



A Study of Ginseng Effects on Spermatogenesis Multiplications (TDI, RI, SI) Proceeding Scrotal Heat Shock Induction in Rat

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Authors' contributions

This work was carried out in collaboration between all authors. Author HM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MN and ES managed the analyses of the study. Author HM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Objective: Ginseng is a plant which has high Antioxidant feature and contains Ginsenosides that has a high affectivity on testicle's tissue. This study is done to increase the survival of germ cells and also to find out its effects on decreasing the restoration time of damaged testicle tissues proceeding heat shock induction.

Methods and Materials: In this study 60 Vistar rats with the weight of 180-220 g are used which were placed in the same environmental conditions (12 hour light, 12 hour darkness – and temperature of 22±2) and were given the same food. Rats were randomly divided into four groups. Except for the group that was in 23c temperature condition and received the normal amount of Saline, other groups were each divided into three subgroups (ginseng, Vitamin E and 43c). Except for the normal group, all groups were kept for 60 days and went under autopsy in day

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15-30-60. Testicles were removed and put into Formalin 10%. After 48-72 hours Lam was made out of samples.

Conclusion: In this study effects of Ginseng on testicle tissues which went under heat shock were visible. And the difference in all groups was meaningful and Ginseng had a better function in the restoration of damaged tissues caused by heat than Vitamin E.

Keywords: Ginseng; Spermatogenesis multiplications (Tubular differentiation index (TDI); Spermiogenesis index (SI) and Repopulation index (RI); heat shock; vitamin E.

1. INTRODUCTION

It's known that if testicles receive heat even in a short process and only once, spermatogenesis may be damaged and restoration proceeding heat shock will be 40-60 days after the incident [1]. Of course, the nature of heat can be different for inducing damage, for example in temperature of 43c and duration of 15 minutes, the damage to testicle tissue will be limited and to the point of damaging spermatocytes. But in the 45c temperature and duration of 15 minutes generalized unlimited and vast damage which will include most of the germ tissues of the testicle will happen. [2] previous studies showed that heat will induce cell death in the testicle, in such a way that death in initial Mitotic spermatocytes and Mitotic spermatogons will be possible [3] and also temporal reduction of testicle weight with temporal or permanent infertility may be caused. Also the sperm quality is under the influence of heat shock, which causes a decrease in sperm movement and reduction of fertility in Oocytes. One of the best known functions of Eukaryotic tissues to environmental stresses like high temperature or being proximate with chemicals is related to HSP (Heat Shock Protein). TheP53 protein, which is produced by the generator genes of P53, is the most important Apoptoze inductor which can be found in generator cells of testicle's tissue in high quantity. This interaction is of two heat dependent proteins and happens exclusively in the inner scrotum temperature (32c). In high temperatures, this compound would not be formed. It means that in scrotal temperature HSP₁₀₅ and P53 would form a compound and in supra scrotal temperature this bound will break [4].

Ginseng is a plant special to East-Asia and North-America. For thousands of years, its root has been known as a mythological drug, and it is used for centuries to boost memory, reduce stress and raise life expectancy and to cure disability, inertia, and depression. Taking the sexual effects of Ginseng into account, we

started this research to determine whether Ginseng can treat the oxidative stress (heat shock) in the testicle or not and we used vitamin E in this study as an antioxidant factor, to compare Ginseng and vitamin E side by side.

2. METHODS AND MATERIALS

60 vistar rats with the weight of 180-220 were prepared. All rats were placed in a standard environment with 12 hours of light and 12 hours of darkness and the temperature of 22±2. Rats were randomly divided into 10 groups of 6. Then all rats were made unconscious by 12 mg Xylazine per kilo and 80 mg Ketamine per kilo [5] and then their scrotum and posterior movement limbs were immersed in Benmurry with the temperature of 43c. [6] Immediately after immersion and induction of heat shock in first, second and third group (n=6) normal saline into peritoneal was prescribed for 60 days and in day 15-30-60 animals went under autopsy and samples were taken. In forth, fifth and sixth group (n=6) Ginseng (Isfahan's Goldarou Pharmaceutical Factory) with the dose of 500mg per kilo [7] was prescribed for 60 days and in day 15-30-60 animals went under autopsy and samples were taken. In seventh, eighth and ninth group vitamin E with the dose of 100 mg was prescribed for 60 days and in day 15-30-60 animals went under autopsy and samples were taken. The tenth group was injected with normal Saline, with the difference that, the temperature for this group was 23c for 15 minutes. After removing the animals' heads the testicles were removed and put into Formalin 10% for 72 hours to be fixed and in the end with H&E coloring Spermatogenesis Multiplications were studied by Light microscope. Statistical studies of this research were done by Tukey and ANOVA software. The study was approved by the local ethic commission. To evaluate the spermatogenesis in seminal tubes, three index tubular differentiation index (TDI) spermiogenesis index (SI) and repopulation index (RI) were used.

