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Effect of ginseng bulk and its nanoparticles in testicular functions of normal rats

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ABSTRACT

Natural herbal medicines such as ginseng have been reported to enhance testicular functions. This study investigated the effects of ginseng bulk and its nanoparticles on male reproduction. A total of forty male albino rats were randomly divided into four equal groups, control (received physiological saline 1ml/day), Ginseng (Gin) 50 (administered with Gin powder 50 mg /kg BW/day), Ginseng 100 (administered with Gin powder 100 mg /kg BW/day) and Ginseng NPs30 (administered with Ginseng powder nanoparticles 30 mg /kg BW/day). We assessed body weight gain, testis weight, semen analysis, testosterone, LH, and FSH levels, as well as antioxidant biomarkers (catalase and malondialdehyde) after 8 weeks. Besides, histological examination, evaluation of spermatogenesis and androgen receptors expression were performed. our results showed increase in sperm concentration in Gin 100 group compared with control. Also, testosterone, LH and FSH levels were elevated significantly after administration of Gin 100 compared with Gin 50. Moreover, rats received Gin100 and Gin NPs 30 showed significant decrease in MDA level compared with the control group. Histologically, all treated rats showed normal structured including seminiferous tubular architectures, presence of different stages of spermatogenesis, normal Sertoli and Leydig cells. However, the number of spermatozoa increased in Gin 50, Gin 100 and Gin NPs 30 respectively compared with control. Also, rats received Gin NPs 30 showed increase in diameter of seminiferous tubules compared with control. It can be concluded that normal testicular function can be structurally maintained and functionally enhanced by ginseng treatment principally by Gin NPs 30 administration.

Introduction

Medicinal plants have been utilized worldwide for maintaining health and treating various diseases (Smith-Hall *et al*., 2012). The demand for herbal medicine has increased due to its positive effects on health, lower side effects, and affordable prices (Sajedipoor and Mashayekhi, 2015). Traditional medicines, such as ginseng, have been reported to enhance testicular functions and enhance fertility (Bardaweel *et al*., 2013; Gray *et al*., 2016).). Ginseng has been used as a general tonic in Asia for thousands of years and is one of the most popular herbal dietary supplements globally (Bahrke and Morgan, 2000; Coleman *et al*., 2003; Xiang *et al*., 2008; Hassan *et al*., 2020). It is believed to counteract fatigue, boost the immune system, improve physical stamina, and stimulate the appetite. Ginseng has also been used for enhancing testicular function, fertility and improving testicular impairment (Kim *et al*., 1999; Xiong *et al*., 2010; Yayeh *et al*., 2012).

Recently, a tissue cultured root of wild *P. ginseng* exhibited the therapeutic effects on spermatogenetic disorder (Park *et al*., 2006). Studies have shown that Korean red ginseng (*Panax ginseng* Meyer; KRG) has multifunctional therapeutic benefits, including its ability to improve spermatogenetic disorder and protect muscles from exercise-induced oxidative stress (McKay, 2004). Ginseng also has beneficial effects against testicular toxicity induced by certain medications such as doxorubicin (Kang *et al*., 2002) and busulfan in male rats therefore, ginseng therapy could potentially aid in the recovery of male fertility during chemotherapy (Ji *et al*., 2007).

The major bioactive constituents of ginseng are steroidal saponins called ginsenosides (Wang *et al*., 2009). These compounds are responsible for many of ginseng's pharmacological actions and have shown potential in treating Alzheimer's disease, aging, immune disorders, cancer, and other ailments (Attele *et al*., 2002; Spelman *et al*., 2006). Ginsenosides also have protective effects on the genital system and have been found to increase spermatozoa count, motility, and testosterone levels (Salvati *et al*., 1996).

Nanotechnology has recently been employed in medicine for therapeutic purposes (Bhattacharyya *et al*., 2010). By reducing the particle size of plant extracts like ginseng, the surface area increases, leading to improved absorption, bioavailability, and release of functional ingredients (Wen *et al*., 2009; Ali *et al*., 2015). Ginseng oral bioavailability is low due to low absorption rate of ginseng saponins (Qi *et al*., 2011). Fadwa *et al*. (2015) found that nanoparticles formulated with ginseng have shown greater effectiveness in enhancing male rat fertility compared to traditional ginseng extract. This nano formulation allows the active ingredients to reach target cells within the hypothalamus-pituitary-testis axis more efficiently, ultimately enhancing male fertility.

The aims of the research topic were to investigate the efficiency of the *Panax ginseng* through using them in the whole plant form or their nanoparticles form as an antioxidant on normal testicular functions, and to explore the underlying mechanisms in adult male Wistar rats administered *P. ginseng* bulk and its nanoparticles for eight weeks. The pattern was be monitored by body weight gain (BWG), testis weight and gonadosomatic index (GSI), hormonal assay, evaluation of antioxidant biomarkers, immunohistochemical investigations of the androgen receptor (AR) expression, complete semen evaluation (Sperm motility, count, abnormality, and viability) and histological examination of testicular tissue.

