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Verapamil versus tamoxifen in treatment of experimentally induced infertility

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ABSTRACT: Clinical and epidemiological studies indicated that there is an increased incidence of male infertility, with many cases that do not respond well to treatments. This study is aimed at evaluating the therapeutic effects of verapamil and tamoxifen against infertility, which may provide an essential strategy for the treatment of this disorder. A case-control study was conducted on 64 male albino rats, divided into four groups (16 for each); group A (control group); group B (infertile group that is induced by the ornidazole); and groups C and D, which are pretreated with verapamil and tamoxifen, respectively, in combination with ornidazole every day for four weeks. Serum levels of testosterone, follicle-stimulating hormone (FSH), luteinizing hormone (LH), glutathione (GSH), nitric oxide (NO), malondialdehyde (MDA), and superoxide dismutase (SOD) were assessed, and sperm parameters, including sperm count, sperm motility percentage, sperm morphology percentage, and sperm viability, were investigated. Furthermore, Motic Image Plus 2.0 ML was used to measure the mean diameter of the seminiferous tubules (SNT), and a morphometric analysis of 20 tubules per rat was also performed. The results revealed that both verapamil and tamoxifen provide a significant improvement in sperm count, motility, morphology, and dead sperm, with a significant improvement in testosterone, FSH, LH, GSH, NO, MDA, and SOD levels compared with the infertile group. Histophotometric results showed that verapamil and tamoxifen improved the number and size of SNT, Leydig cells, and Sertoli cell counts, which may indicate the promise of using these agents in the treatment of male infertility.

KEYWORDS: Infertility; Ornidazole; Verapamil; Tamoxifen.

1. INTRODUCTION

Male infertility is a complex condition that affects a significant portion of the community that caused from any disruption in the spermatogenesis sequence [1]. One of the most disruptions caused by the increment in the oxidative stress which is originate from the excessive generation of free radicals which in turn affects unsaturated fatty acids by attacking them, resulting in lipid peroxidation in the cell membrane, enzymatic dysfunction, and ultimately cellular damage and necrosis.[2]. Reactive oxygen species (ROS) have the ability to disrupt the cell cycle and trigger apoptosis, which reduces the quantity and quality of sperm generation [3]. The World Health Organization estimates that 8 percent of couples in the reproductive phase are infertile, with male factors accounting for 20 percent of these cases. In developed countries, the prevalence of male infertility ranges from 10 to 25 percent, which can reach 30 percent [4]. Inadequate epididymal maturation, disorders in sperm production and transport, and dysfunction of accessory sex glands can also result in male infertility [5]. Furthermore, aberrant epididymal functions can alter the characteristics of semen and the integrity of sperm DNA, which raises proinflammatory mediator levels and increases the amount of immature germ cells and debris in the reproductive tract [6]. According to reports, disorders of spermatozoa such as azoospermia/oligospermia, asthenospermia, and teratospermia are the main causes of male infertility globally [7]. Additionally, a number of risk factors have been proposed for male infertility; these include exposure to environmental toxins, alcohol, smoking, drugs, obesity, testicular infections, exposure of the testicles to excessive heat, hormonal disorders, testicular trauma, and erectile disorders [8]. Furthermore, metabolic diseases such as diabetes are associated with deleterious effects on sperm parameters [9]. Diabetes can affect genes involved in sperm DNA repair expression, which can lead to a substantial rate of nuclear DNA fragmentation [10], mitochondrial DNA deletion [10–12], changes to the mitochondrial respiratory chain, and ultimately a decrease in sperm motility [13]. Although there are many drugs have been used in treatment of male infertility. However, to date, there has been limited success that

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focus the light on the treatment of potential causes and risk factors in ways to enable the identification of simple preventive and treatment strategies.

