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Metformin improves semen profile and hormonal levels in experimental varicocele in the rat

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Abstract

Objective: This study aimed to determine the effect of metformin (MET) on semen profile and hormonal levels in a model of varicocele in rats.

Materials and Methods: Sixty male Wistar rats were allocated into 5 treatment groups as control and varicocele groups. In groups 3-5, varicocele animals received MET (25, 50 and 100 mg/kg) for 42 days. At the end of the study, semen evaluations and serum testosterone and luteinizing hormone and follicle stimulating hormone levels were measured.

Results: Experimental varicocele significantly decreased total sperm count and sperm motility and increased non-motile sperms compared to the control group ($p<0.05$). Metformin administration (50 and 100 mg/kg) significantly increased total sperm count and sperm motility and decreased non-motile sperm as compared to the varicocele group ($p<0.05$). In addition, experimental varicocele significantly decreased testosterone and luteinizing hormone and follicle stimulating hormone levels as compared to the control group ($p<0.05$). Metformin administration (50 and 100 mg/kg) significantly increased testosterone and luteinizing hormone and follicle stimulating hormone levels as compared to the varicocele group ($p<0.05$).

Conclusion: These results suggested that metformin has positive role against varicocele in the rat.

Keywords: Metformin, Semen, Testosterone, Luteinizing hormone, Follicle stimulating hormone, Varicocele

1. Introduction

Spermatogenesis is the production of sperm cells, by which the germ cells produce haploid spermatozoa. Sperm is produced within the seminiferous tubules, a convoluted cluster of tubes located within the testes (22). The hypothalamic-pituitary-gonadal axis (HPGA) is a crucial regulator of testosterone and gonadal activities, which potentiates spermatogenic processes and sustenance of male reproductive function. Male steroidogenesis involves the conversion of cholesterol moiety to the production of male steroid hormones through complex biochemical pathways and involves several enzymatic pathways (27). However, cholesterol homeostasis is a significant factor in the male reproductive function, which is involved in the normal sperm production (20). The gonadal trophic releasing hormone-(GnRH) regulates the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) through the anterior portal system. Luteinizing hormone mainly acts on the Leydig cells to increase testosterone production, with testosterone having a limit on its secretion via negative feedback. Gonadal steroids and pituitary gonadotropins play a significant physiological role in establishing homeostatic mechanisms that maintain male reproductive functions (19).

Varicocele is an abnormal vascular dilatation of the

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pampiniform plexus and is commonly observed on the left side. 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 (11). Based on the literature, varicocele causes a progressive decline in fertility and can continue to induce the impairment of spermatogenesis despite prior fertility (13).

Metformin (N, N-dimethylbiguanide; MET) is a known biguanide-derivative antidiabetic drug most widely used as an oral agent in humans and used in the treatment of polycystic ovarian syndrome. It is a reliable drug used in treating tumor cells and aging cells and with cardioprotective effect and neuroprotective effects (14). Despite its antidiabetic activities, reports have shown that MET enhances antioxidant levels of glutathione, superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferase (9) and improves spermatic function through enhancement of the activity of adenosine monophosphate kinase (AMPK) pathway (4).

In our recent work, we found that MET (50 and 100 mg/kg) improves testis damage grade in experimental varicocele in rats. Also, MET decreased tissue malondialdehyde while increased superoxide dismutase, glutathione peroxidase and total antioxidant levels (18). MET possesses a non-genomic action; it could be an interesting molecule for the treatment of sperm which can improve fertility (6). MET improves semen characteristics in men with metabolic syndrome (21). Based on literature, MET has positive roles and there is no report for its possible protective effect in varicocele. Thus, this study aimed to determine effects of MET on semen profile and hormonal levels in varicocele in rats.

2. Materials and Methods

2.1. Animals

Sixty male Wistar rats (body weight: 230-250 g) were allocated into 5 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 (32). 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.

