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EFFECT OF *PANAX GINSENG* ROOT POWDER ON FERTILITY AND ANTIOXIDANT ENZYMES IN MALE RABBITS

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ABSTRACT

To investigate the effects of *Panax ginseng* root powder on reproductive functions and antioxidant enzymes, Glutathione-S Transferase (GST), Catalase (CAT) activities in normal adult male rabbits. Powder suspension of Panax ginseng root were administered orally to two groups of male rabbits at 150mg/kg b.w. or 300mg/kg b.w. A third group served as control and received the treatment vehicle, distilled water. Treatment lasted for 15 days before sacrifice. Serum testosterone sperm counts, plasma GST, CAT activities and testicular sections were determined. The administration caused a non-significant increase ($P<0.05$) in the serum testosterone. There was a statistically significant increase in the sperm count in rabbits which admnistered with *Panax ginseng* root powder at dose 150mg/kg or 300mg/kg when compared with control. There was also a significant increase ($P<0.05$) in plasma GST and CAT activities. The histological findings in animals treated with *Panax ginseng* at dose 150mg/kg or 300mg/kg showed an improvement of the cellular activity and spermatogenesis in the testicular sections. Our results indicated that *Panax ginseng* root powder possesses pro-fertility properties in male rabbits which might be a product of both its potent antioxidant properties and androgenic activities.

KEY WORDS: *Panax ginseng*, testosterone, antioxidant enzymes, sperm count, testicular sections, rabbits.

INTRODUCTION

As per estimation of World Health Organization that 80% of people worldwide rely on herbal medicines for some part of their primary health care.[1] Herbal medicines had become an integral part of life in many communities and considered as a promising avenue for the discovery of new drugs due to its easy access and relatively low cost.[2] Two major species of Ginseng are *Panax ginseng* C.A. Meyer (Asian ginseng), and *Panax quinquefolius* L. (NorthAmerican ginseng). Both species contain active saponin glycosides, such as ginsenoside and panaxoside, but have significant differences in their identity and distribution. *Panax quinquefolius* plants grow in rich woods throughout eastern and central North America, especially along the mountains from Quebec and Ontario to Georgia. *Panax ginseng* plants are slightly larger, native of Korea and China and cultivated in Korea, Japan, China, and Russia. Other less frequently encountered species include Panax notoginseng and Panax japonicas.[3] In the Asian world, Ginseng (*Panax ginseng* C.A. Meyer, family Araliaceae) root has been used for centuries as a traditional herb to enhance physical strength and resistance[4] and enhancing body strength, recovering physical balance, and stimulating metabolic function.[5] When it is steamed, it is called Red Ginseng (RG).[6] And it used for thousands of years in, particularly in China, Korea and Japan, for its wide spectrum of medicinal effects such as tonic, immunomodulatory, adaptogenic, and anti-aging activities.[7,8] Many of its medicinal effects are attributed to the triterpene glycosides known as ginsenosides. the best known Asian species is *Panax ginseng* whereas the American species belongs to *Panax quinquefolius*. Both species are widely used in Asian countries for a variety of purposes. Chemical analysis of ginseng revealed the presence of many ingredients, including organic acids, vitamins, sugars, inorganic salts, sterols, oligopeptides, polysaccharides, volatile oils, and saponins. Of these, the saponins (commonly known as ginsenosides) are well studied for their biological properties.[9] ginsenosides such as Rg3, Rb1, Rb2, and Rc as main constituents.[10]

Infertility is one of the problems of human society. According to the World Health Organization (WHO) , 15-10 percent of couples have experienced some forms of infertility problems which 40% of these problems are due to male factor.[11,12] Reproductive ability in the male contain the production of semen containing normal spermatozoa (quality) in the adequate number (quantity), together with the desire and ability to mate.[13] Different reasons are involved in the occurrence of male infertility such as genetic disorders, genital duct obstruction, varicocele, decreased sperm production, decreased semen quality parameters, erectile dysfunction and male

impotence.[11] Ginseng enhanced mounting behaviour of male rats[14,15] and increased increased the serum testosterone level and inhibited prostate weight in male rats.[16]

This study was aimed to investigate the effect of *Panax ginseng* root powder on the fertility and antioxidant enzymes, Glutathione-S Transferase (GST) and Catalase (CAT) activities given orally in normal adult male rabbits.

