for 42 days; metformin (50 mg/kg) group: abdominal cavity opened, varicocele induction, and 50 mg/kg of metformin for 42 days; metformin (100 mg/kg) group: abdominal cavity opened, varicocele induction, and 100 mg/kg of metformin for 42 days; different letters (i.e., a-d) in each column indicating significant differences between treatments (P<0.05)

Table 1. Effect of different doses of metformin on tissue values of malondialdehyde, superoxide dismutase, glutathione peroxidase, and total antioxidant status on the 42nd day in experimental testicular varicocele-induced rats

Group	MDA (nmol/g tissue)	SOD (U/mg tissue)	GPx (U/mg tissue)	TAS (mmol/ml)
Control	111.02±1.24 [d]	5.32±0.13 [a]	6.24±0.26 [a]	16.15±1.54
Sham	110.15±1.35 [d]	5.18±0.20 [a]	6.17±0.33 [a]	16.11±1.21
Varicocele	175.05±2.32 [a]	1.25±0.11 [d]	2.35±0.16 [d]	13.21±1.62
Metformin (25 mg/kg)	165.02±2.25 [b]	1.26±0.16 [c]	2.17±0.23 [c]	13.22±1.14
Metformin (50 mg/kg)	158.23±2.13 [b]	3.34±0.21 [c]	3.42±0.16 [c]	14.26±1.27
Metformin (100 mg/kg)	120.36±1.24 [c]	4.27±0.18 [b]	4.54±0.11 [b]	13.11±1.18

Control group: no medication and surgery; sham group: no medication, abdominal cavity opened, but no varicocele induction; varicocele group: abdominal cavity opened, varicocele induction, and no medication; metformin (25 mg/kg) group: abdominal cavity opened, varicocele induction, and 25 mg/kg of metformin for 42 days; metformin (50 mg/kg) group: abdominal cavity opened, varicocele induction, and 50 mg/kg of metformin for 42 days; metformin (100 mg/kg) group: abdominal cavity opened, varicocele induction, and 100 mg/kg of metformin for 42 days; MDA: Malondialdehyde; SOD: Superoxide dismutase; GPx: Glutathione peroxidase; TAS: Total antioxidant status; different letters (i.e., a-d) in each column indicating significant differences between treatments ($P<0.05$)