To compute the tube differentiation term, abbreviated as TDI, the percentage of seminal tubes containing 3 and more than 3 differentiated spermatogenesis cells from the A spermatogonial cell were calculated. These cells were composed of intermediate spermatogonial, type B spermatogonia, spermatocyte and spermatid cells. This is the life index and the differentiation of stem cells of seminal tubes namely as Aspermatogonia.

To calculate TDI index for each testicle, at least 200 cross sections of seminal tubes were examined and counted. To calculate repopulation index (RI), the ratio of active spermatogonial cells to inactive spermatogonial cells in seminal tubes was calculated. To do this, more than 200 counts of seminal tubes were counted. To calculate spermiogenesis index (SI), the ratio of sperm semeners containing sperm to tubes without sperm was counted. In 200 sections, seminal tubes were counted.

3. RESULTS

3.1 Statistical Results

Maximum effects of heat shock and Ginseng treatment starts from day 30 and for this reason spermatogenesis multiplications in all groups were studied in day 30 and at the end of day 60 testicle's tissue automatically starts the restoration progress to some level. In this part, we study the items from day 30. Spermatogenesis multiplications of all groups in day 30 are shown in Table 1. TDI index on day 30 among ginseng groups and group 43 C had a meaningful difference ($p=0/000$) but between Ginseng group and vitamin E, there was no such a difference ($p=0/471$). RI and SI multiplication in day 30 among Ginseng groups and group 43 degree had a meaningful difference ($p=0/000$) and also SI multiplication among Ginseng groups and vitamin E there was a meaningful difference ($p=0/004$).

Table 1. Comparing groups according to spermatogenesis multiplications in day 30, multiplications unit %

Items groups	23C	43C	Ginseng	Vitamin E
TDI	74.3	*59.47	*86.72	85.1
RI	47.8	47.9*	*85.5	81
SI	74.7	54.1*	*80.5	*71

(TDI) the meaningful difference between ginseng group and 43C. $p<0.001$. also, RI and SI multiplication in day 30 among Ginseng groups and group 43 degree had a meaningful difference. $p<0/001$. SI meaningful difference between ginseng group and Vitamin E**

3.2 Histology Results

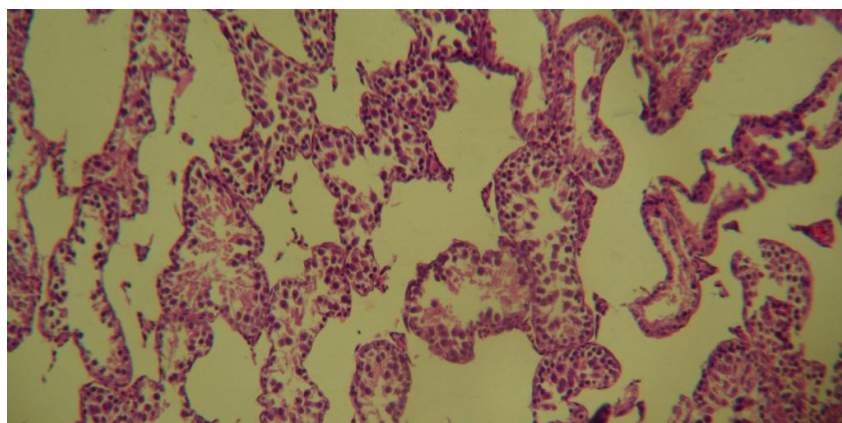


Fig. 1. Testicle's tissue group 43c in day 30: decrease in spermatogenesis cell levels has happened. Development of connective intermediate tissue is high. Magnification X100, coloring H&E

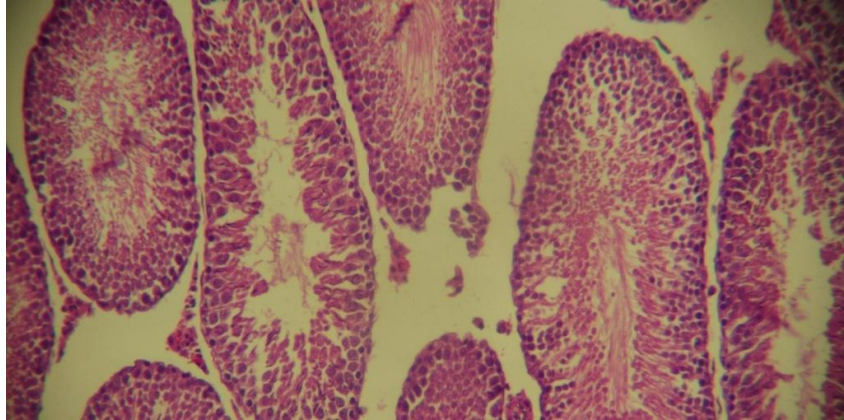


Fig. 2. Testicle's tissue of Ginseng group on day 30. Most pipes contain sperm and different levels of spermatogenesis cells are clearly visible in pipes walls. Magnification X100, coloring H&E