MATERIALS AND METHODS

Experimental Animals: A total of 15 local domestic adult male rabbits weighing 2.00 -2.32 kg were used in the present study. The animals were grouped and housed in cages (100 *x* 85*x* 45 cm) at the laboratories of the zoology department, Omar AL- Mukhtar University. The photoperiod was regulated at 12 hours light / 12 hours dark cycle and temperature was adjusted at 25±1˚C. The rabbits were fed on commercial standard pellet and offered drink water *ad libitum.* The animals were acclimatized to laboratory conditions for one week before commencement of the experiment.

Plant Material and Preparation: The dry root of *Panax ginseng*, were obtained from a local herbal market. The root were identified and authenticated by the Herbarium of Botany Department, Faculty of Science, Omar AL-Mukhtar University. The root were powdered using a commercial blender. Known weight of their powders was used as suspension in constant distilled water volume.

Experimental groups and protocol

Rabbits were randomly distributed into three groups (five rabbits/group).

- Group I: control (G1) normal control rabbits were given distilled water orally daily.
- Group II: rabbits were given 150mg /kg b.wt. *Panax ginseng*[17] in 2 ml distilled water orally daily.
- Group III: rabbits were given 300mg /kg b.wt. *Panax ginseng*[17] in 2 ml distilled water orally daily.

All groups of rabbits were experimented for 15 days.

Blood, semen fluid and organ sampling: At the end of the experimental period (15 days), overnight fasting rabbits were deprived of food but allowed for free access of drinking water. Animals were sacrificed by decapitation and the shed blood was collected in two cleaned vials, one was without anticoagulant and used for serum separation. The serum was analyzed to determine the testosterone. The second vial (with EDTA) for plasma separation. The plasma was analyzed to determine the Glutathione-S-Transferase (GST) and Catalase (CAT) activities. Both plasma and sera were obtained by centrifugation at 3000 rpm for 10 min.

Serum total testosterone hormone: The testosterone was estimated using Elecsys Testosterone Enzyme Immune Assay Test Kit, Cat. No.: BC – 1115 , By : Digital and Analog Systems (dos).

Sperm count estimation: The testes and epididymes from all animals were dissected out, washed with normal physiological saline solution, dried with filter paper. The cauda epididymis from both sides were removed and washed repeatedly in 10 ml of normal physiological saline. Spermatozoa were counted by using 1-ml aliquots of sperm suspension with the help of a haemocytometer.[18] After wards the testes samples were transferred into buffer formalin 10% (fixative solution) and kept for histological study .

Catalase activity measurement in plasma

Catalase reacts with a known quantity of H_2O_2. The reaction is stopped after exactly one minute with catalase inhibitor. In the presence of peroxidase(HRP), remaining H_2O_2 reacts with 3,5-Dichloro-2 hydroxybenzene sulfonic acid (DHBS) and 4-aminophenazone (AAP) to form a chromophore with a color intensity inversely proportional to the amount of catalase in the original sample.[19]

Glutathione-S-Transferase activity measurement in plasma: Glutathione-S-Transferase assay Kit (biodiagnostic) measures total GST activity (cytosolic and microsomal) by measuring the conjugation of 1-chloro- 2,4-dinitrobenzene (CDNB) with reduced glutathione. The conjugation is accompanied by an increase in absorbance at 340 nm. The rate of increase is directly proportional to the GST activity in the sample.[20]

Preparing the samples of testis tissue: Autopsy samples were taken from the testis of rabbits in different groups and fixed in 10% formalin saline for twenty four hours. Washing was done in tap water then serial dilutions of alcohol (methyl , ethyl and absolute ethyl)were used for dehydration . Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for twenty four hours . Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin & eosin stain for routine examination through the light microscope.[21]

Statistical Analysis

The results were expressed as mean ± standard error (SE) for five animals in each group. The data were analyzed using IBM computer and SPSS statistical package. The One-way analysis of variance (ANOVA) and Student's t test were used to detect differences between the control group and the other experimental groups of animal. The significant differences were considered at $P<0.05$.[22]

