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Effect of Ginseng on Blood Lipid Profile, Testosterone Level and Epididymal Sperm Quality of Aged BALB/C Mice

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ABSTRACT

It is well known that aging in males leads to male infertility; therefore, the current study determines the impact of ginseng on blood lipid profile, testosterone level, and epididymal sperm quality in aged balb/c mice. Aged male mice (n=48) were assigned to three groups (n=16 in each) i.e., control (CON), high dose ginseng (HDG) and low dose ginseng (LDG). Mice were treated as CON (normal saline), HDG (500 mg/kg/day) and LDG (250 mg/kg/day) for 5 weeks. Daily feed intake, weekly weight, blood lipid profile, testosterone level, epididymal sperm quality, and testicular tissue were evaluated during and 5 weeks after the end of treatment. Results revealed that weight gain and feed intake decreased ($P< 0.05$) in mice treated with HDG and LDG than CON during and 5 weeks after the treatment. During the treatment, levels of testosterone, cholesterol, and low-density lipoproteins increased ($P< 0.05$) in mice treated with HDG and LDG than CON; however, cholesterol, triglyceride, and LDL-C were increased in CON than HDG and LDG treated mice after 5 weeks of treatment. Motility and viability (%) of spermatozoa increased ($P< 0.05$) in HDG and LDG treated mice than those of CON mice whereas sperm concentration was more ($P< 0.05$) in LDG than HDG and CON treated mice during the treatment. Five weeks after the treatment, motility and viability of spermatozoa were greater ($P< 0.05$) in HDG and LDG than CON treated mice. In conclusion, Ginseng treatment in aged BALB/c mice enhances the epididymal sperm quality, lipid profile and blood testosterone level during and even five weeks after the end of treatment period.

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Key words
Ginseng, Sperm quality, Lipid profile, Testosterone, BALB/c, *Panax ginseng*

INTRODUCTION

Male infertility is the least studied issue worldwide (Agarwal *et al*., 2015). There are about 48.5 million infertile couples worldwide (Mascarenhas *et al*., 2012) of which ~40% couples have male origin infertility (Smith *et al*., 2010; Medicine, 2013). In recent times, age-related infertility is commonly due to socioeconomic and societal reasons as couples wait until the age of 30-35 to begin their families. Using a rodent's model, researchers have documented that age-related changes occur in both male and female reproductive organs. In male rats, these changes include a decline in the number of germ cells, thinning of seminiferous tubular epithelium, testicular atrophy, sperm motility, and production.Furthermore, the incidences of gene mutations, sperm aneuploidy (Lowe *et al*., 1995; Walter *et al*., 1998), fertilization failures, and neonatal deaths (Serre and Robaire, 1998) are greater in aged male rats when mated to healthy young females.

Ginseng (*Panax ginseng)* is a medicinal plant having aphrodisiac and anti-aging properties (Hu, 1976). The pharmacological actions of ginseng are attributed to one of its ingredients ginsenosides. Red ginseng is known to improve age-related decline in learning and memory (Lee and Oh, 2015). In aged mice, it improves cognition through the regulation of cholinergic and antioxidant activity (Lee *et al*., 2017). The therapeutic use of ginseng is well documented in frogs, rabbits, rats, and chickens to promote the development of reproductive organs, spermatogenesis, ovulation, female receptivity, and egg-laying (Hong *et al*., 2002; Rahim, 2014). In addition to its aphrodisiac properties, ginseng is often used to cure diabetes, skin and hair problems, obesity, anxiety, and stress (Cooke, 2019).

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Several studies emphasize the beneficial effects of ginseng on male fertility in animal models such as mice (Yoshimura *et al*., 1998), rabbits (Kim *et al*., 1998), guinea pigs (Kim *et al*., 1999), and rats (Tsai *et al*., 2003). These effects of ginseng may not be due to changes in hormonal secretions, but directly effects or its ginsenoside components on the central nervous system and gonadal tissue (Hong *et al*., 2002; Murphy and LEE, 2002)

There has been no previous study reported in scientific literature to describe the effect of ginseng on blood lipid profile, testosterone level, and sperm quality analysis of aged BALB/c mice. Therefore, the objective of this study was to evaluate the effect of ginseng on blood lipid profile and semen quality in aged BALB/c mice strain.