Verapamil, which got approval from the Food and Drug Administration (FDA) as a first-generation calcium channel blocker in 1981 for the treatment of atrial fibrillation, supraventricular tachycardia, angina, and hypertension [14]. In vascular smooth muscle and the heart, verapamil blocks the entry of calcium ions into long-acting calcium channels (L-type) [15]. Additionally, verapamil has been shown to significantly protect against ischemic acute renal injury in an experimental model [16]. Moreover, previous research has indicated that verapamil may also have anti-inflammatory and anti-angiogenesis properties [17]. Furthermore, verapamil significantly increases the generation of nitric oxide (NO), which promotes the growth of fibroblasts, keratinocytes, endothelial cells, and epithelial cells and also has antioxidant properties [18]. On the other hand, Tamoxifen, which is a selective estrogen receptor modulator (SERM), has been used to treat non-obstructive azoospermia, oligospermia, and idiopathic male infertility [19]. While hormonal therapy is useful for treating infertility resulting from gonadotropin insufficiency, there are limited benefits for idiopathic male infertility [20]. Tamoxifen has antioxidant and free radical scavenging effects that confirm its protective role in male infertility [21]. Moreover, tamoxifen can induce the production of antioxidant enzymes such as glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD), and it can also stimulate nitric oxide synthase (NOS), which can convert L-arginine into NO [22]. Furthermore, tamoxifen prevents neutrophil infiltration and the production of hydrogen peroxide by neutrophils [23].

This study is aimed at evaluating the therapeutic effects of verapamil and tamoxifen against infertility by assising the sperm parameters, hormones, oxidative stress markers and histophotometric markers which may provide an essential strategy for the treatment of this disorder.

2. RESULTS

2.1. Sperm Parameters

Results illustrated in Table 1 summarized the results obtained for the sperm parameters in all groups as following

2.1.1. Sperm counts

Results of this study showed that Ornidazole induced infertility group was associated with highly significant reduction in sperm count as compared with normal control group ($P< 0.01$). On the other hand, both verapamil and tamoxifen groups provide protective effect against Ornidazole and they elicited no significant difference in sperm counts as compared with control group ($P> 0.05$).

2.1.2. Percent sperm motility

Current study showed that sperm motility was considerably lower in the group of Ornidazole-induced infertility as compared with control, verapamil and tamoxifen groups ($P<0.01$). on the other hand, the sperm motility of verapamil and tamoxifen groups were non-significantly differ from that of controls.

2.1.3. Mean sperm morphology

Abnormal sperm shape percent was highly significantly highest in Ornidazole induced infertility group as compared with control, verapamil and tamoxifen groups ($P <0.01$). While abnormal sperm shape percentage of verapamil and tamoxifen were not significantly different as compared with control group ($P>0.05$)

2.1.4. Mean Dead sperm

The results of the present work showed that the group that received Ornidazole-induced infertility had the greatest mean percentage of dead sperm. as compared with control, verapamil and tamoxifen groups ($P <0.01$). While Mean Dead sperm percentages of verapamil and tamoxifen were not significantly different as compared with control group ($P > 0.05$).

Additionally, results also revealed that Verapamil group showed non-significant changes in all sperm parameters when compared with Tamoxifen group.

Table 1. Comparison of sperm parameters among control and treatment groups.

Characteristic	Control	Ornidazole induced infertility	Verapamil	Tamoxifen
Sperm count	25.2± 2.3 a	16.6± 2.4 b	22.5 ± 2.5 a	23.5± 3 a
Sperm motility (%)	91 ± 2.4 a	66.4 ± 4.8 b	80.4 ± 8 a	85.2 ± 9 a
Abnormal sperm shape (%)	8.2 ± 1.2 a	15.2 ± 3.9 b	12 ± 4.2 a	11 ± 3.3 a
Dead sperm (%)	7.8 ± 5.4 a	15 ± 4.9 b	8.9 ± 3 a	8.3 ± 4 a

Lowercase letters within the same row indicate significant differences.

2.2. Hormonal Study

The results of hormonal studies tabulated in Table 2 showed that the mean serum testosterone level was significantly lower in the ornidazole-induced infertility group as compared with the control group ($P<0.01$). Although the mean blood testosterone level in the Verapamil group has improved, it is still considerably lower than in the Tamoxifen and control groups ($P > 0.05$). On the other hand, there was no discernible difference between the Tamoxifen group and the control group ($P > 0.05$). When comparing the control, verapamil, and tamoxifen groups to the ornidazole-induced infertility group, the mean serum follicle-stimulating hormone (FSH) was considerably lower ($P<0.01$). The verapamil and tamoxifen groups' mean serum FSH did not differ significantly from the control group's ($P > 0.05$). Nevertheless, in all groups of control, ornidazole-induced infertility, and the examined drugs, there was no significant difference in mean serum LH ($P > 0.05$).