RESULTS

Effect of *Panax ginseng* root powder on testosterone assay and sperm count: There was not significantly

different (p>0.05) the serum levels of testosterone (ng/ml) in group 2and 3which was administered 150mg/kg or 300mg/kg *Panax ginseng* root powder (11.11±0.15) and (11.05±0.24) respectively when compared to control (10.97±0.06). On the other hand, there was a statistically significant increase in the sperm count (million/ml) in group 2 (742±15.94) and group 3(813.40±6.21) when compared with control (613.40±6.58) as shown in Table 1 and figures 1,2.

Table 1: Effect of *Panax ginseng* root powder on testosterone hormone and sperm count in control and treated rabbits.

Groups / Parameters	(G1) Control	(G2) *Panax ginseng* 150mg/Kg	(G3) *Panax ginseng* 300mg/Kg
Testosterone (ng/ml)	**10.97±0.06**	**11.11±0.15**	**11.05±0.24**
% of Change from G1		**1.28**	**0.73**
% of Change from G2			**- 0.54**
Sperm count (million/ml)	**613.40±6.58**	**742±15.94** [a]	**813.40±6.21** [ab]
% of Change from G1		**21.04**	**32.63**
% of Change from G2			**9.57**

Values are given as mean ± SE for 5 rabbits in each group.
[a] significant (P< 0.05) as compared with control group (G1).
[b] significant (P< 0.05) as compared with the (G2).

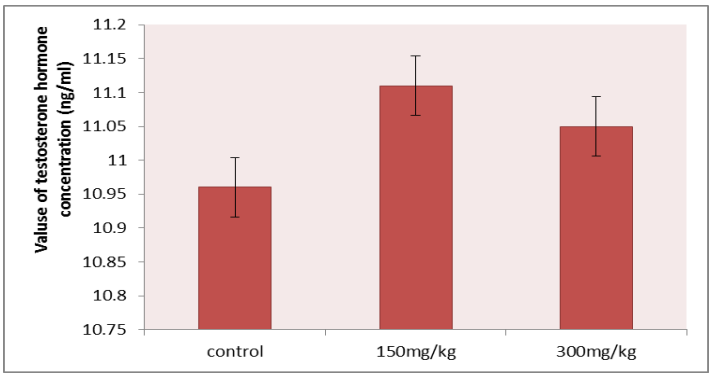

Figure 1: Values of testosterone hormone levels (Means ± SE) for control and treated groups of rabbits.

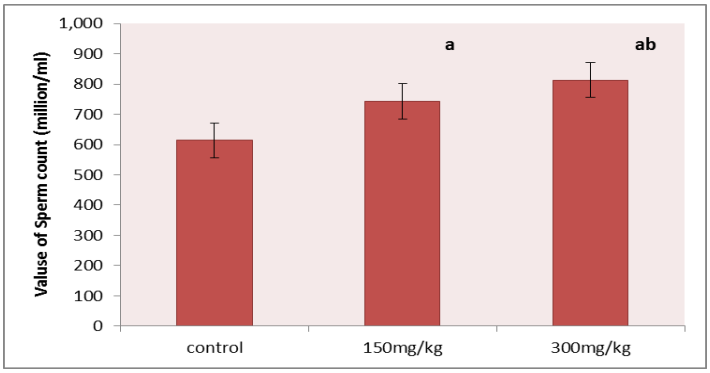

Figure 2: Values of sperm count (Means ± SE) for control and treated groups of rabbits.

Effect of *Panax ginseng* root powder on antioxidant enzymes: Table 2 and Figures 3,4 shows the Administration of *Panax ginseng* root powder at dose 150mg/kg to the animals resulted in a marked non-significant increase in GST activity (673.91±44.95), when compared with control group (424.61±13.83). However, oral administration of *Panax ginseng* root powder at dose 300mg/kg to the animals caused a significant increase in GST activity (1264.25±133.85), comparison with the control animals (424.61±13.83). There was a statistically significant increase in the CAT activity in group 2 (406.92±46.19) and group 3 (782.83±86.15) when compared with control (155.58±19.84) as shown in table 2and figures 3,4.