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Materials and methods

Ethical approval

The experimental protocol followed institutional animal care and was approved by the Veterinary Medical Ethics Research Committee- Faculty of Veterinary Medicine, Sohag University, Egypt (Approval number: Soh. un.vet/00044R).

Reagents

Ginseng was purchased in the commercial form of Korean Red Ginseng (100% natural *Panax ginseng* Roots Powder) (IMTENAN, Assiut, Egypt). Ginseng nanoparticles (Gin NPs) were prepared by Nano Tech company (NanoTech, 6th October city, Egypt) by ball milling technique.
Characterization of prepared Gin NPs

Ginseng NPs characterization was carried out by using X-ray diffraction (XRD) and the morphology of the prepared nanoparticles was investigated by using scanning electron microscopy (SEM) and transmission electron microscopy (TEM) (JEOL, JEM 2100) and their elemental quantification was performed by energy-dispersive spectroscopy (EDS) attached to SEM. Optical properties were studied by using UV/VIS spectroscopy (Shashanka, 2021; Hassan *et al*., 2024). Ball milling was done by using Planetary Ball Mill PM 400 "4 grinding stations". The ball milling process results in the reduction of the size of particles and improve the specific surface area (SSA), adding new active edge sites to enhance the ability of adsorption of the ball milled materials (Wei *et al*., 2020; Amusat *et al*., 2021).

Animals

Forty mature male Wistar albino rats weighing 250.0±10.0 g and of age 8 weeks (Sengupta, 2011). The animals were obtained from the animal house lab, Faculty of Medicine, Sohag University, Egypt. Animals were housed in a specific clean and pathogen free plastic cages in the animal house at Physiology Department, Faculty of Veterinary Medicine, Sohag university with a 12 h light/dark cycle and at temperature of 23±2°C humidity 50:55%, with ad libitum access to standard rodent pellets food and water. Animals were adapted for two weeks to the experimental site (Ma and Lightman, 1998).

Experimental Design

After the end of acclimatization period, rats were randomly distributed into four equal groups (10 rats per each group) and dosed according to Hassan *et al*. (2024) as following:
Group I (control): received physiological saline (1ml/day).
Group II (Gin 50 group): administered with Korean red ginseng roots powder 50 mg /kg BW.
Group III (Gin 100 group): administered with Korean red ginseng roots powder 100 mg /kg BW.
Group III (Gin NPs 30 group): administered with Korean red ginseng roots powder nanoparticles 30 mg /kg BW.

The dry powder of Gin NPs was first suspended in deionized water and then sonicated at room temperature for 10 minutes. This process was done to achieve a homogeneous suspension. To prevent aggregation of the NPs before administration, the time interval from preparation to oral gavage was limited to 20 minutes. Throughout the eight-week duration, all rats were given physiological saline and the drug solution orally using a drenching tube.

Recording of the body weight gain and reproductive organs weight

The initial body weights (I.W) of all groups were measured before starting the treatment. The weighing of rats was done continuously weekly throughout the experimental period. Additionally, the final body weights were recorded at the time of sacrificing. Furthermore, the testes from each rat were quickly excised out and weighed to calculate the gonadosomatic index. This index was calculated using the formula: testes weight (g) divided by Final body weight (g) multiplied by 100, as suggested by Kumari and Singh (2013).

Sample collection

The experimental procedures involved the anesthesia of rats using intraperitoneal dose of sodium thiopental (50 mg/kg BW) at the end of the experimental period. Following the anesthesia, the testes were promptly excised for epididymal semen analysis and histological examination.

Blood samples collection

At the end of the experimental period, the rats were sedated using diethyl ether. Individual blood samples were collected from the retro-orbital venous plexus of each group in evacuated plain tubes. The blood was allowed to coagulate before being centrifuged at 3000 rpm for 15 minutes. Sera were carefully collected and stored in separate Eppendorf tubes at a temperature of -80°C until hormonal assay including serum testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH).

Semen analysis

Semen was collected from the epididymis and diluted in warmed physiological saline at 37°C according to D'Souza (2003). Sperm motility was evaluated by placing a drop of semen on a warmed, dry, and clean slide. To assess the sperm's vitality (dead and live percent), one drop of semen was applied simultaneously on a dry, clean slide that had been prewarmed then stained by Eosin-Nigrosin stain and the percent of dead and live sperms were calculated (Esteso *et al*., 2006). According to Wyrobek and Bruce (1978), sperm abnormalities were detected by combining one drop of diluted semen with an Eosin stain, the percent of normal and abnormal sperms were calculated. Finally, the epididymal sperm concentration was assessed by dilution with sodium bicarbonate solution and formalin, followed by sperm counting using Neubauer hemocytometer as described by Srinivasulu and Changamma (2017).