MATERIALS AND METHODS

Experimental animals and location

The current research was conducted at mice facility of Theriogenology Department, University of Veterinary and Animal Sciences, Lahore. All the procedures performed in the study were approved by Animal Care and Ethical Review Committee of the university. For this study, aged male BALB/c mice (n=24; age: 6 months ± SEM 6 and weight: 40 g ± SEM) were kept under uniform and optimum environmental conditions i.e., 20-26˚C room temperature, and 10:14 h of light and dark cycle. All mice were offered broiler starter ration (National Feeds, Pvt. Ltd., Pakistan) containing 23% crude protein, <5% crude fiber, 6.9% crude fat, and 3000 Kcal/Kg of metabolizable energy. All mice had free access to water *ad libitum*.

Experimental design, testicualar histology, semen processing, and blood sampling

In this study, aged mice (n=48) were randomly subjected to a high dose of ginseng (HDG; 500mg/kg/day; n=16), a low dose of ginseng (LDG, 250mg/kg/day, n=16), and normal saline (Control, n=16) for 5 weeks. Both ginseng (Vitamin Company, Lahore, Pakistan) and normal saline were administered in a total of 0.2 ml volume through gastric intubation. Weekly bodyweight, feed intake and sexual behaviors of mice were recorded through out the study. Lipid profile and plasma testosterone concentration were analyzed by obtaining blood from heart after killing mice at the end of 5-weeks treatment and then 5-weeks later (n=8 mice per group at each time point). Similarly, both testes were recovered for histological examination after secluding caudal epididymides for semen evaluation.

For histological analyses, testes were fixed (10% formal saline for 24 h), dehydreated in series of alocohol baths, paraffinized into wax blocks. 5 µm thick sections were cut and stained by hematoxylin and eosin method (Bancroft and Stevens, 1996) for examination under the light microscope

For semen analyses, caudal epididymides were sliced open in HTF media containing 4 mg/ml of BSA and incubated in a CO_2 incubator (5% CO_2 in air at 37ºC) for 10 min, allowing sperm to swim into the medium. To determine the sperm concentration, semen was diluted 100 times with PBS and 10 µl of semen was used to charge the haemocytometer. Heads of the sperms were counted at 400X magnification under a light microscope. To evaluate the sperm motility (%), 5µl of diluted semen was aliquoted on prewarmed glass slide, covered with a coverslip and observed at 400X magnification unser the phase contrast microcope with a heated stage (37°C). At least 3-5 various fiels were observed to estimate the percent motiliy of each sample. To evaluate viability of spermatozoa, 20 µl of the diluted semen was mixed with 0.05% eosin and smeared on glass slide. After air drying the smear at room temperature, at least 200 live (unstained) or dead (stained as pink) spermatozoa were counted under the light microscope at 400X maginification.

Statistical analysis

Effect of ginseng on weekly weight, daily feed intake, serum testosterone, lipid profile and semen parameters were analyzed by one way ANOVA using IBM Statistical Package for Social Sciences (SPSS Version 20). The comparisons among treatments and after withdrawal period were analyzed by paired t-test. A probability level $P \leq 0.05$ was considered as significant.

RESULTS

The data of the effect of ginseng treatment on daily feed intake, weekly weight gain, testosterone, cholesterol, triglyceride, high density lipoprotein (HDL) and low density lipoprotein (LDL) concentrations in aged male BALB/c mice during treatment has been depicted in Table I. Results reveal that plasma concentration of testosterone was greater ($P< 0.05$) in HDG group as compared LDG and CON. However, cholesterol concentration was was greater ($P< 0.05$) in LDG group as compared to LDG and CON.