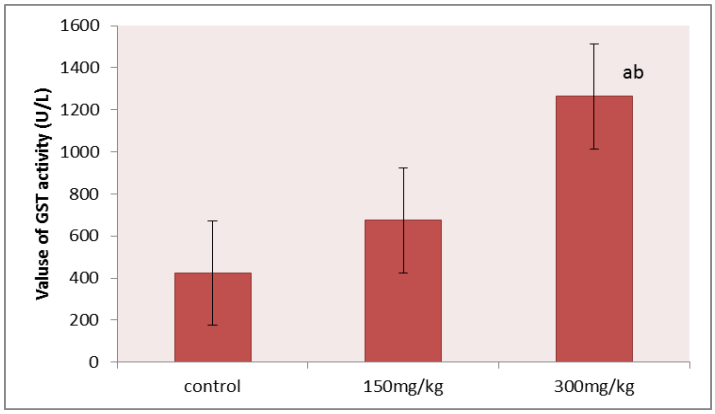

Figure 3: Values of GST activity (Means ± SE) for control and treated groups of rabbits.

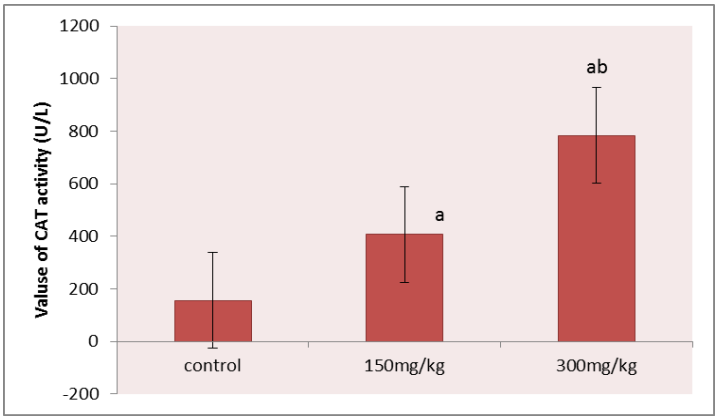

Figure 4: Values of CAT activity (Means ± SE) for control and treated groups of rabbits.

The histological study: The prepared slides were examined under the light microscope and sections photographed. The histological findings in animals treated with *Panax ginseng* at dose 150mg/kg or 300mg/kg showed an improvement of the cellular activity and spermatogenesis in the testical sections and the clear function of the epithelium and seminifirous tubules which indicated by the increasing of the concentration of the spermatozoa in the lumen of the testes (Figure 5 A and B) of these animals sections compared to that of control group(Figure 6).

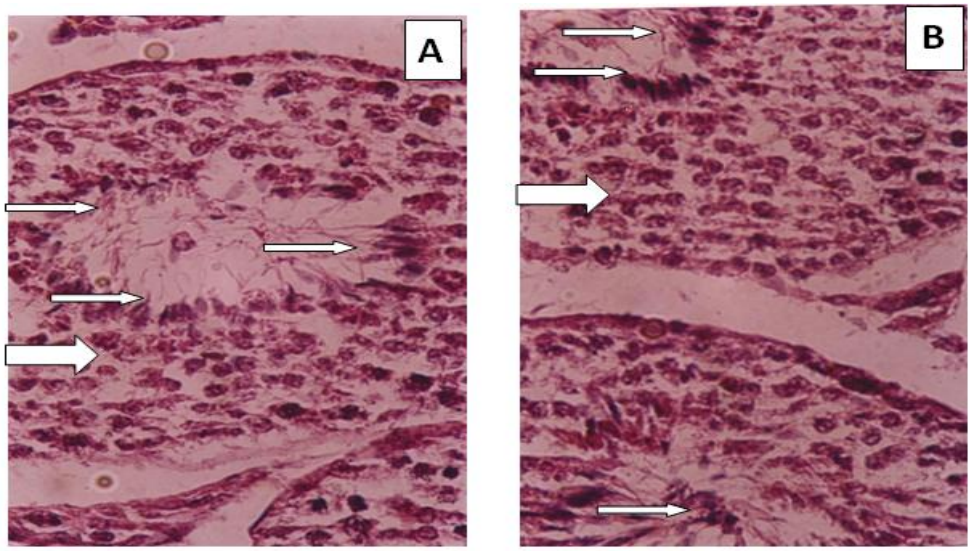

Figure 5: Histological sections of treated mature testis(A=150mg/kg and B=300mg/kg) show the spermatogenesis activity clear improvement (thick arrow) and lumen with many spermatozoa(thin arrow)(H. & E. x 400)