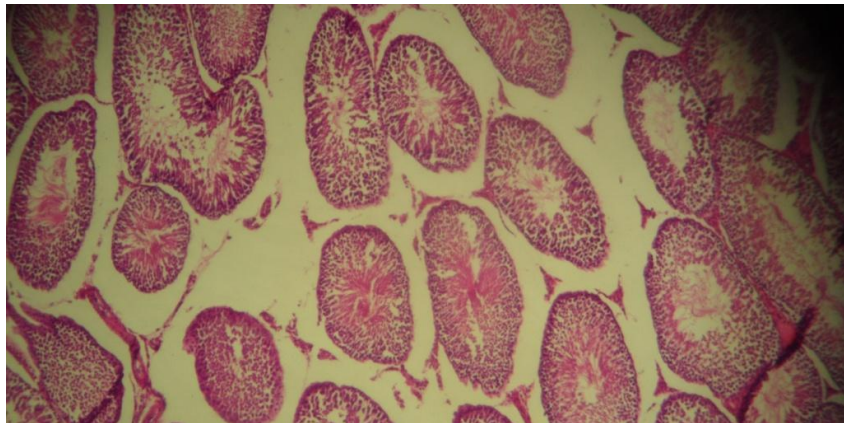


Fig. 3. Testicle's tissue of vitamin E group in day 30: spermatogenesis' generating cells are visible in pipes walls. In the central cavity of some pipes masses of sperm can be seen. Magnification X100, coloring H&E

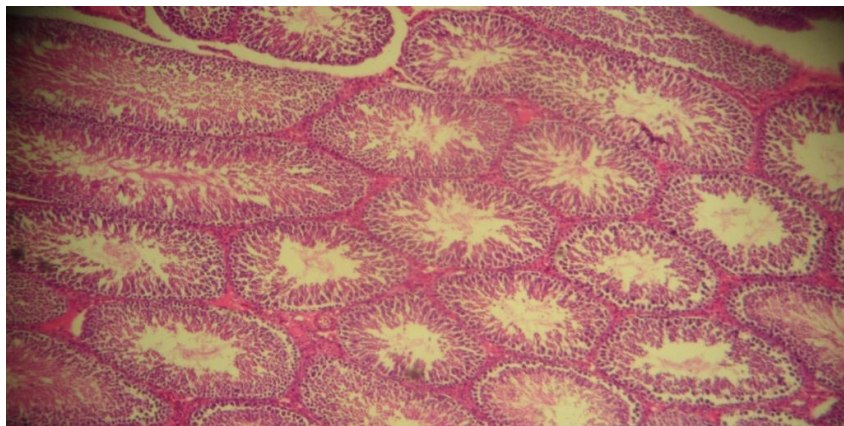


Fig. 4. Testicle's tissue of Ginseng group day 60: Pipes walls thickened completely, the intermediate tissue is low and diagonal of semen generator pipes is high and increase in a number of breeding cells is visible. Magnification X100, coloring H&E

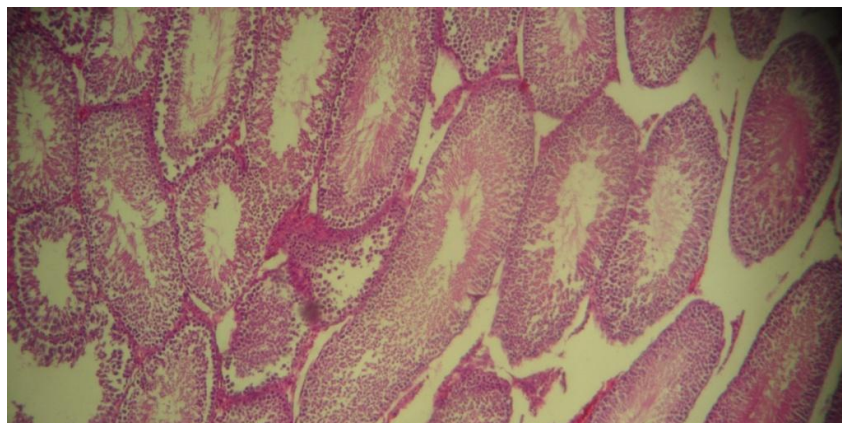


Fig. 5. Testicle's tissue of vitamin E group on day 60. There is a clear difference with Ginseng and Ginseng had a better performance in decreasing the thickness of intermediate tissue. Magnification X100, coloring H&E

4. DISCUSSION

Ginseng is a medicinal plant which is being used for centuries as an anti-stress, booster of sexual power, etc. it is also one of the oldest and well known medicinal plants which prevent sexual disorders. It has several medical, antioxidant features and protects the organs from free radicals and environmental stresses [8].