In Group 1 (control), rats received no medication and underwent no surgery. In group 2 (varicocele), the abdominal cavity was opened and the rats underwent varicocele induction and received no medication. In group 3, the abdominal cavity was opened and the animals received 25 mg/kg of MET for 42 days and varicocele induced. Groups 4 and 5 were similar to group 4 except that the rats received 50 and 100 mg/kg of MET, respectively. At the end of the study, semen evaluations were done.

2.2. Surgical procedure

Procedures were performed under anesthesia by an intraperitoneal (IP) injection of 60 mg/kg of ketamine hydrochloride (10%) and 10 mg/kg of 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) (26). 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 0.4-0.85 mm 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 (10).

2.3. Sperm motility

Sperm cells was obtained from the epididymis' caudal end, placed on a clean glass slide, and mixed with a physiological solution of 990 µl (paraformaldehyde and sodium citrate) in the ratio of 1-20. About 5.0 µl of supernatant containing the sperm was placed between the slide and coverslip and observed at 100 X in a negative phase contrast microscope. The evaluation of the movement of the sperm was done in three different fields, and motility was expressed from the middle of the fields in the percentage of motile sperm of the total sperm counted (3).

2.4. Testosterone test procedure

The serum was used to assay for testosterone levels in different groups using the ELISA method described by the Manufacturer's manual using the commercial ELISA based kits.

2.5. Luteinizing hormone and follicle stimulating hormone test

Serum samples was used to assay luteinizing hormone and FSH using the enzyme immunoassay (EIA) technique as described by the Manufacturer's manual using the commercial ELISA based kits.

2.6. Statistical analysis

Data were analyzed by one-way analysis of variance using SPSS software (version 24.0) and expressed as mean ± standard error. The differences between groups were analyzed using Duncan's Multiple Range test. P< 0.05 was regarded as a significant difference between the groups.

3. Results

As seen in figure 1, experimental varicocele significantly decreased sperm motility compared to control group (p<0.05). MET administration (50 and 100 mg/kg) significantly increased sperm motility compared to varicocele group (p<0.05).

Based on figure 2, experimental varicocele significantly increased non motile sperm compared to control group (p<0.05). Metformin administration (50 and 100 mg/kg) significantly decreased non motile sperm compared to varicocele group (p<0.05).

According to figure 3, experimental varicocele significantly decreased total sperm count compared to control group (P<0.05). MET administration (50 and 100 mg/kg) significantly increased total sperm count compared to varicocele group (P<0.05).

As shown in figure 4, experimental varicocele significantly decreased testosterone levels compared to control group (p<0.05). Metformin administration (50 and 100 mg/kg) significantly increased testosterone levels compared to varicocele group (p<0.05).

In figure 5, experimental varicocele significantly decreased luteinizing hormone levels compared to control group (p<0.05). MET administration (50 and 100 mg/kg) significantly increased luteinizing hormone levels compared to varicocele group (p<0.05).

