The role of ginseng and vitamin C in protection against damage induced by ketoconazole in male gonad of laboratory rats, *rat rattus*

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Abstract
Ketoconazole (KCZ) is antifungal used for fungal contagion and is used for some prostate cancer. Although it’s therapeutic activity several side effect in human body system has been recorded, one of these side effect was its effect in the androgens. The present study designed to evaluate the protective role of an herbal formula of ginseng and vitamin C against the toxic damage of KCZ. Forty male rats were used in this study divided into four groups the first treated orally with kcz the second kcz-gin. The third kcz-vit. and the control group. The reproductive performance, and testosterone levels and MDA, SOD concentration, sperm count, and sperm malformation were measured also histological sections for the testes were studied. The Results showed that both ginseng and vitamin were able to significantly reduce the toxic effect of kcz in all mentioned reproductive parameters except the MDA and SOD concentrations.

Keywords: KCZ, protective role, ginseng, vitamin C, reproduction parameter

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Introduction
Ketoconazole (KCZ) is a synthetic wide-ranging antifungal agent used to treat serious fungal or yeast infections. Moreover, it is indicated for the treatment of the following systemic fungal infections: Chronic Mucocutaneous Candidiasis, Candiduria, this medicine used only when you cannot use other antifungal medications. Ketoconazole is commonly used for the treatment of advanced Prostate cancer [1, 2, 3]. However, the adverse effects of Kcz on the male reproductive system in both humans and animals have been documented. Significantly, Kcz was reported to decrease the weight of male reproductive organs, especially the testes, and to reduce epididymal sperm concentration and serum testosterone levels Moreover, it was also reported to damage in rat testes induced by Kczintraperitoneal injection [4]. Interestingly, such pathological changes in Kcz-treated rat testes could be prevented by pretreatment and co-administration with an antioxidant plant extract [5].

Recently, there have been studies searching for non-toxic medicinal plants and one of the promising plants is the Panax ginseng which is a traditional herbal medicine that has been used therapeutically for more than 2000 years. It is the most valuable of all medicinal plants. it is a highly valued herb in the Far East and has gained a reputation in the West during the last decade. There is extensive literature on the beneficial effects of ginseng and its constituents [6]. The herb contains saponins or soap-like materials that have been named with various numbers and letters. Numerous studies focus on the research of individual ginsenosides instead of using whole ginseng extract against various diseases [7].

Several clinical studies indicated the beneficial effects of ginseng on male fertility in different animal models such as mice [8, 9]. In addition, human studies indicated a beneficial effect of ginseng on healthy human subjects [10]. Another important antioxidant material is Vitamin C it is a major water-soluble antioxidant in the plasma [11]. Vitamin C is an essential nutrient involved in the repair of tissue and the enzymatic production of certain neurotransmitters [12]. Indicate that vitamin C is an important independent antioxidant in protecting cells against death from oxidative stress [13]. Despite the benefit of Ketoconazole against fungus infection, it has many side effects. So the present study aimed to evaluate the protective role of ginseng and vitamin C against the toxicity induced by ketoconazole treatment in the gonad of laboratory male rat’s laboratory.
Materials and methods

Experimental animals

Forty adult, and sexually mature male of albino Wistar rats aged 12 weeks. The experiment executed 5 weeks. These rats were housed in the animal house of the Faculty of Pharmacy at the University of Kufa. Standard environmental conditions such as temperature (20-25°C) and relative humidity (45-55%), 12hrs dark/ light were maintained in the room. The study had approval from the Animal ethical Committee of Kufa University. Animals were properly fed with rodent pellet diet, and water was allowed freely ad libitum.

Doses preparation:
The doses were calculated according to [14]. All rats were treated once daily for five weeks using gastric gavage tube for kcz group. The animal was divided into four groups each of ten male as the following:-
1- Control group male rats treated with 0.5 ml of distal water orally.
2- Ketoconazole (kcz group) this group treated with 6 mg/kg of ketoconazole suspended in 0.5ml of distal water.
3- kcz- ginseng group these animals treated orally with 10 mg/kg ginseng mixed with 6mg/ kg of kcz in 0.5 ml of distal water.
4- kcz -vitamin group this group the animals were treated orally with 10mg/ kg of vitamin C mixed with 0.6mg of kcz in 0.5 ml of distal water. After four weeks of treatment, the following procedures were achieved.