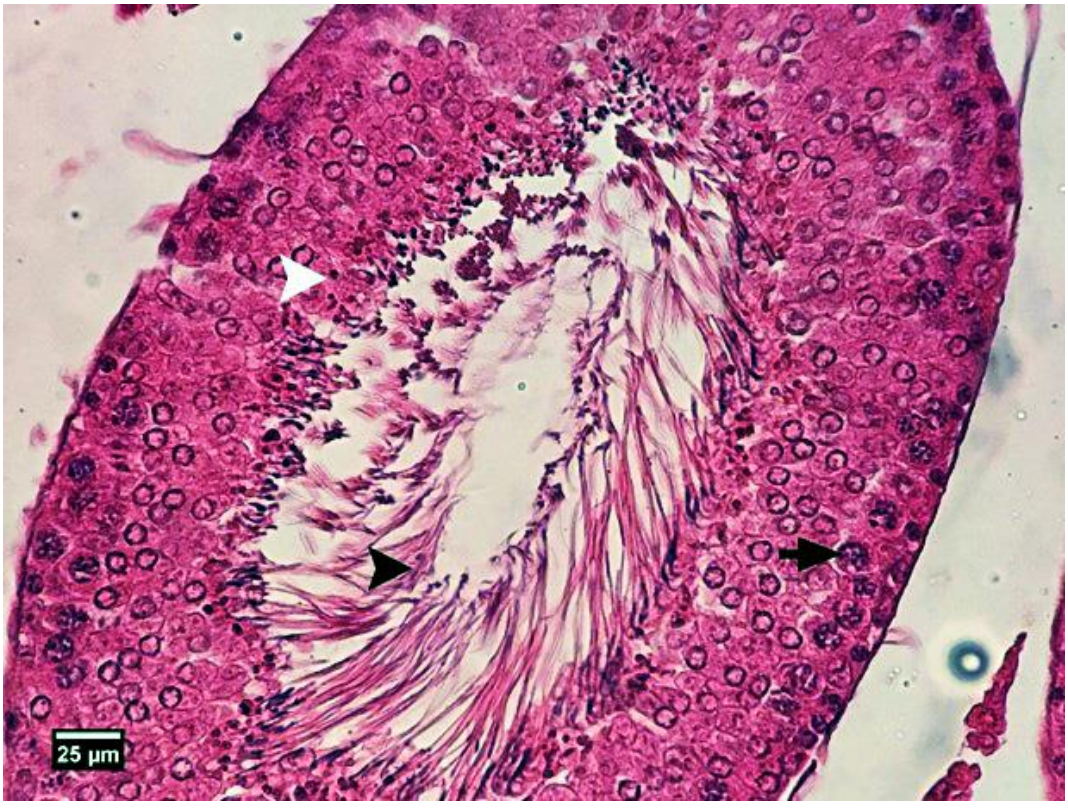

Figure 3. Testis section of left testis in control and sham rats on the 21st day showing normal seminiferous tubules (arrow) and interstitial cells (arrowhead) between tubules (hematoxylin and eosin)

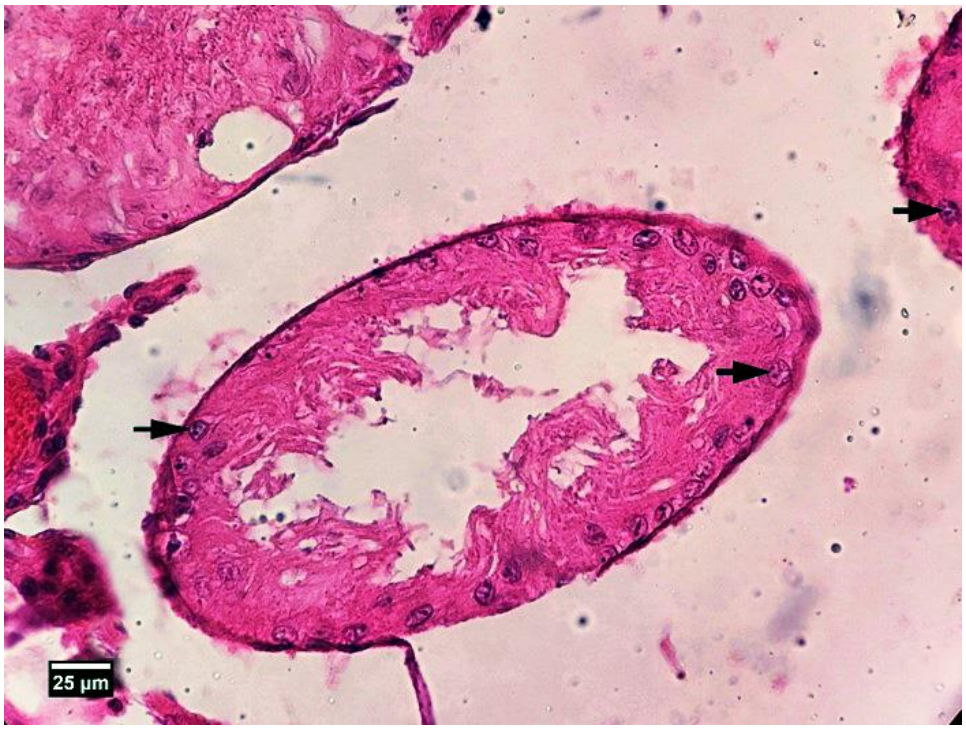

Figure 4. Testis section of left testis in varicocele rats on the 21st day showing degenerated seminiferous tubules (arrow) and loss of spermatogenesis (hematoxylin and eosin)