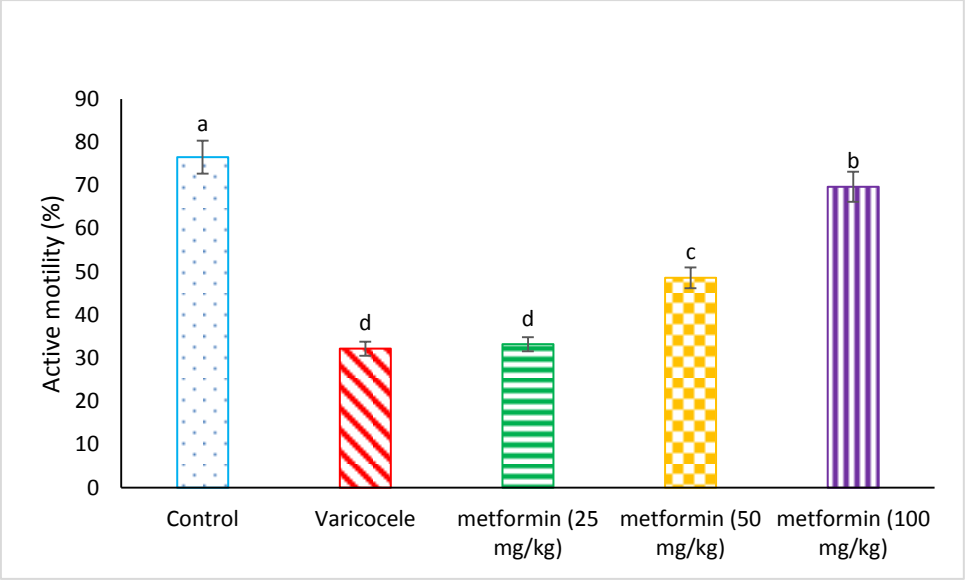

Figure 1. Effects of metformin on sperm active motility (%) in experimental varicocele rats. Different letters (a-d) in each column indicating significant differences between treatments (P<0.05).

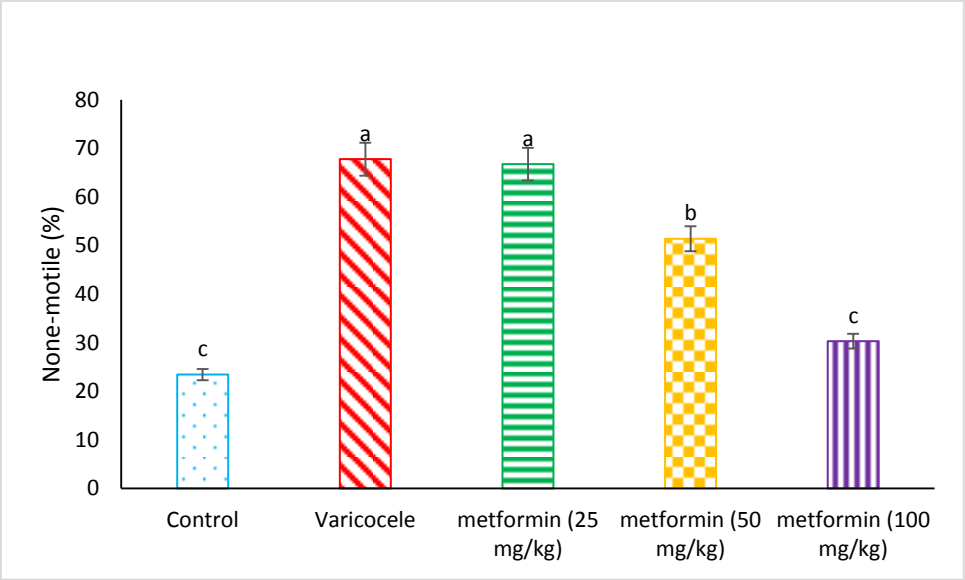

Figure 2. Effects of metformin on sperm non-motile (%) in experimental varicocele rats. Different letters (a-d) in each column indicating significant differences between treatments (P<0.05).