Hormonal Assay

The frozen serum was thawed for measuring serum testosterone, follicle stimulating hormone (FSH) and luteinizing hormone (LH) by enzyme-linked immunosorbent assay (ELISA) kits according to manufacturer's instructions (Chen *et al*., 1991; Qiu *et al*., 1998 and Frank *et al*., 1996, respectively) Catalogue NO. (TE187S), (FS232F) and (LH231F) (Calbiotech, El Cajan, CA, USA) respectively, using microplate reader (Infinite 50, Männedorf, Switzerland) at wavelength 450 nm.

Testicular Oxidative/Antioxidant Status

Immediately after anesthesia by intraperitoneal injection of sodium thiopental, testes were collected. Testicular tissue homogenate was prepared according to Behairy *et al*. (2020). In cold phosphate buffered saline (PBS, 0.01 mol/L, pH 7); the right testis for every rat was homogenized using the glass homogenizer (1:9 W/V). The resulting homogenates were centrifuged for 5 min at 5000 ×g; the supernatants were filtered with a Millipore filter (0.45 µm) to eliminate TES's tissue debris hormone evaluation and oxidative stress. Catalase (CAT) activity and Malondialdehyde (MDA) levels were estimated by using kits reagents according to the methods (Aebi, 1984; Ohkawa *et al*., 1979) Catalogue NO. (CA 25 17) and

(MD 25 29) (Bio-diagnostic Co, Al-Sadat city, Egypt) respectively. Concentrations were determined via the spectrophotometric method using T80 UV/VIS spectrophotometer according to the manufacturer's protocol.

Histological examination

Testes of all rats in the experimental groups were dissected from epididymis, and quickly fixed in neutral buffered paraformaldehyde (PFA) 4%, processed through the conventional paraffin embedding technique (Bancroft and Gamble, 2002), sections were dewaxed with xylene and rehydrated through a descending alcohol series. Slides were stained with Harris hematoxylin and eosin (H&E) (Carleton *et al.*,1980 and , Bancroft *et al.*, 1996). All sections were examined and photographed using light microscope OLYMPUS CX43 microscope and photographed with an OLYMPUSDP72 camera adapted to the microscope (Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, Sohag University).

Morphometric study

ImageJ versus 1.48 software (NIH), was used for measuring the diameter of seminiferous tubules/µm2, height of spermatogenic cells (SGs). Five non-overlapping sections from each paraffin block were taken and examined at low-power fields (× 200), except for SC and SG counts which were taken from high-power fields (× 400) (de Siqueira Bringel *et al.*, 2013 and Soliman *et al.*, 2014).

Evaluation of spermatogenesis

Spermatogenesis was assessed using the Johnsen-like score (Johnsen, 1970) which is the usual tool for evaluating human spermatogenesis, and it was adapted to be used in the rat spermatogenesis analysis. The adapted Johnsen score was called the Johnsen-like score (Oliveira Filho *et al.*, 2010). In each biopsy, 50–100 cross sections of seminiferous tubules were evaluated (Filipiak *et al.*, 2012 and Soliman *et al.*, 2014) according to criteria presented in previous studies (Flegr *et al.*, 2014).

Immunohistochemical investigation of androgen receptor expression in the Sertoli and Leydig cells

Testes were obtained after whole body perfusion of rats with 4% paraformaldehyde and stored until embedded in paraffin. Testes from four animals per group were analyzed in this procedure. Consecutive five-micron thick testicular sections (one section/animal) were prepared from the formalin-fixed paraffin-embedded blocks, then stained for androgen receptor and analyzed by immunohistochemistry for androgen receptor (AR) detection. Following deparaffinization and hydration, endogenous peroxidase was blocked with 0.9% H_2O_2, and background blocking was performed with normal goat serum. The tissue sections were then incubated with primary antibody overnight at 4°C or one hour at room temperature in PBS. Sections were incubated with the secondary antibody, biotinylated goat-anti-mouse, and an ABC staining kit was used as the detection system. Peroxidase activity was revealed in 0.03% 3,3 diaminobenzidine tetra dihydrochloride (DAB) under a light microscope. Images were taken using a Canon digital camera. Randomly selected five, non-duplicated 40× microscopic fields/animal were taken, and the AR immunoexpressing was recorded by counting the numbers of positive Sertoli and Leydig cells/image. The negative control slides were processed like others except that PBS was used in place of the primary antiserum. Signal intensity for AR protein was analyzed using Image J (NIH) (Abdel-Maksoud *et al.*, 2019).