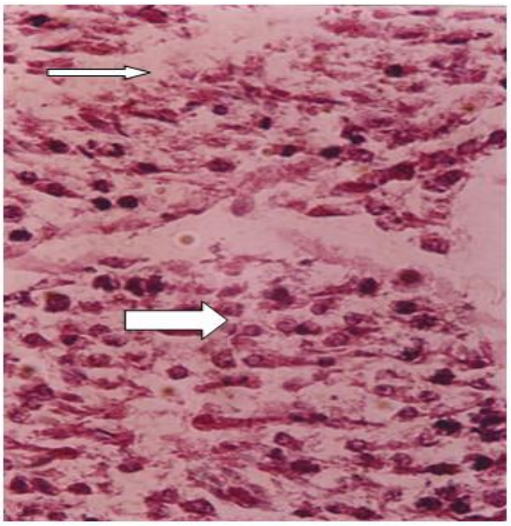

Figure 6: Histological sections of control mature testis show active spermatogenesis (thick arrow) with a lumen seminifeoous tubule contain spermatozoa(thin arrow)(H. & E. x 400).

Table 2: Effect of *Panax ginseng* root powder on antioxidant enzymes (Glutathion-S-transferase and Catalase) in control and treated rabbits.

Groups **Parameters**	**(G1)** **Control**	**(G2)** ***Panax ginseng* 150mg/Kg**	**(G3)** ***Panax ginseng* 300mg/Kg**
GST (U/L)	424.61±13.83	673.91±44.95 **58.80**	1264.25±133.85[ab] **197.74** **87.60**
% of Change from G1			
% of Change from G2			
Catalase(U/L)	155.58±19.84	406.92±46.19[a] **161.55**	782.83±86.15[ab] **402.88** **92.38**
% of Change from G1			
% of Change from G2			

Values are given as mean ± SE for 5 rabbits in each group.
[a] significant (P< 0.05) as compared with control group (G1).
[b] significant (P< 0.05) as compared with the (G2).

DISCUSSION

Panax ginseng was known to have protective and therapeutic effects against the testicular atrophy and other damages induced the most potent environmental pollutants toxic to reproductive organs.[23]

The best known Asian species is *Panax ginseng* whereas the American species belongs to *Panax quinquefolius.* Both species are widely used in Asian countries for a variety of purposes. In our study, administration of *Panax ginseng* root powder at dose 150 or 300 mg/kg to the animals caused non-significant increase in serum testosterone concentration and, Interestingly, caused significant increase in sperm count when compared with control group. These results agree with the findings recorded[14] which reported that ginseng increased sperm counts in rabbits, It has been reported that there is no sex hormone-like function but probably gonado tropin-like action in ginseng. The previous studies showed that no significant change in testosterone and luteinizing hormone (LH) was observed in the ginseng supplemented athletes[24] or male rats.[15] However, Fahim *et al*.[16] showed a decreased blood testosterone level in 5% ginseng-received rats.[16] Ginseng saponin abolished morphine induced release of plasma corticosterone and apoptosis of thymocytes.[25] *Panax ginseng* normalized FSH, LH and testosterone levels this may be due to a direct or an indirect effect on the HPG axis.[26] Hassan and Abdel-Wahhab; Kumar *et al* [27,28] reported that Ginseng causes a significant decrease in total chromosomal aberrations, sperm abnormalities and increase in testosterone concentration and sperm numbers and motility .Hwang *et al*,[29] indicated that ginseng improves the survival rate and sperm quality in guinea pigs exposed to 2, 3, 7, 8-tetrachlorodibenzop-dioxin (TCDD) and stimulates the spermatogenesis.[29] The initial evidence that ginseng may have positive effects on spermatogenesis was first published in 1977. Here it was demonstrated that the stimulatory effect of ginseng extracts on DNA and protein syntheses in rat testes.[30] Later studies in both rodents and humans have shown that ginseng can increase sperm count. Ginseng-treated rats have demonstrated an increased rate of spermatogenesis via glial cell-derived neurotrophic factor (GDNF) expression elevation in Sertoli cells[31], and activation of testicular cAMP-responsive element modulator (CREM).[32] GDNF is a possible regulator of the survival and cell fate decision of undifferentiated spermatogonial cells[33,34], and CREM is essential for spermatid maturation.[35] Ginseng is also found to help preserve the ejaculated sperms. It has been shown that