Fertility performance of treated male

The treated male was kept in an individual cage with one untreated sexually mature female for 10 days and with contentious of the treatment of the male. at the end of the tenth day the fertility rate was calculated by counting the number of a pregnant female to a number of the total female in the group [15].

Collection of blood sample

At the last day of the fifth weeks, 5 ml of blood were collected from the heart directly, using disposable syringe and divided into the following: 4ml of blood, placed in gel tube, left to stand for 30 seconds, and then centrifuged 5000 rpm for 15, then the serum was collected and frozen at (-20 C°) and subsequently used for determination of oxidation parameter (MDA and SOD) and measurements of testosterone hormone.

Analysis of sperms

Determination of total sperms count

The caudal epididymis was cut to release all sperms in sperm collection vial containing 5ml PBS solution and the fluid in the vial was filtered through nylon mesh sieve. The resulting stock filtered was then diluted with formalized saline (0.1 ml of filtrate in .09 ml saline) and the number of sperms was determined with a hemocytometer. The measured sperm number was multiplied by the dilution factor to yield the total sperm count[16]. Then a drop of the solution on the slide to form smears then stained with Eosin Y and 500 sperms were evaluated for head and tail abnormalities and classified as normal or abnormal sperms.

Histological preparation.

At the end of the experiment, the animals were sacrificed after euthanized the right testes were removed to the process of sectioning protocol to form 5μ thickness slides and histological. 30 cross-sectional seminiferous tubule was examined under the light microscope for the quantitatively assessing the germ cell elements and the relationship of spermatogenesis to the sperm density. The average of no cells only Sertoli cell, only spermatogonia few spermatocytes many spermatocytes no spermatids many spermatocytes few spermatids many spermatids no spermatozoa many spermatids few spermatozoa disorganized spermatogenesis perfect seminiferous tubules were taken as final maturations score and the range of the score (8.9- 9.3) were consider as normal sections and below this range were considered as affected sections [17].

Hormonal assay

For hormonal assays special testosterone Elisa kit for rats (Cusabio Biotech co., ltd.) were used to testosterone assay ng/ml. Malondialdehyde (MDA) ELISA Kit (Elabscience biotech co.ltd) was used to determine the MDA concentration ng/m.

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Statistical analysis
All data were presented as Mean ± S.E and analyzed via SPSS version 25.000 (2018) software using one - way ANOVA with standard error the p≤ 0.05. The value level was considered significant in comparisons between the treatments.

Result
Male fertility performance
Result in figure (1) showed significant p≤0.05 reduction in the number of fertile males in the treated groups when compared with control group and the group treated with kcz-gin and kcz-vit. The result in this figure also showed there were no significant differences between these group and control groupor between them.

![Figure (1): Effect of kcz in fertility rate of male rats n=10](http://doi.org/10.36295/ASRO.2020.232122)

Hormonal assay.
The result in figure (2) showed a significant p≤0.05 decrease in testosterone level in the group treated with kcz drug compared with the control group and the groups treated with kcz-gin. And kcz-vit. Also, there was a considerable increasing p≤0.05 in testosterone levels in a kcz-gin group compare with the control group. While there were no major differences between this group and the kcz-vit group.

![Figure (2): Effect of kcz in testosterone level of male rat’s n=10](http://doi.org/10.36295/ASRO.2020.232122)

Enzyme concentration
The result in figure (3) appears no important reduction in MDA enzyme concentration in the treated group when compared with the control group. While kcz-gin. Showed significant p of ≤ 0.05 reduction in enzyme concentration when compared with control while no mentioned differences with other groups and kcz-gin and kcz-vit. Groups. While there were no significant differences in kcz-vit and control and kcz groups. Figure (4) showed no mentioned differences in SOD enzyme concentration.
between kcz group and control and the group treated with kcz-gin and kcz- vit. Although there was no significant increase in the kcz- gin group compared with the control group.

Figure (3): Effect of kcz in MDA concentration of male rat’s n=10

Figure (4): Effect of kcz in SOD concentration of male rat’s n=10

Sperm analysis
The result in figure (5) shows a significant p≤0.05 reduction in sperm count in the group treated with kcz compared control group while there was a significant improvement in the sperm count in the group treated with kcz-gin and kHz vit. Compared with the group treated with kcz drug. Also, there were no mentioned differences between these group and control group.
Figure (5): Effect of kczz in sperm count of male rat’s n=10

Sperm malformations

The result in figure (6) showed there a significant increase in the sperm malformation number in the group treated with kczz compared with a control group and the group treated with kczz-gin and kczz-vit also there were significant reduction in the sperm malformation number in these groups. The result in figure (7) reflect the comparison between histological examination score for 30 slide section for each treatment by studying following parameter the number of cells, only Sertoli cell-only spermatogonia, few spermatocytes, many spermatocytes no spermatids, many spermatocytes few spermatids, many spermatids no spermatozoa, many spermatids few spermatozoa, disorganized spermatogenesis, perfect seminiferous tubules. The study result of this parameter indicates that the section of the kczz group showed a low score value of 5.6 while the score value of control, kczz-gin, and kczz-vit, which are (9.7, 9.4, 9.5) respectively. Figures (8, 9) refer to the sections of control and treatment.