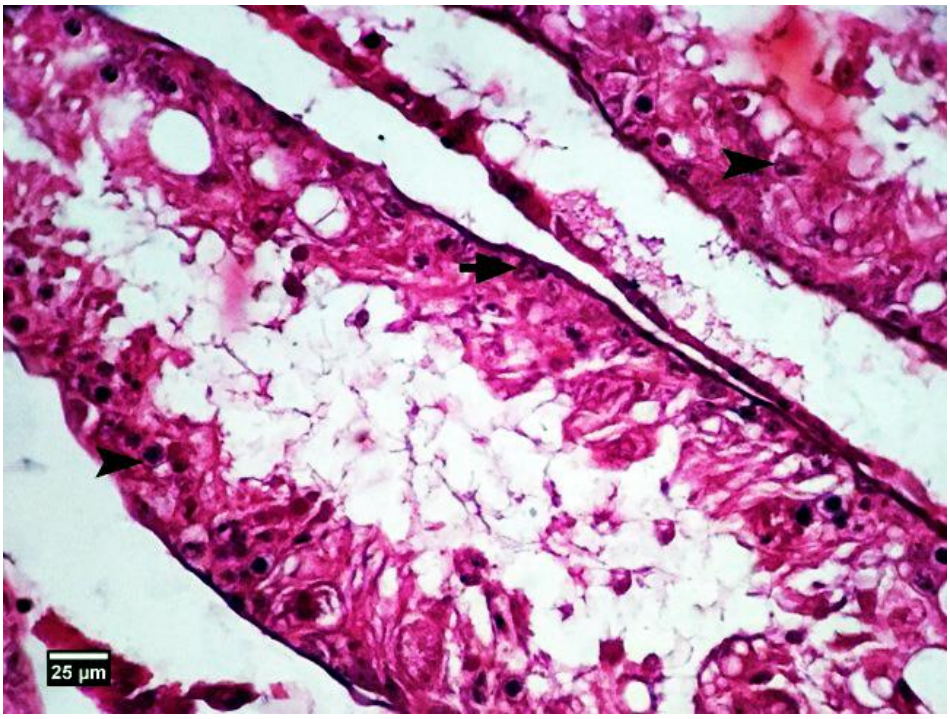

Figure 5. Testis section of left testis in metformin (25 mg/kg) group followed in varicocele rats on the 21st day showing seminiferous tubules (arrow) with few spermatocytes and interstitial cells (arrowhead) between tubules (hematoxylin and eosin)

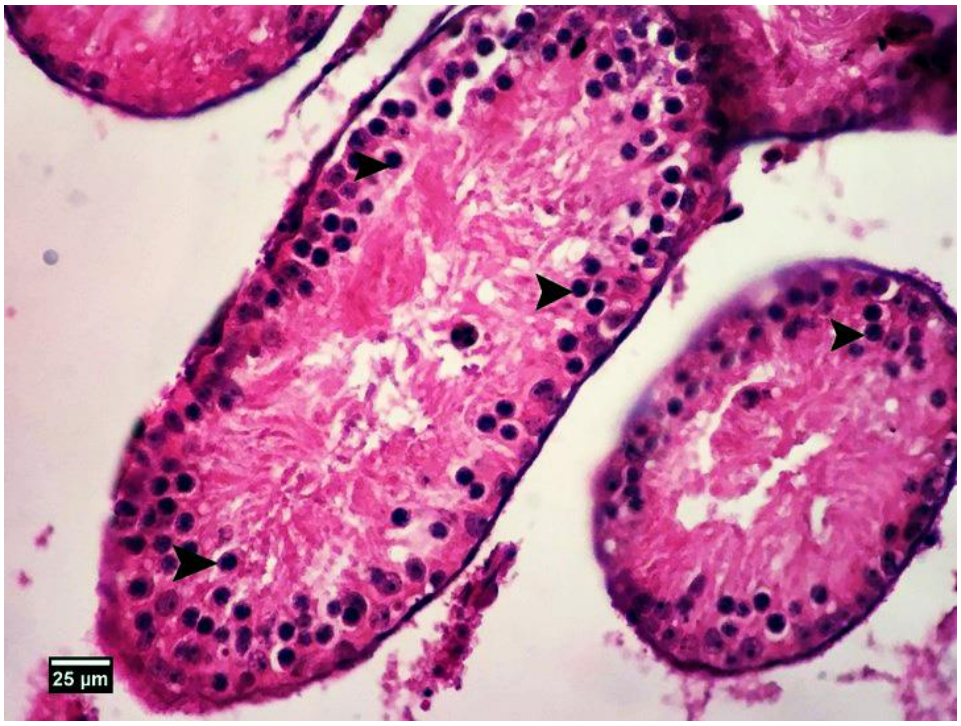

Figure 6. Testis section of left testis in metformin (50 mg/kg) group followed in varicocele rats on the 21st day showing seminiferous tubules **(**arrow) with few spermatocytes and interstitial cells (arrowhead) between tubules (hematoxylin and eosin)