The effect of ginseng treatment on daily feed intake, weekly weight gain, testosterone, cholesterol, triglyceride, HDL-C and LDL-C concentrations in aged male BALB/c mice 5 weeks after end of treatment period has been presented in Table I. Results reveal that plasma concentration of testosterone was greater ($P< 0.05$) in HDG group as compared LDG and CON. In contrast, Cholestrol concentration was greater ($P< 0.05$) in CON group as compared to HDG and LDG.

Table I. Effect of ginseng supplementation on weight, feed intake, blood lipid profile and testosterone during 5 weeks and after 5 weeks treatment period.

Variables	Control (n=8)	Low dose (n=8)	High dose (n=8)
During 5 weeks treatment			
Feed intake* (g)	23.35± 0.43^{b}	22.13 ± 0.36^{a}	21.78 ± 0.37^{a}
Weight* (g)	40.18 ± 1.64^{b}	29.36 ± 0.7^{a}	30.21 ± 0.77^{a}
Testosterone (ng/dl)	4.98±0.71^{c}	9.55±0.35^{b}	12.99±1.64^{a}
Cholesterol (mg/dl)	149.75±6.17^{ab}	164±4.63^{b}	135.75±8.52^{a}
Triglyceride (mg/dl)	147.75±6.43^{bc}	155±8.93^{b}	116.75±10.42^{a}
HDL-C[1] (mg/dl)	19±0.91^{ac}	13.31±1.26^{a}	15.93±0.88^{ac}
LDL-C[2] (mg/dl)	93.75±7.04^{c}	118.75±7.46^{ad}	116.75±5.76^{ab}
After 5 weeks treatment			
Feed intake (g)	22.40±0.38^{b}	12.97 ± 0.26^{a}	12.76±0.91^{a}
Weight (g)	41.0 ± 1.31^{b}	34.69 ± 0.81^{a}	35.65±0.93^{a}
Testosterone (ng/dl)	4.36 ± 0.81^{c}	29.88 ± 1.50^{b}	116.25±1.49^{a}
Cholesterol (mg/dl)	175 ± 2.34^{c}	134.5 ± 1.32^{b}	89.5±2.21^{a}
Triglyceride (mg/dl)	210 ± 9.30^{c}	68.5 ± 1.70^{b}	116.5±2.10^{a}
HDL-C[3] (mg/dl)	60.25±3.88^{c}	83±4.60^{b}	37.31±0.64^{a}
LDL-C[4] (mg/dl)	125±6.06^{c}	80.93±3.10^{b}	58.75±2.56^{a}

[a,b,c] Values in the same rows with different superscripts differ significantly (P<0.05). Values are mean ± SEM. No treatment group (CON) representing control group. [3]HDL-C: high density lipid cholesterol. [4]LDL-C: low density lipid cholesterol.

Table II. Effect of ginseng supplementation on semen parameters and testis weight during and after the 5 weeks treatment period.

Parameter	Control (n=8)	Low dose (n=8)	High dose (n=8)
During 5 weeks treatment			
Sperm motility (%)	57.73±3.37^{b}	70.2±2.13^{a}	69.8±1.39^{a}
Viability (%)	47.58±2.3^{b}	59.1±2.13^{a}	58.2±2.14^{a}
Concentration (×10$^{6/ml}$)	14.7±3.40^{c}	15.6±3.40^{b}	14.24±2.24^{a}
Testes weight (g)	0.62±0.14^{ab}	0.61±0.12^{b}	0.64±0.17^{a}
After 5 weeks of treatment			
Sperm motility (%)	64.2±3.37^{b}	77.8±3.53^{a}	79.8±3.29^{a}
Viability (%)	44.92±23^{b}	55.1±1.45^{a}	56.2±1.14^{a}
Concentration (×10$^{6/ml}$)	13.7±2.40^{c}	15.6±2.40^{b}	18.4±2.24^{a}
Testes weight (g)	0.59±0.16^{ab}	0.56±0.18^{b}	0.60±0.13^{a}

[a, b, c] Values in the same rows with different superscripts differ significantly (P<0.05). Values are mean ± SEM.

The effect of ginseng treatment on testes weight, sperm motility percentage, viability and sperm concentration in aged male BALB/c mice during treatment has been illustrated in Table II. Results reveal that sperm motility and viability was greater (P< 0.05) in HDG and LDG groups as compared to CON.