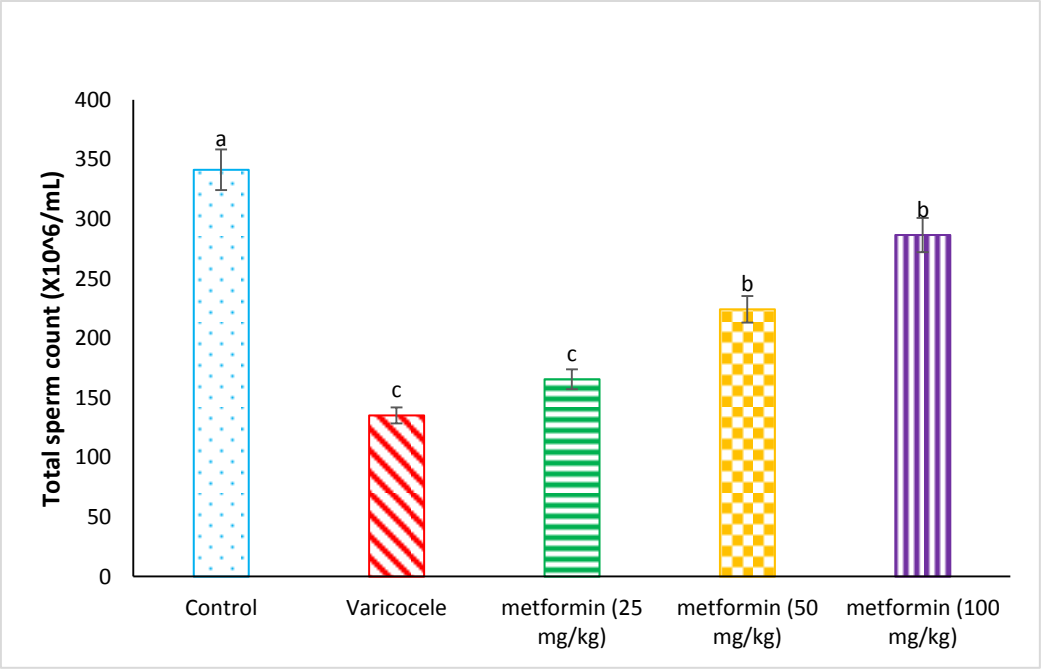

Figure 3. Effects of metformin on sperm total sperm count in experimental varicocele rats. Different letters (a-d) in each column indicating significant differences between treatments (P<0.05).

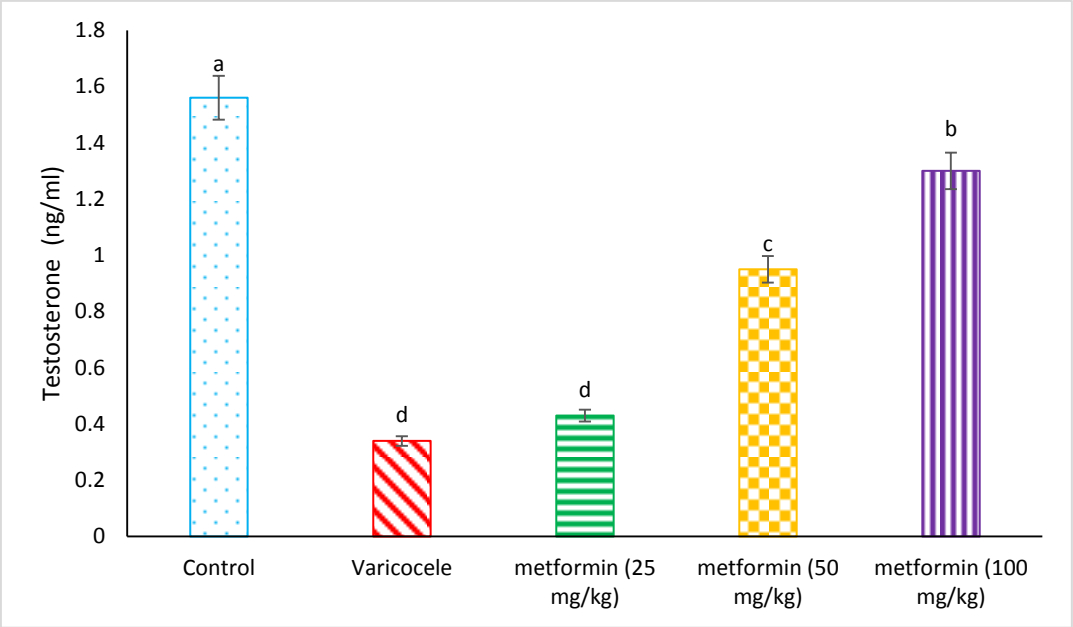

Figure 4. Effects of metformin on testosterone levels in experimental varicocele rats. Different letters (a-d) in each column indicating significant differences between treatments (P<0.05).