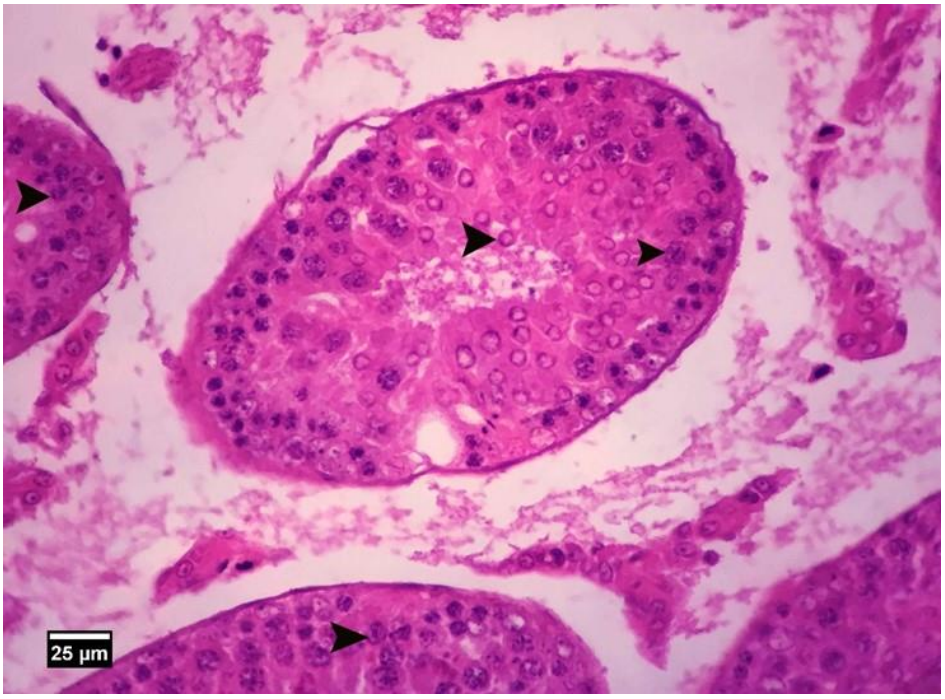

Figure 7. Testis section of left testis in metformin (100 mg/kg) group followed in varicocele rats on the 21st day showing many normal seminiferous tubules (arrow) with few spermatocytes (arrowhead) (hematoxylin and eosin)