Moreover, except for the levels of testosterone, which showed significantly higher levels in the tamoxifen group compared with the verapamil group, the results of all other hormones studied showed no significant difference between tamoxifen and verapamil.

Table 2. Comparison of testosterone, FSH, LH among control and treatment groups.

Characteristic	Control	Ornidazole induced infertility	Verapamil	Tamoxifen
S. Testosterone (ng/ml)	2.65± 0.8 a	1.2 ± 0.85 b	1.7 ± 0.5 b	2.70± 0.9 a
S.FSH (IU/L)	2.9 ± 0.5 a	1.17 ± 0.15 b	2.1 ± 0.4 a	2.5 ± 0.5 a
S. LH (ng/ml)	2.3± 0.73 a	2.14 ± 0.67 a	2.15 ± 0.7 a	2.45 ± 0.7 a

Lowercase letters within the same row indicate significant differences.

2.3. Marker of oxidative Stress

According to this study, the results postulated in Table 3 elucidate that the mean blood glutathione levels in the ornidazole-induced infertility group were significantly lower than those in the tamoxifen, verapamil, and control groups ($P<0.01$), whereas non-significant changes were obtained when the tamoxifen, verapamil, and control groups were compared with each other. When comparing the ornidazole-induced infertility group to the tamoxifen, Verapamil, and control groups, the mean serum SOD decreased significantly ($P <0.01$). However, there was no discernible difference between the Tamoxifen and Verapamil groups and the control group ($P > 0.05$). In a similar manner, comparing the ornidazole-induced infertility group showed significantly higher levels of malondialdehyde (MDA) when compared with tamoxifen, verapamil, and control groups ($P <0.01$). When comparing the Ornidazole-induced infertility group to the tamoxifen, verapamil, and control groups, the mean serum NO was considerably lower ($P <0.01$), while the mean serum NO of the tamoxifen and verapamil groups showed improvement to significantly higher levels than those of the Ornidazole-induced infertility group but remained considerably lower than that of the control group ($P<0.05$). However, it was improved significantly when compared to the Ornidazole-induced infertility group ($P<0.05$).

Finally, it was noticed that the levels of the studied oxidative markers in the Verapamil group did not differ significantly from those in the Tamoxifen groups.

2.4. Histophotometric study (Table 4)

The current study showed that the mean number of seminiferous tubules (SNT) was significantly reduced in the ornidazole-induced infertility group as compared with control groups ($P <0.05$). When compared to the ornidazole-induced infertility group, the mean number of SNT improved significantly with verapamil and tamoxifen treatment ($P <0.05$); nevertheless, it was still significantly lower than that of the control group ($P <0.05$).

Table 3. Comparison of mean serum marker of oxidative stress among control and treatment groups.

Characteristic	control	Ornidazole induced infertility	Verapamil	Tamoxifen
S. GSH (µg/ml)	4.14± 0.2 a	2.63± 0.4 b	3.5 ± 0.5 a	3.9 ± 0.7 a
S.SOD (u/ml)	17.13± 2.6 a	10.2 ± 2.2 b	16.5± 1.2 a	17.5 ± 1.1 a
S.MDA (nmol/Ml)	6.34 ± 0.86 a	15.12 ± 2 b	9.2 ±1.5 a	8.1 ±1.2 a
S.NO (µmol/L)	6.1 ± 0.77 a	1.80 ± 0.43 c	3.2± 0.4 b	4.2 ± 0.3 b

Lowercase letters within the same row indicate significant differences.