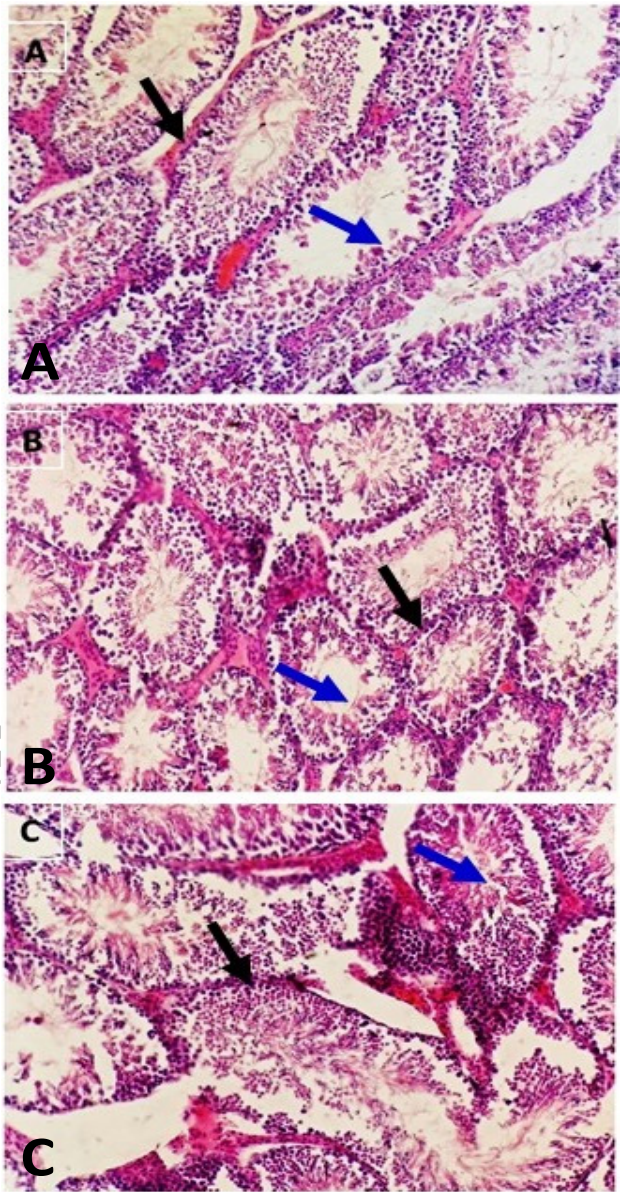

Fig. 1. The histological structure of testies of BALB/c mice. It representing that histologically there is no difference among groups. (A) representng high dose group testis. (B) demonstarting the low dose group testies. (C) Depicting the control group testies. Black arrow showing seminiferous tubules and blue arrow is showing germinal epithelial cell layer.

The effect of ginseng treatment on testes weight, sperm motility percentage, viability, and sperm concentration in aged male BALB/c mice 5 weeks after the end of treatment period has been shown in Table II. Results reveal that sperm motility and viability was greater (P<0.05) in HDG group as compared both of other

groups LDG and CON. The effect of ginseng on testes histological examination has been depicted in Figure 1. The normal appearance of the testicular structure of mice was determined by evaluation of group 1(CON) (Fig. 1C), which had a normal arrangement of germinative cells in the seminiferous tubules. The appearance of group 2 and 3 (Fig. 1) was similar to that of group 1. The germinal epithelial layer appeared nearly normal. In figure black arrow showing seminiferous tubules and blue arrow is showing germinal epithelial cell layer.