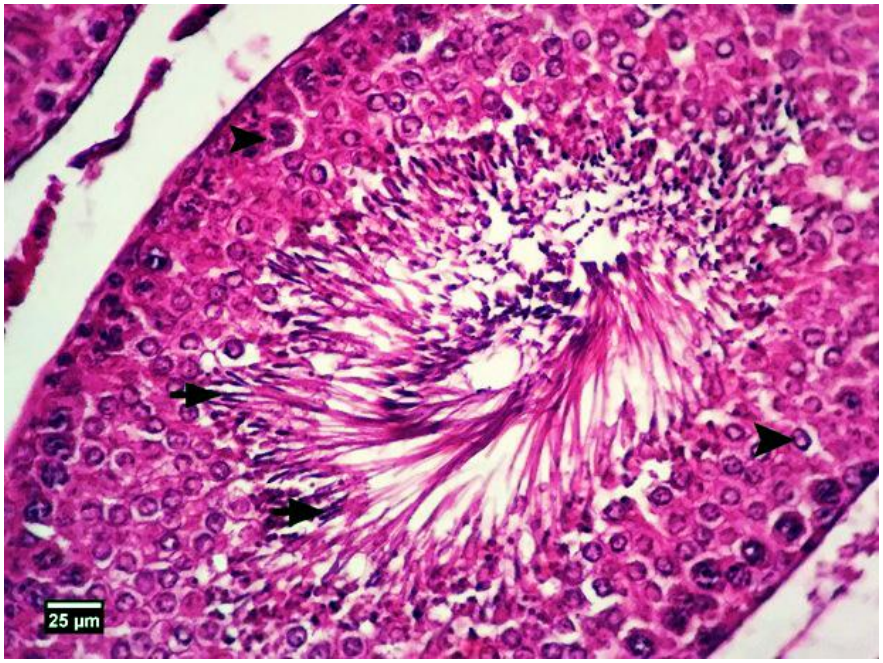

Figure 8. Testis section of left testis in control and sham rats on the 42nd day showing normal seminiferous tubules (arrow) and interstitial cells (arrowhead) between tubules (hematoxylin and eosin)

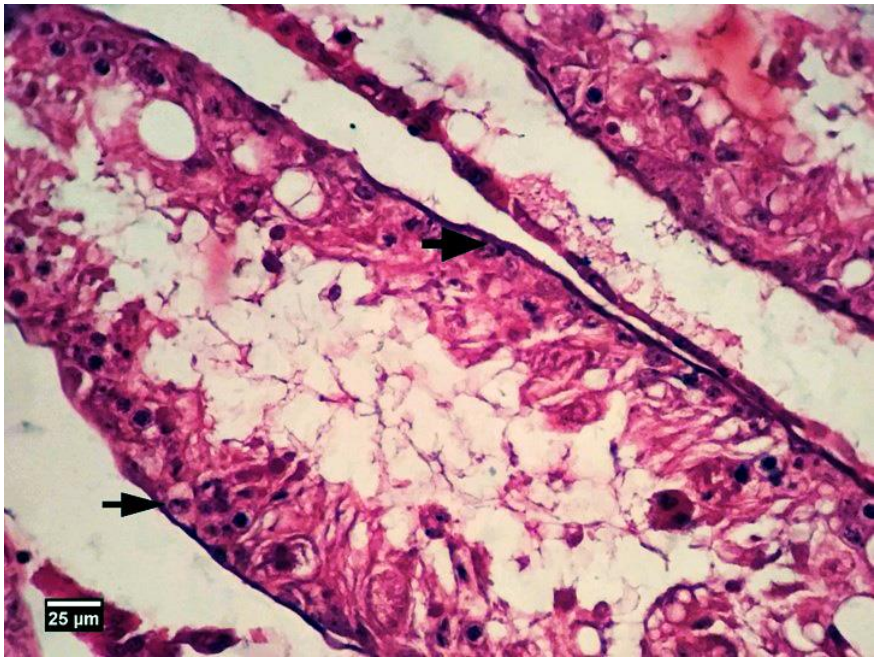

Figure 9. Testis section of left testis in varicocele rats on the 42nd day showing degenerated seminiferous tubules (arrow) and loss of spermatogenesis (hematoxylin and eosin)