Table 4. Histomorphometry of section through animal testes

Characteristic	control	Ornidazole induced infertility	Verapamil	Tamoxifen
No. of SNT	22.4 ± 2.24 a	13.7± 1.3 c	16 ± 2.7 b	18 ± 3.7 b
Size SNT (µm)	284.2± 12 a	176 ± 5 c	220 ± 7.2 b	250 ± 6 b
Leydig cell count	54.2 ± 4.2 a	27 ± 2.2	38 ± 3.9 b	44 ± 3 b
Sertoli cell count	27 ± 2.7 a	18 ±1.9 b	23 ± 2.6 a	24 ± 3 a

Lowercase letters within the same row indicate significant differences.

The ornidazole-induced infertility group had a considerably smaller mean size of SNT as compared with the control group (P<0.05). The mean size of SNT in the verapamil and tamoxifen treatment groups was significantly smaller than in the ornidazole-induced infertility group (P<0.05), although it was still significantly larger as compared with the control group (P<0.05). When comparing the ornidazole-induced infertility group to the control group, the mean Leydig cell count was considerably lower (P<0.05), while the Leydig cell count of the verapamil and tamoxifen-treated groups was significantly lower than that of the control group (P<0.05); however, it was dramatically improved when compared to the ornidazole-induced infertility group (P<0.05). When comparing the ornidazole-induced infertility group to the control group, the mean Sertoli cell count decreased considerably (P<0.05). Verapamil and tamoxifen treated groups showed significant improvement in Mean Sertoli cell count as compared with Ornidazole induced infertility group (P<0.05) which is non-significantly differ from that in controls.

In a pattern similar to that obtained in other studied markers, the levels of all histophotometric studies in the group of rats that received Verapamil did not significantly differ from those in the group of rats that received Tamoxifen.

3. DISCUSSION

The male infertility model in the current study has been induced by ornidazole, which exerts its spermatotoxic effect through the generation of hydrogen peroxide and hydroxyl radicals, leading to impeding epididymal sperm motility in terms of reducing sperm velocity [24, 25]. The oxidative stress mechanism results from disequilibrium between reactive oxygen generation and the protective biological mechanisms. Free radicals may attack a diversity of biomolecules, like carbohydrates, lipids, proteins, nucleic acids, and macromolecules of connective tissue, which cause potential cellular damage [26]. The current study depicted that the sperm count, motility, and morphology of sperm cells in the ornidazole-induced infertility group decreased substantially compared to the control group. In addition, the reduction in the concentration of serum antioxidant activity in the infertile group can generate superoxide inions that are associated with testicular injuries and the extensive generation of cellular reactive radicals [27]. These radicals have a deleterious effect on the biosynthesis of DNA and RNA in sperm through inhibition of the function of spermatic mitochondria [28]. Oxidative stress can disrupt divisions and differentiation, leading to a reduced number of spermatids, spermatocytes, and mature sperm [29]. The oxidative stress process can inhibit sperm biosynthesis through the formation of gametes associated with modified chromatin that will be targets for free radicals' attacks [30]. The current study is in accordance with Khan et al.'s study, which showed arsenic-mediated oxidative stress in rats testes caused oxygen radical generation, which has deleterious effects on all sperm [31]. Reduction of sperm motility (asthenospermia) may be partly attributed to glutathione reduction [32].

The current study showed that the ornidazole-induced infertility group is significantly associated with decreased mean serum GSH as compared with the normal control and verapamil groups. Also, asthenospermia is associated with decreased SOD levels that have a protective role in sperm motility by inhibiting lipid peroxidation [33].

In the present study, the mean serum SOD was lower in the ornidazole-induced infertility group, while both the verapamil and tamoxifen-treated groups were not significantly different as compared with