DISCUSSION

In the present study, the administration of ginseng for 5 weeks in BALB/c mice decreased daily feed intake, weekly weight gain, serum triglycerides (LDL) whereas increased serum testosterone and HDL levels (Jorge *et al*., 1998; Lauridsen and Mortensen, 1999). The reduction in triglyceride, LDL-C, and cholesterol might be due to hypolipidemia caused by ginseng administration (Prasad, 1999). Besides, the ginseng due to its antioxidative potential may have decreased cholesterol and LDL-C. Several mechnisms have been described in previous reports for the decline in cholesterol after ginseng treatment. These include extraction of cholesterol through bile acid formation, and accelerated degradation of cholesterol through feces. It seems that all mechanisms of action of ginseng might be involved in the present study. Furthermore, the results of our study are under different studies conducted by Kim *et al*. (2017) who concluded that long term administration of ginseng in obese women resulted in a significant reduction of cholesterol, LDL-C, and enhanced production of HDL-C. However, the association between ginseng antioxidant property and lipid metabolism were not discussed in previous studies. Controversy still exists in the current study that HDL-C production was enhanced in the low dose treatment group as compared to the control group which supported the previous studies, but it was reduced in high dose group, which is an exception in our study. This discrepancy in results may be attributed to different pre-body weight, dosage variation associated with body weight, and type of ginseng used.

The findings of the current study also revealed that ginseng also had a profound positive effect on serum testosterone, libido, resulting in enhanced plugs formations. The active ingredient in ginseng is ginsenoside which is triterpenoid saponin and structurally resembles with steroid hormones. So, this constituent of ginseng may be associated with the enhanced sexual function, reproductive behavior by activating the steroid receptors (androgen receptors) which are abundantly present in the male reproductive tract, genital organs, and spermatozoa (Solakidi *et al*., 2005). Similar observations regarding the effects of ginseng have been described by other researchers who explained that ginseng acts on steroid receptors and enhances the production of testosterone and ultimately the libido Matsumoto (Travison *et al*., 2006). Moreover, behavioral studies have suggested that ginseng effects on the central nervous system (Bhattacharya and Mitra, 1991; Watanabe *et al*., 1991; Benishin, 1992). It is further suggested that ginseng enhances the spontaneous motor activity which inturns inhibit daily feed intake (Yoshimatsu *et al*., 1993). For this reason, a decline in the weekly weight gain of mice treated with ginseng was observed in the present study (Aitken and Clarkson, 1987).

In this study semen analysis revealed greater testicular damage in aged mice but that was recovered by ginseng administration. Moreover, improvements in sperm motility, viability, and concentration in response to ginseng administration have been described by previous researchers testing the efficacy of ginseng in fertile and asthenozoospermic men. The success rate of fertilization is directly related to number of motile spermatozoa (Gist *et al*., 2000). Activities of Na^+-K^+-ATPase is highly sensitive to ROS which cause depletion of Na^+-K^+-ATPase and results in loss of motility of spermatozoa. Consequently, the observed protective influence of ginseng against age-related oxidative damage potentially contributes to the restoration of reproductive hormone production.

Additionally, ginseng demonstrated an enhancement in sperm production through its stimulatory effect on testosterone synthesis and the complete mechanisim for antoxident activity of ginseng has been previously described by (Ku *et al*., 2020). Another plausible mechanism underlying the beneficial effects of ginseng pertains to its conjectured association with the activation of testicular angiogenesis. Ginseng notably elevated the expression levels of two angiogenic factors, VEGF and visfatin. This observation aligns with the understanding that angiogenesis plays a role in the context of spermatogenesis and spermiogenesis, potentially contributing to ginseng's observed effects on reproductive health (LeCouter and Ferrara, 2002). The effect of ginseng on antioxidation and sexual hormone production significantly contributed to increased sperm production and motility by fostering spermatogenesis (Kilinç *et al*., 2004). The role of ginseng in minimizing ROS damage to sperm as evident from current study is similar to Chen *et al*. (2001) who reported that ginseng acts as free radical scavengers (Yun *et al*., 2016) and improve reproductive hormone receptors expression which are declined by production of ROS (Kim *et al*., 2017) and avoid further damage to spermatozoa. Some of ginsenosides (Rg3) are well documented possess the many

biological properties (Lee *et al*., 2019). Secondly, decrease in cholesterol and LDL-C which may be attributed towards antioxidative potential of ginseng and thirdly, the reduction in daily feed intake and hence, weekly weight gain possibly caused by the effect of ginseng on central nervous system in enhancing spontaneous motor activity.