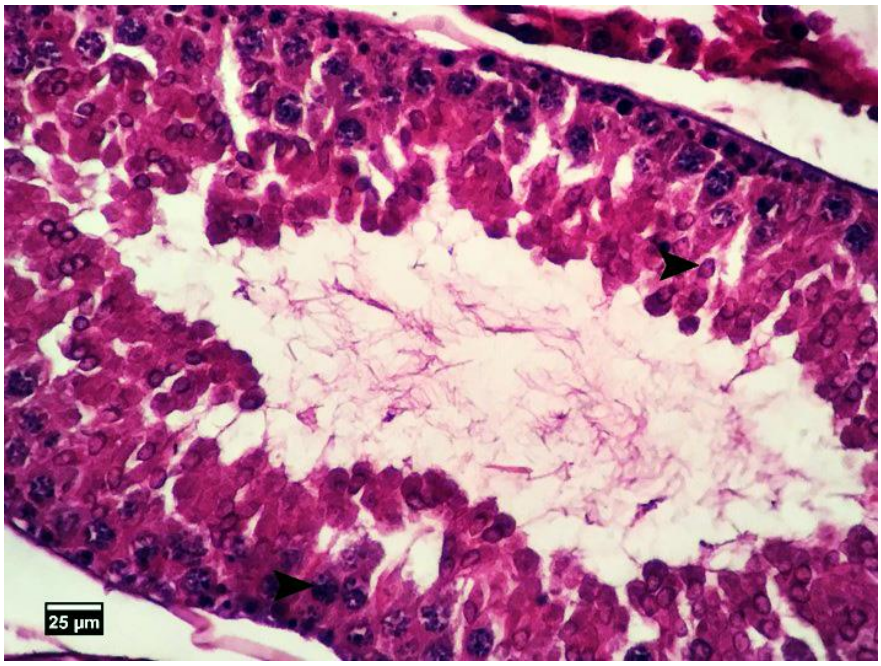

Figure 10. Testis section of left testis in metformin (25 mg/kg) group followed in varicocele rats on the 42nd day showing seminiferous tubules (arrow) with few spermatocytes and interstitial cells (arrowhead) between tubules (hematoxylin and eosin)

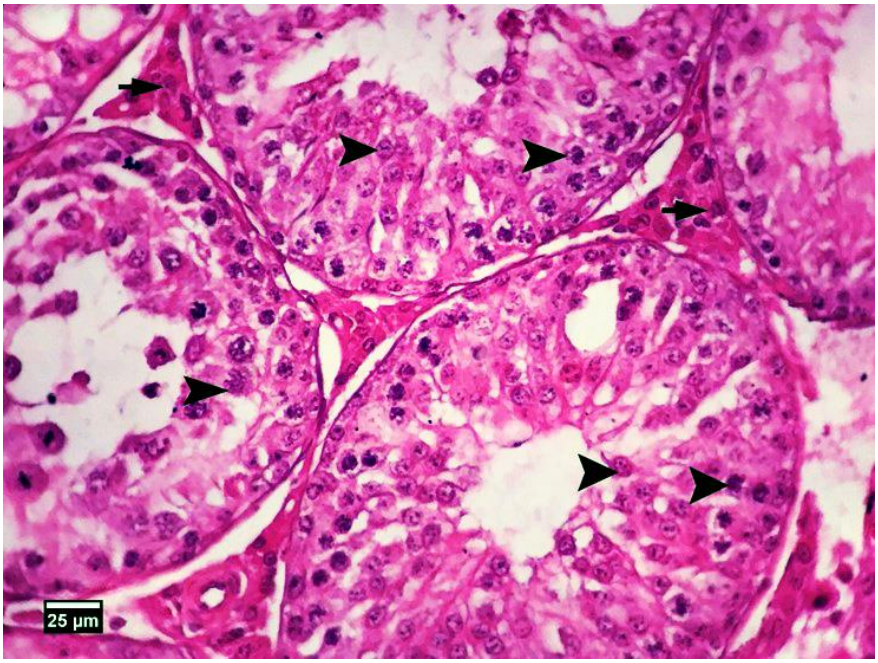

Figure 11. Testis section of left testis in metformin (50 mg/kg) group followed by varicocele rats on the 42nd day showing seminiferous tubules (arrow) with few spermatocytes and interstitial cells (arrowhead) between tubules (hematoxylin and eosin)