Figure (6): Effect of kczz in sperm in malformation count of male rat’s n=10
Figure (7): Effect of kcz in the testes sections of male rat’s n=10

Figure (8): shows normal spermatogenesis included the presence of sterol and primary and secondary spermatocyte with normal interstitial tissue in control testes 200x.

Figure (9): shows the protective effect of kcz- gin in the testes included the presence of spermatogenesis phases and the presence of many spermatides .200x.
Discussion.
Figure (1) showed the effect of kcz in reproductive performance which can be affected by various factors. It started from the hypothalamus-pituitary axis and its control on reproductive performance to the secretion of the hormone. Then the response of the target tissue which manages the sexual desire or behavior and odor of sexual receptivity in the animal there was more than one study to the effect of kcz on different sexual parameters. It appeared from the figure (2), there were significantly decreasing in testosterone level in the group treated with kcz the mechanism of this reduction may due different factors one of this factor is suggested by [20]. Who they suggest that ketoconazole can inhibit testosterone synthesis only inadequate concentration.

Since the action of kcz effect on fungi may occur from the drug's capability to restrain the conversion of lanosterol to ergosterol [21] or, it possible binding onto cytochrome P-450, which plays a major role in steroid metabolism [22]. The reduction of testosterone may have resulted from the direct effect of Kcz on Leydig cells. Kcz may inhibit testosterone secretion in rats partially [23] or, by reduced secretion of hormone in Leydig cells [24]. Figures (3, 4) showed a significant reduction in sperm count and an increase in sperm malformations because of the kcz toxicity and there was a protective role in the group kcz-gin and kcz-vit. It appears from this study that ginseng herb may have a protective role against the toxic effect of kcz several studies showed that ginseng Herb may act as an effective proposal to treat infertility. Ginseng has also used to recover sex performance and approval.

It has indicated that ginseng herb may have appositive effects on spermatogenesis, protein syntheses and DNA. Ginseng has appeared to improve sperm factors [22, 24, 25, 9]. The protective role of vitamin C also has been proved by several studies. The antioxidant activity of Vitamin C was indicated and has been shown that the vitamin may inhibit free-radical which induced damage to sensitive cell membranes of the testis [12]. The ascorbic acid is a known antioxidant present in the testis with the precise role of protecting the latter from the oxidative damage [26]. Vitamin C plays a role to support spermatogenesis at least in part through its ability to keep this antioxidant in an active state. Vitamin C is itself maintained in a reduced state by a GSH-dependent dehydroascorbatereductase, which is abundant in the testes. Vitamin C has been shown to improve sperm motility and enhances semen quality and fertility of treated rats [27].

Vitamins C able to reduced oxidative stress in the testes that improve both spermatogenesis and the production of testosterone [28]. Suggest that Vitamin C supplementation improves the stress-induced reproductive infertility due to both their testosterone increase effect and their antioxidant effect and has significant effect improvement in sperm count. The effect of kcz in oxidative enzyme the MDA and SOD does not show any significant effect although there was no considerable reduction in MDA in the group treated with kcz-gin while there was no significant increase in SOD enzyme.

The histological examination to the section depends on that all progeny of spermatogenesis are held together by a narrow cytoplasmic bridge and several methods have been proposed for quantitatively assessing for the germ cell elements and the relationship to of spermatogenesis to sperm density. The sections of treated kcz group showed highly affected tissue this appear from the score value that mentioned in methods which consider the value below 8.3 represents that sectioned were affected. Figures (8, 9) showed that the score of kcz group was 5.6 which indicate the effect of kcz in the sections while the protective role of ginseng herb and vitamin c reflected by the raising of the sections score to 9.4 for kcz-ginseng and 9.5 for kcz-vit. Which indicates the protective role of ginseng herb and vitamin C against the oxidative agent's treatment in laboratory rats [29, 30].

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