It is concluded from the results of current study that treatment with ginseng for 5 weeks improved sperm parameters in aged BALB/c mice, enhances the male fertility during treatment period and even five weeks after the end of treatment period and concluded that ginseng acts as a potent antioxidant in the protection of mice sperm cells against oxidative stress and may be useful to treat male infertility. However, further studies may be planned in the future to study integrating diverse methodologies, including molecular biology, cellular physiology, and pharmacology, will be instrumental in unraveling the intricate mechanisms behind ginseng's effects on male reproductive health. Such insights hold promise not only for understanding ginseng's role but also for potential therapeutic applications in addressing fertility issues.

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IRB approval

IRB approval was granted by the Ethical Review Committee of University of Veterinary and Animal Sciences, Lahore, with Approval No. DR/1320 dated 21/12/2017.

Ethical statement

This study was approved by the university of veterinary and animal Sciences Lahore, local ethical review Committee (Approval No. DR/1320).

Statement of conflict of interest

The author declares that he has no conflict of interests.

REFERENCES

Agarwal, A., Mulgund, A., Hamada, A. and Chyatte, M.R., 2015. A unique view on male infertility around the globe. *Reprod. Biol. Endocrinol*., **13**: 37. https://doi.org/10.1186/s12958-015-0032-1

Aitken, R.J. and Clarkson, J.S., 1987. Cellular basis of defective sperm function and its association with the genesis of reactive oxygen species by human spermatozoa. *Reproduction*, **81**: 459-469. https://doi.org/10.1530/jrf.0.0810459

Bancroft, J. and Stevens, A., 1996. *Wiley-liss. Theory and practice of histological techniques*, 4th ed. Churchill Livingstone, New York/London/San Francisco/Tokyo.

Benishin, C.G., 1992. Actions of ginsenoside Rb1 on choline uptake in central cholinergic nerve endings. *Neurochem. Int*., **21**: 1-5. https://doi.org/10.1016/0197-0186(92)90061-U

Bhattacharya, S. and Mitra, S., 1991. Anxiolytic activity of *Panax ginseng* roots: An experimental study. *J. Ethnopharmacol*., **34**: 87-92. https://doi.org/10.1016/0378-8741(91)90193-H

Chen, J.-C., Chen, L.-D., Tsauer, W., Tsai, C.C., Chen, B.-C. and Chen, Y.-J., 2001. Effects of Ginsenoside Rb2 and Rc on inferior human sperm motility in vitro. *Am. J. Chinese Med*., **29**: 155-160.

Cooke, J., 2019. *Also known as Siberian ginseng, this herb is specific for reducing the negative effects of stress, and improving athletic performance. It was popularized in the 1970's by* May 8, 2019 endocrine, immune. endocrine.

Gist, D., Turner, T. and Congdon, J., 2000. Chemical and thermal effects on the viability and motility of spermatozoa from the turtle epididymis. *J. Reprod. Fertil*., **119**: 271-278. https://doi.org/10.1530/reprod/119.2.271

Hong, B., Ji, Y.H., Hong, J.H., Nam, K.Y. and Ahn, T.Y., 2002. A double-blind crossover study evaluating the efficacy of Korean red ginseng in patients with erectile dysfunction: A preliminary report. *J. Urol*., **168**: 2070-2073. https://doi.org/10.1016/S0022-5347(05)64298-X

Hu, S.Y., 1976. The genus Panax (ginseng) in Chinese medicine. *Econ. Bot*., **30**: 11-28. https://doi.org/10.1007/BF02866780

Jorge, P.A.R., Neyra, L.C., Ozaki, R.M. and de Almeida, E., 1998. Improvement in the endothelium-dependent relaxation in hypercholesterolemic rabbits treated with vitamin E. *Atherosclerosis*, **140**: 333-339. https://doi.org/10.1016/S0021-9150(98)00137-3