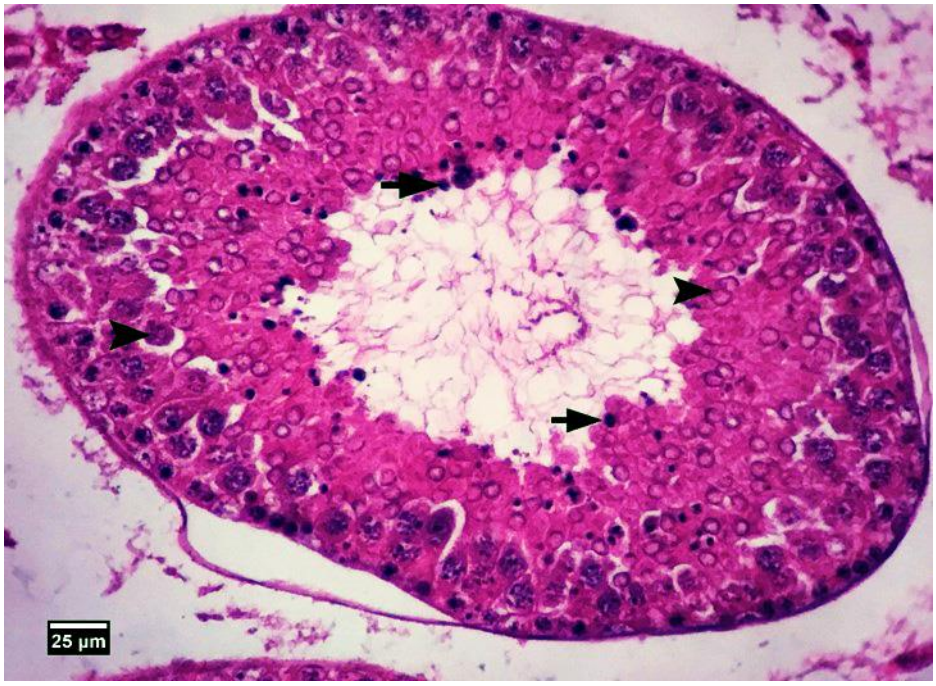

Figure 12. Testis section of left testis in metformin (100 mg/kg) group followed in varicocele rats on the 42nd day showing many normal seminiferous tubules (arrow) with few spermatocytes (arrowhead) (hematoxylin and eosin)

4. Discussion

According to the results, the dose-dependent difference was noticed in testis damage grade in the MET treated groups, compared to that reported for the varicocele group. No difference was observed between 25 and 50 mg/kg of MET. As demonstrated in the current study, the dose-dependent difference was noticed in testis damage grade in MET treated groups, compared to that reported for the varicocele rats. As observed in the histological results, the testis section of the left testis in varicocele rats showed degenerated seminiferous tubules. The testis section of the left testis in the MET (25 mg/kg) group followed in varicocele rats on the 21st day demonstrated seminiferous tubules with few spermatocyte and interstitial cells.

In addition, MET (50 mg/kg) leads to seminiferous tubules with few spermatocytes and interstitial cells between tubules. The MET (100 mg/kg) improved varicocele injury which leads to many normal seminiferous tubules with few spermatocytes. In a recent report, Gungor-Ordueri et al. (2019) revealed that oral gavages of MET (300 mg/kg per day for 8 weeks) improved spermatogenesis and seminiferous tubule integrity and reduced apoptotic activity as manifested by the decreased expression of cleaved caspase 3 in rats with varicocele, which is similar to the results of the current study.

The MET improved the semen parameters related to its effects on weight loss, increased testicular weight, and reduced testicular cell apoptosis (Yan et al., 2015). On the other hand, Tartarin et al. (2012) reported that MET at a concentration 10 times higher than therapeutic levels decreased testosterone secretion and number of Sertoli cells in rats when it was administered during pregnancy. Faure et al. (2016) demonstrated that the reduction in testicular weight and testosterone level

were was observed in 6-week-old chickens treated with MET for 3 weeks.

As observed in the current study, tissue MDA levels significantly increased in the varicocele rats ($P<0.05$); nevertheless, MET (25, 50, and 100 mg/kg) in a dose-dependent manner decreased varicocele-induced MDA. Experimental varicocele significantly decreased SOD activity, compared to that reported for the control group. The administration of MET (25, 50, and 100 mg/kg) significantly increased tissue SOD activity in the varicocele rats. The MET (25, 50, and 100 mg/kg) in a dose-dependent manner increased GPx activity in the varicocele rats.

Sperm membranes contain large amounts of unsaturated fatty acids which provide fluidity, a process that is necessary for membrane fusion (Hwang and Lamb, 2012). Oxidative stress occurs when there is an imbalance between ROS and antioxidants that scavenge surplus free radicals (Hwang and Lamb, 2012). The ROS are natural products of cellular metabolism which, in physiological amounts, are the essential requirements of spermatozoa for sperm processes leading to successful fertilization, such as capacitation, hyperactivated motility, and acrosomal reaction (Agarwal et al., 2014).