the sperm count of ejaculated sperms that were incubated with ginseng extract was significantly higher than those treated with vehicle.[36] Treatment with ginsenoside Rg1 (50 µg/ml) significantly increases sperm motility and membrane integrity of post-thawed sperms as compared with fresh and untreated thawed sperms.[37] These findings suggest that the addition of ginseng extract to the cryogen for sperm storage could enhance fertility. There is evidence to support the use of *Panax ginseng* in the treatment of male sexual dysfunction.[38] Taking *Panax ginseng* orally may enhance male fertility by increasing sperm count, quality, and movement, as it activates the body system that increases production of certain hormones.[38] The use of *Panax ginseng* extract showed an increase in spermatozoa number/ml and progressive oscillating motility, an increase in serum testosterone, FSH and LH levels. It is suggested that ginsenosides may have an effect at different levels of the hypothalamuspituitary-testis axis.[39] The aqueous, organic, and polysaccharide fractions of *Panax notoginseng* have been shown to enhance the directional motility of human sperms in 60–120 min.[40] Similarly, ginsenosides Rc and Rb2 (0.01 mg/ml) have been shown to enhance sperm progression in vitro.[41] Both oligoastenospermic patients and age-matched healthy counterpart showed an increase in spermatozoa density and motility after the use of *Panax ginseng*.[42] Asthenospermia patients treated with ginseng also showed a significant increase in progressive sperm motility.[43]

Ginseng roots contain multiple active constituents, ginsenosides being the major biologically active compounds.[44] The potential therapeutic effects of ginseng have been attributed to its immunostimulatory, antioxidant, vasorelaxant, anti-neoplastic and anti-inflammatory activities.[45] The antioxidant enzyme activities of GST and CAT in this study were significantly increase in the rabbits ($p < 0.05$), after administration with *Panax ginseng* when compared with control group. These results agree with the findings recorded by Kim and Park [46]which reported that the activity of SOD and CAT increased by 31% and 24%, respectively, when ginseng extract was fed for 8 weeks to humans. Jin and Chang[47] stated that red ginseng extract fed to gamma-irradiated mice increased the activity of SOD, peroxidase, and CAT. Liu *et al*.[48] reported that saponin purified from ginseng has either no or only weak antioxidant activity, leading to the suggestion that phenolic compounds rather than saponins are responsible for ginseng's antioxidant properties[59] Chemical analysis of ginseng revealed the presence of many ingredients, including organic acids, vitamins, sugars, inorganic salts, sterols, oligopeptides, polysaccharides, volatile oils, and saponins. Of these, the saponins (commonly known as ginsenosides) are well studied for their biological properties.To date, more than 30 ginsenosides have been found in the roots and other parts of *P. ginseng* , and a total of over 60 ginsenosides were isolated from members of the *Panax* genus (7).

The function of male reproductive organs is under the control of androgens.[50]This is in concert with our histological study as improvement of the cellular activity and spermatogenesis in the testical sections and the clear function of the epithelium and seminifirous tubules. Chang and But,[14] reported that gensig increased sperm counts in rabbits, It has been reported that there is no sex hormone-like function but probably gonadotropinlike action in ginseng.

CONCLUSIONS

Our current study suggest that *Panax ginseng* root powder possesses pro-fertility properties in male rabbits which might be a product of both its potent antioxidant properties and androgenic activities.

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