Kilinç, F., Kayaselcuk, F., Aygun, C., Guvel, S., Egilmez, T. and Ozkardes, H., 2004. Experimental varicocele induces hypoxia inducible factor-1α, vascular endothelial growth factor expression and angiogenesis in the rat testis. *J. Urol*., **172**: 1188-1191. https://doi.org/10.1097/01.

ju.0000135455.97627.15

Kim, H., Woo, D., Lee, G. and Kim, J., 1998. The relaxation effects of ginseng saponin in rabbit corporal smooth muscle: Is it a nitric oxide donor? *Br. J. Urol*., **82**: 744-748. https://doi.org/10.1046/j.1464-410x.1998.00811.x

Kim, M.K., Cha, K.M., Hwang, S.Y., Park, U.K., Seo, S.K., Lee, S.H., Jeong, M.S., Cho, S., Kopalli, S.R., Kim, S.K., 2017. Pectinase-treated *Panax ginseng* protects heat stress-induced testicular damage in rats. *Reproduction*, **153**: 737-747. https://doi.org/10.1530/REP-16-0560

Kim, W., Hwang, S., Lee, H., Song, H. and Kim, S., 1999. *Panax ginseng* protects the testis against 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin induced testicular damage in guinea pigs. *BJU Int*., **83**: 842-849. https://doi.org/10.1046/j.1464-410x.1999.00046.x

Ku, J.Y., Park, M.J., Park, H.J., Park, N.C. and Joo, B.S., 2020. Combination of Korean red ginseng extract and hydrogen-rich water improves spermatogenesis and sperm motility in male mice. *Chin. J. Integr. Med*., **26**: 361-369. https://doi.org/10.1007/s11655-019-3047-1

Lauridsen, S.T. and Mortensen, A., 1999. Probucol selectively increases oxidation of atherogenic lipoproteins in cholesterol-fed mice and in Watanabe heritable hyperlipidemic rabbits. *Atherosclerosis*, **142**: 169-178. https://doi.org/10.1016/S0021-9150(98)00234-2

LeCouter, J. and Ferrara, N., 2002. EG-VEGF and the concept of tissue-specific angiogenic growth factors. *Semin. Cell Dev. Biol*., (Elsevier), **13**: 3-8. https://doi.org/10.1006/scdb.2001.0284

Lee, M.R., Ma, J.Y. and Sung, C.K., 2017. Chronic dietary ginseng extract administration ameliorates antioxidant and cholinergic systems in the brains of aged mice. *J. Ginseng Res*., **41**: 615-619. https://doi.org/10.1016/j.jgr.2017.06.002

Lee, S.H., Choi, K.H., Cha, K.M., Hwang, S.Y., Park, U.K., Jeong, M.S., Hong, J.Y., Han, C.K., In, G. and Kopalli, S.R., 2019. Protective effects of Korean Red ginseng against sub-acute immobilization stress-induced testicular damage in experimental rats. *J. Ginseng Res*., **43**: 125-134. https://doi.org/10.1016/j.jgr.2017.09.002

Lee, Y. and Oh, S., 2015. Administration of red ginseng ameliorates memory decline in aged mice. *J. Ginseng Res*., **39**: 250-256. https://doi.org/10.1016/j.jgr.2015.01.003

Lowe X, Collins B, Allen J, Titenko-Holland N, Breneman J, Van Beek M, Bishop J, Wyrobek A. 1995. Aneuploidies and micronuclei in the germ cells of male mice of advanced age. *Mutat. Res. DNAging*, **338**: 59-76. https://doi.org/10.1016/0921-8734(95)00012-U

Mascarenhas, M.N., Flaxman, S.R., Boerma, T., Vanderpoel, S. and Stevens, G.A., 2012. National, regional, and global trends in infertility prevalence since 1990: A systematic analysis of 277 health surveys. *PLoS Med*., **9**: e1001356. https://doi.org/10.1371/journal.pmed.1001356

Medicine ECotASfR. 2013. Fertility preservation and reproduction in patients facing gonadotoxic therapies: A committee opinion. *Fertil. Steril*., **100**: 1224-1231. https://doi.org/10.1016/j.fertnstert.2013.08.041