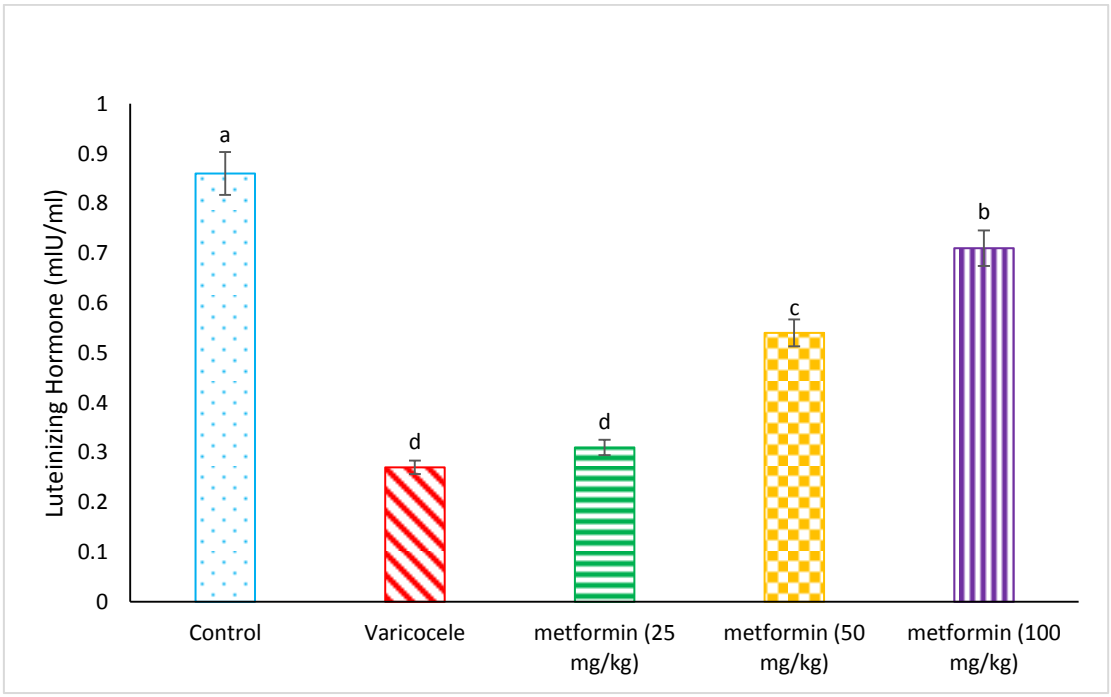

Figure 5. Effects of metformin on luteinizing hormone levels in experimental varicocele rats. Different letters (a-d) in each column indicating significant differences between treatments (P<0.05).

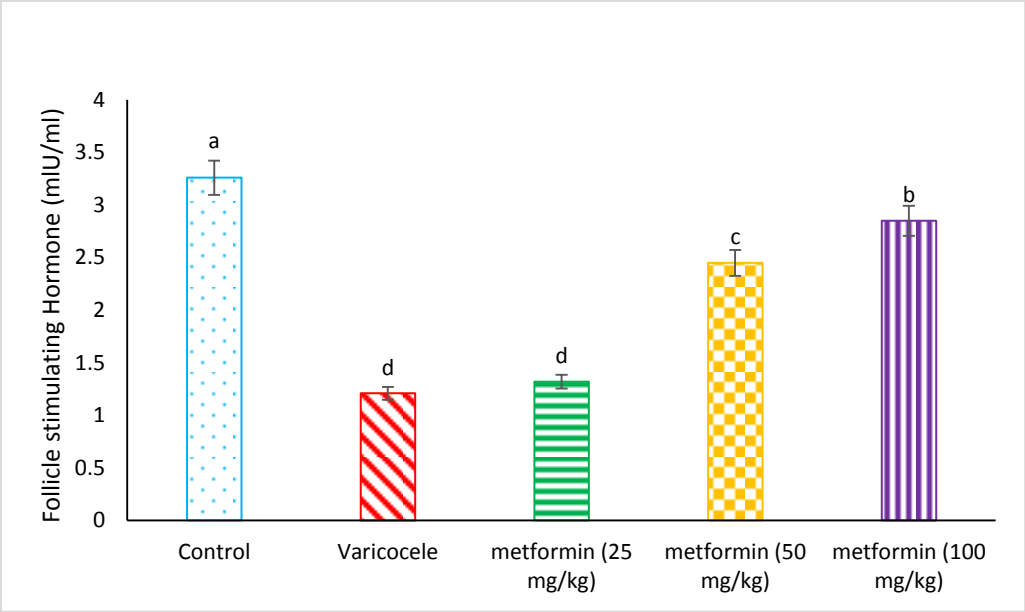

Figure 6. Effects of metformin on follicle stimulating hormone levels in experimental varicocele rats. Different letters (a-d) in each column indicating significant differences between treatments (P<0.05).