the control group. Moreover, the current study showed that verapamil and tamoxifen treatment improved serum levels of antioxidant activity as compared with the ornidazole-induced infertility group. In present research, the increased levels of antioxidant activity induced by verapamil and tamoxifen focus the light on the antioxidant and anti-lipid peroxidation activities of these drugs. Furthermore, verapamil and tamoxifen treatment increase the levels of NO, which improves antioxidant activity [34, 35]. Nitric oxide has diverse biological activity in spermatogenesis and sperm function through regulation of Sertoli cell-germ function and also supports the blood-testis barrier [36, 37]. Lipid peroxidation is a process that involves the oxidative conversion of fatty acids to metabolites that are commonly referred to as free radicals and can be represented by malondialdehyde [38]. Malondialdehyde (MDA) is the most important indicator of oxidative stress and lipid peroxidation [39]. The current study shows that significantly increased MDA levels in the ornidazole-induced infertility group as compared with the control group were obtained. Verapamil and tamoxifen treatments showed antioxidative activity that caused a reduction in lipid peroxidation, that appears clearly in the significant decrease in MDA levels as compared with ornidazole-induced infertility group. The present research showed that the SNT number and testosterone hormone levels in the ornidazole-induced infertility group substantially decreased compared to the normal group. However, verapamil and tamoxifen treatment significantly improve the morphology of sperm, ideally SNT, and the testosterone hormone. All these changes produced by verapamil may be attributed to anti-ischemic, antioxidant, and nitric oxide donor actions that exert substantial functional, cellular, and morphological protection against ischemia and improve blood supply to the cells, which may be the protective role of verapamil and tamoxifen against fertility disorders [35, 40].

4. CONCLUSION

Results of the current research elucidate the powerful role of verapamil and tamoxifen in protecting against male infertility, which can be demonstrated by their anti-inflammatory, anti-angiogenesis, and antioxidant properties, which lead to a restoration in the sperm parameters, hormones, oxidative stress markers, and histophotometric markers to levels nearly comparable to those of controls, which may indicate the promise of using these agents in the treatment of male infertility.

5. MATERIALS AND METHODS

5.1. Study design

Sixty-four healthy male albino rats, weighing 210–320 grammes, were purchased from the Iraqi Centre for Drug Evaluation and Research, with an age range of 12–14 weeks. The animals are housed in ideal laboratory settings with a 12-hour light/dark cycle. They are fed rodent pellets and high-fat diets, and they have unrestricted access to water. Each of the six rats had a decent cage. Experimental rats are divided randomly into four groups (16 rats in each group) as follows:

- Group A (control group, receive vehicle of citrate buffer).
- Group B (infertile group that induced by administration of Ornidazole (400 mg/kg/day for 4 weeks)
- Group C and group D pretreated with Verapamil (5 mg/kg /d) and Tamoxifen (0.6 mg/kg/day) respectively in combination with infertility inducing agent Ornidazole (400 mg/kg/day) daily for four weeks.

All drugs have been freshly prepared, immediately used within 5 minutes of preparation, and given orally by gavage. Experimental infertility in rats was induced by oral administration of Ornidazole (400 mg/kg/day) for 4 weeks [41]. Ornidazole is dissolved in citrate buffer, and the pH is adjusted to 4.5 [42]. Verapamil and tamoxifen doses were chosen depending on previous research that has investigated it in different disease models in rats. After 4 weeks of experimental work, all animals have been sacrificed, and biochemical and histological investigations have been done. Using assay kits and the manufacturer's instructions, ELISA was used to assess the levels of testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) in the serum. Conversely, measurements were also made of sperm parameters, including sperm count, sperm motility percentage, sperm morphology percentage, and sperm viability. Testes were also dissected through an abdominal incision, and specimens were fixed in a buffer of 10% neutral formalin overnight, and the histological procedure was done. Additionally, the software Motic Image Plus 2.0 ML was used to measure the mean diameter of the SNT and perform morphometric analysis on 20 tubules per rat. Additionally, to assess spermatogenesis, twenty of the roundest tubules per testicular section were selected and calculated. In addition, oxidative stress markers, including GSH, NO, MDA, and SOD, in serum were assessed by the ELISA technique according to the manufacturer's instructions.

5.2. Statistical analysis

Software tools (Statistical Package of Social Sciences, version 23) and Microsoft Office Excel 2010 were used to evaluate the data. Numerical data are shown as mean and standard deviation, while categorical variables are presented as a number and percentage. When there were more than two groups and the data was normally distributed, a one-way ANOVA and a post hoc test were conducted, considering $P < 0.05$ as a significant threshold for the differences among the studied groups [43, 44].

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