Murphy, L.L. and Lee, T.J.F., 2002. Ginseng, sex behavior, and nitric oxide. *Ann. N.Y. Acad. Sci*., **962**: 372-377. https://doi.org/10.1111/j.1749-6632.2002.tb04081.x

Prasad, K., 1999. Reduction of serum cholesterol and hypercholesterolemic atherosclerosis in rabbits by secoisolariciresinol diglucoside isolated from flaxseed. *Circulation*, **99**: 1355-1362. https://doi.org/10.1161/01.CIR.99.10.1355

Rahim, S.A., 2014. Protective effect of Panax ginseng on Flutamide-induced spermatogenesis impairment in adult rats. *J. Univ. Babylon*, **22**: 2423-2410.

Serre, V. and Robaire, B., 1998. Paternal age affects fertility and progeny outcome in the Brown Norway rat. *Fertil. Steril*., **70**: 625-631. https://doi.org/10.1016/S0015-0282(98)00259-3

Smith, J.F., Eisenberg, M.L., Millstein, S.G., Nachtigall, R.D., Shindel, A.W., Wing, H., Cedars, M., Pasch, L., Katz, P.P. and Group IOPP. 2010. The use of complementary and alternative fertility treatment in couples seeking fertility care: Data from a prospective cohort in the United States. *Fertil. Steril*., **93**: 2169-2174. https://doi.org/10.1016/j.fertnstert.2010.02.054

Solakidi, S., Psarra, A.G., Nikolaropoulos, S. and Sekeris, C.E., 2005. Estrogen receptors α and β (ERα and ERβ) and androgen receptor (AR) in human sperm: Localization of ERβ and AR in mitochondria of the midpiece. *Hum. Reprod*., **20**: 3481-3487. https://doi.org/10.1093/humrep/dei267

Travison, T.G., Morley, J.E., Araujo, A.B., O'Donnell, A.B. and McKinlay, J.B., 2006. The relationship between libido and testosterone levels in aging men. *J. Clin. Endocr. Metab.,* **91**: 2509-2513.

Tsai, S.C., Chiao, Y.C., Lu, C.C. and Wang, P.S., 2003. Stimulation of the secretion of luteinizing hormone by ginsenoside-Rb1 in male rats. *Chin. J. Physiol*., **46**: 1-8.

Walter, C.A., Intano, G.W., McCarrey, J.R., McMahan, C.A. and Walter, R.B., 1998. Mutation frequency declines during spermatogenesis in young mice but increases in old mice. *Proc. natl. Acad. Sci*., 95: 10015-10019. https://doi.org/10.1073/pnas.95.17.10015

Watanabe, H., Ohta, H., Imamura, L., Asakura, W., Matoba, Y. and Matsumoto, K., 1991. Effect of Panax ginseng on age-related changes in the spontaneous motor activity and dopaminergic nervous system in the rat. *Jpn. J. Pharmacol*., **55**: 51-56. https://doi.org/10.1016/S0021-5198(19)39977-9

Yoshimatsu, H., Sakata, T., Machidori, H., Fujimoto, K., Yamatodani, A. and Wada, H., 1993. Ginsenoside Rg1 prevents histaminergic modulation of rat adaptive behavior from elevation of ambient temperature. *Physiol. Behav*., **53**: 1-4. https://doi.org/10.1016/0031-9384(93)90002-W

Yoshimura, H., Kimura, N. and Sugiura, K., 1998. Preventive effects of various ginseng saponins on the development of copulatory disorder induced by prolonged individual housing in male mice. *Methods Find. Exp. Clin. Pharmacol*., **20**: 59-64. https://doi.org/10.1358/mf.1998.20.1.485633

Yun, S.J., Bae, G.S., Park, J.H., Song, T.H., Choi, A., Ryu, B.Y., Pang, M.G., Kim, E.J., Yoon, M., Chang, M.B., 2016. Antioxidant effects of cultured wild ginseng root extracts on the male reproductive function of boars and guinea pigs. *Anim. Reprod. Sci*., **170**: 51-60. https://doi.org/10.1016/j.anireprosci.2016.04.002