In this study, it became clear that heat shock affects the testicle's tissue and Ginseng can treat the effects of it at some point and recovers the testicle's tissue. In fact, ginseng acts as a phagocyte free radical and causes such changes. According to histological findings, the difference was significant in all cases, however; the RI index was not significant on day 30 in the comparison between vitamin E and ginseng groups, On day 30, the TDI index between ginseng and vitamin E was not significant. On day 15 The diameter of the conjugated capsule was not significant in both ginseng and vitamin E groups. There was no significant difference between the two groups of interstitial tissue gravity on day 30 in both vitamin E and control groups. Eventually epithelium diameter on day 60 was not significant between control and vitamin E groups. $p > 0/05$.

On the Contrary to the remarkable results in the histology section in which ginseng showed better performance than vitamin E, testosterone was superior to vitamin E compared to ginseng. The results also indicated that spermatogenesis indexes in the under treatment group with ginseng compared to the control group has reached to a desirable condition that confirms the

results of the ginseng study on thermal shock as an oxidative stress with investigations on ginseng and oxidative stress.

[9] Ginseng showed treating function on items and in most cases the difference between the groups was meaningful and the results of this study are the same as other studies on oxidative stresses. Vitamin E is an indicator of antioxidants. The Ginseng which is more effective than vitamin E can be the presence of ginsenoside beside antioxidant effects. Seock-Yeon and co-workers in 2010, studied improvement of function of testicle tissue preceding the usage of Ginseng which showed that testicle's tissue performance is increased compared to the old group [10]. Young-Hokim et al. in 2010 worked on Ginseng effects on testicle's Torsion and found out an increase in hormones level and restoration of tissue after Ginseng usage [11]. Ahmad A. Hendawy and co-workers did a research on antioxidant effects of Ginseng on testicle tissue under effect of chlorpyrifos and understood that usage of chlorpyrifos and Ginseng together will rise the testosterone level and the number of sperms comparing to usage of only chlorpyrifos [12] Several mechanisms of improving effects of Ginseng on function of under stress tissues has been introduced, which some will be mentioned below.

With improving the production of NO in Endothelium of lungs, heart, kidney, corpus cavernosum [13] Ginseng plays its antioxidant and protective role. Higher NO synthesis causes dilation of vessels and this might be the cause of boosting sexual instinct. Although many

researchers have reported that Ginseng causes disorder in erection [14,15]. Ginseng contains phenolic, polyacetylenes, alkaloids, polysaccharides compounds [16]. Discovering saponins as an active explosive shed light on medicinal features of Ginseng [17]. Raw part of saponins of Korean Red Ginseng (KRG), stops the production of NADPH super oxide in rats [18]. Polyacetylenes which is of Ginseng compound stops the growth of cancer cells [19]. Phenolic compounds comparing to saponins have more responsibility about antioxidant effect [20]. Ten phenolic acids including Frulike, Synamyk and Caffeic have been found in Ginseng. Maltul is an especial material in processed red Ginseng from white Ginseng which has a strong antioxidant activity [21]. Also Phenolic compounds like Maltul, Salicylic Acid and Anylyk acid which have a lower molecular weight than Ginsenosides Rb1, Re, Rg1 have stronger antioxidant effect [22]. Ginseng also increases the activity of Endogenous antioxidant enzymes like SOD, Catalase, Peroxidase and Glutathione peroxidase [23]. Up to now near 30 different types of Ginsenosides were found in Ginseng [24]. These compounds are categorized as Diols or Triols or according to structural features of Glycans. Diols or Triols count as majority of Ginsenosides and only Ginsenosides RO (from %0/6 Ginsenosides) is categorized as oleacane [25]. The most common Ginsenosides are diol-type, Rb1, Rb2, Rc and Rd. Rb1 and Rg1 are main and important materials in Ginseng. Zhang reported that Ginsenosides increase the sperm speed in zygosis [26]. The number and speed of sperms are affective in zygosis success [27]. Treatment by Ginseng prevents testicle disorders by reducing oxidize and NADPH super oxide production.

5. CONCLUSION

According to the studies Ginseng has a key role in the restoration of testicle's tissue and vitamin E showed less effectiveness compared to Ginseng. Based on this we can point to the Ginsenosides function in the restoration of testicle's tissue in addition to the antioxidant role, Whereas vitamin E is considered as an indicator of antioxidants, these changes can be explained with the existence of Ginsenosides in Ginseng and also anti oxidant role of Ginseng.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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