As observed in figure 6, experimental varicocele significantly decreased follicle stimulating hormone levels compared to control group (P<0.05). Metformin administration (50 and 100 mg/kg) significantly increased follicle stimulating hormone levels compared to varicocele group (p<0.05).

4. Discussion

Based on main finding of the current study, experimental varicocele decreased total sperm count and sperm motility and increased non-motile sperm. MET administration (50 and 100 mg/kg) increased total sperm count, sperm motility and decreased non-motile sperm. Experimental varicocele decreased testosterone, luteinizing hormone and follicle stimulating hormone levels. MET administration (50 and 100 mg/kg) significantly increased testosterone and luteinizing hormone and follicle stimulating hormone.

Male infecundity has been linked to environmental toxicant exposure, which has contributed immensely to infertility in animal and human studies resulting from oxidative stress processes (8). However, exposure to environmental carcinogenic substances or endocrine disruptors has been significantly associated with infertility and, thus, has a substantial influence on hormones of reproductive function in males, which alters spermatogenesis and impairs glycolytic pathways and impairment of sperm capacitation resulting from oxidative stress (16). In our previous work, we found that MET (50, and 100 mg/kg) improves testis damage grade in experimental varicocele in rats. Also, MET decreased tissue malondialdehyde while increased superoxide dismutase, glutathione peroxidase and total antioxidant levels (18). MET improved the semen parameters related to its effects on weight loss, increased testicular weight, and reduced testicular cell apoptosis (30). In the other hand, Tartarin et al (2012) reported that MET at a concentration 10 times higher than therapeutic levels decreases testosterone secretion and number of Sertoli cells in rats when it was administered during pregnancy (28). demonstrated that the reduction in testicular weight and testosterone level were was observed in 6-week-old chickens treated with MET for 3 weeks (12).

The attenuated effect of MET is attributed to its ability to combat lipid peroxidation in the testicular membrane and its tissues. MET treatment significantly improved total sperm count, which is linked to the decline in oxidative stress and lipid peroxidation, enhancement of 5'-AMP-activated protein kinase activity, and restoration to the expected levels of pituitary-gonadal hormones (5). The study corroborates the report of Attia et al (2), Nna et al (23) and Pourheydar et al (24), revealing an increase in sperm count following MET in testicular dysfunction. The report of Zaidi et al (31) is inconsistent with the study findings showing a non-significant difference in sperm count following MET administration in testicular dysfunction. Adaramoye et al (1) indicated a significant decline in sperm count with respect to MET administration, which refutes the study findings. Thus, the physiology linked to MET ameliorative and protective phase is associated with the enhancement of 5'-AMP-activated protein kinase activity and restoring expected levels of pituitary-gonadal hormones (7).

Gonadotropins secreted by the brain oversee male fertility, and their regulations have significance in the continuity of species. However, LH and FSH

hormones are determinants of testosterone function in steroidogenesis (15). The mechanism of action following the significant decline in testosterone and FSH activity after arsenic trioxide exposure is associated with an inhibition of the hypothalamic-pituitary axis, which caused changes in LH and FSH plasma concentrations. Thus, the decline of plasma LH could impair Leydig cell function and result in a consequent reduction in testosterone production (17). The mechanism of action linked to MET is poorly understood. Wang et al. (29) indicated that MET had an insignificant increase in the testicular index in triptolide toxicity, which refutes the study outcome. Pourheydar et al (25) showed a significant increase in testicular weight in MET treated diabetic model, which contradicts the study findings.

In conclusion, these results suggested MET has positive role against varicocele in the rat. We think the positive effects of MET on hormonal levels and sperm parameters is related to its antioxidant activity in varicocele rat.

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