A correlation was observed in varicocele and semen oxidation where elevated ROS levels lead to diminished antioxidant capacity in the semen of varicocele-induced animals (Zhang et al., 2006). The aforementioned changes lead to abnormal sperm function and infertility (Masson and Brannigan, 2014). A correlation was shown between the increasing percentage of motile thawed spermatozoa stimulated by MET and the best success of in vitro fertilization in our findings (Bertoldo et al., 2014). Several studies have already described that during freezing and thawing, cell organelles, such as mitochondria, can suffer from injury via a considerable reduction in high membrane potential (Bertoldo et al., 2014).

The MET therapy has been shown to normalize total and free testosterone and may therefore explain the beneficial effect of MET on semen parameters in oligo-terato-asthenozoospermic men (Morgante et al., 2011). It was reported that MET has a positive effect on the proliferation and migration of human umbilical vein endothelial cells. Recently, new mechanisms have supported the effect of MET; nonetheless, the accuracy of them still remains controversial (Esfahanian et al., 2012).

For instance, Zhou et al. (2001) suggested that most of the beneficial effects of MET are mediated through its ability to activate adenosine monophosphate-activated protein kinase (AMPK). Various biological effects have been attributed to the activation of AMPK by MET. It interferes with the action of the mammalian target of rapamycin functioning as a part of the cellular signaling processes regulating cell growth, cell proliferation, cell motility, transcription, and protein synthesis. Possibly, some effects of MET are mediated via this pathway. However, the identification of these effects needs further investigation. Vascular endothelial growth factor reduced apoptosis in varicocele-induced rats by decreasing caspase-3 positive cells (Tek et al., 2009).

Sperms in the epididymis are vulnerable to oxidative damage during the maturation and storage stage. It has been proven that ROS have a key effect on sperm maturation and capacitation, and high ROS production leads to sperm dysfunction (Zhang et al., 2006). Seminal plasma is endowed with frequent enzymatic antioxidants, including SOD, GPx, and MDA. Therefore, the neutral levels of ROS are critical for normal fertilization, capacitation, hyperactivation, and motility (Agarwal et al., 2009). Varicocelectomy reduces the ROS levels with an increase in the antioxidant capacity of semen in infertile men (Masson and Brannigan, 2014). However, this also makes spermatozoa vulnerable to ROS attack. Seminal fluid is an important source of antioxidants in semen, as the lack of cytoplasm and deoxyribonucleic acid compaction in spermatozoa leaves very little space for translation or antioxidant defenses. Lipid peroxidation has also been associated with a decrease in sperm motility (Agarwal et al., 2008).

The MET stimulates lactate production which plays a key role in germ cell development and anti-apoptotic

effect in Sertoli cells (Ghasemnejad-Berenji et al., 2018). It was reported that the anti-apoptotic effect of MET is mediated via caspase-3 in rat testis (Gungor-Ordueri et al., 2019). The findings of the present study are consistent with the results of previous studies regarding the fact that MET reduced apoptosis in testis with varicocele (Gungor-Ordueri et al., 2019). Cryopreservation in the presence of MET can increase the viability without the effect of lipid peroxidation, compared to the control group in mouse spermatozoa (Bertoldo et al., 2014).

The discrepancy between biochemical and protective actions of MET is not an isolated observation. Cisplatin-induced functional and histological nephropathy was not prevented by MET in a rat model in vivo, although MET significantly attenuated drug-induced lipid peroxidation and reactive oxidant species and preserved enzymatic and non-enzymatic antioxidants (Sahu et al., 2013). In contrast, MET prevented experimental gentamicin-induced nephropathy in rats (Morales et al., 2010).

5. Conclusion

In conclusion, the results of the current study suggested that MET treatment had beneficial effects on varicocele. The MET prevents the progression of varicocele-induced infertility by decreasing elevated MDA and free radical scavenging activity through increasing SOD and GPX levels in varicocele-induced rats.

Authors' Contribution

Study concept and design: A. A.
Acquisition of data: H. K.
Analysis and interpretation of data: P. M.
Drafting of the manuscript: A. A.
Critical revision of the manuscript for important intellectual content: Gh. A.
Statistical analysis: A. J.
Administrative, technical, and material support: H. K.

Ethics

The authors declare that all ethical standards were respected in the preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

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