Statistical analysis

Data for each group were shown as mean ± standard error of mean (SEM) and differences between groups were evaluated by using one-way analysis of variance (ANOVA). Values of $P<0.05$ and $P<0.001$ and $P<0.0001$ were considered significant compared with control. Results were analyzed statistically by the computer program GraphPad prism 8 (GraphPad Software, San Diego, California USA) (Brown, 2005).

Results

Physical characteristics of Gin NPs

Transmission electron microscopy (TEM) was performed on a JEOL JEM-2100 high resolution transmission electron microscope at an accelerating voltage of 200 KV. The resulting Gin NPs are a pale brown colored powder that disperses in H_2O. The average size of the nanoparticles, as determined by TEM, is 60 ± 10 nm in length and 25±5 nm in diameter. The nanoparticles have a rod-like shape. (Fig. 1 A & B).

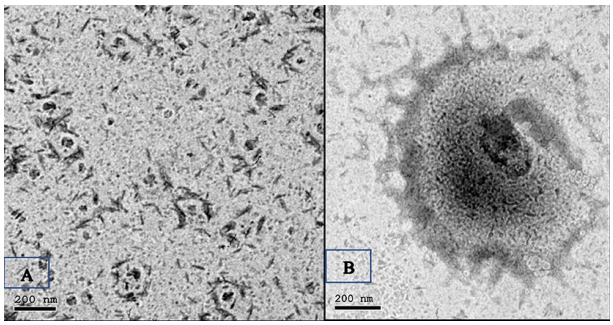

Fig. 1. Scanning electron microscopy (SEM) images of cross-sections of powder particles produced after different milling times.

Effect of ginseng bulk and its nanoparticles on body and reproductive organ weights.

The data showed a non-significant change in body weight gain, testis weight and the gonadosomatic index in all treated groups compared with their control (Tables 1 and 2).

Table 1. Effect of ginseng bulk and its nanoparticles on body and reproductive organ weights.

Group	Body weight gain (g)	Testis weight (g)	GSI
Control	84.83±16.70	2.54±0.08	0.93±0.03
Gin 50	95.00±12.00	2.43±0.09	0.80±0.03
Gin100	104±11.81	2.37±0.05	0.77±0.04
Gin NPs 30	81.60±16.63	2.33±0.09	0.82±0.04

Data are expressed as mean± standard error.

Table 2. Effect of ginseng bulk and its nanoparticles on some sperm parameters.

Group	Concentration (10^6 /ml)	Abnormal sperms (%)	Dead sperms (%)	Motility (%)
Control	215.64±22.94	3.65±0.42	3±0.42	81.80±2.43
Gin50	258.20±37.84	4.00±0.45	3.40±0.43	87.70±1.64
Gin100	387.60 ±43.05*	2.70±0.54	3±0.56	89±2.23
Gin NPs 30	267.47±44.36	6.70±1.36	3.9±0.46	90.2±3.70

Data are expressed as means ± standard error. Significant differences vs. the control group are marked by different asterisks: * $p \leq 0.05$ vs. control group.

Ginseng bulk and its nanoparticles effect on some sperm parameters

As shown in Table 3 administration of Gin100 mg/kg B.W daily for 8 weeks resulted in significant increase at ($p < 0.05$) in sperm concentration and no significant changes in all other treated groups and recorded non-significant changes in abnormal sperm %, dead sperm % and motili-

ty% between all treated groups and control group (Table 2).

Table 3. Effect of ginseng bulk and its nanoparticles on levels of testosterone (ng/ml), FSH (MIU/mL) and LH (MIU/mL) in treated rats.

Group	Testosterone	FSH	LH
Control	8.83±00.67	18.89±1.02	5.90±00.66
Gin 50	7.97±00.28	17.34±1.66	6.11±00.64
Gin100	10.82±00. 63$	19.53±2.01	8.41±1.04
Gin NPs 30	10.59±00.46	17.94±1.59	8.18±1.08

Data are expressed as means ± standard error. Significant differences vs. Gin50 group are marked by: $ $p \leq 0.05$ vs. Gin50.

Effect of ginseng bulk and its nanoparticles on levels of testosterone (ng/ml), FSH (MIU/mL) and LH (MIU/mL) in treated rats.

Table 3 cleared the variation of levels of reproductive hormones in the experimental groups. There are non-significant differences in testosterone, FSH and LH levels in all treated groups compared with their control. And there is significant increase ($p < 0.05$) in testosterone level in rats received Gin100 mg/kg BW compared with rats received Gin50 mg/kg BW.

Effect of ginseng bulk and its nanoparticles on testicular oxidative/antioxidants status.

As cleared in Table 4 daily administration of Gin 100 and Gin NPs 30 mg/kg BW for 8 weeks to adult male rats significantly ($p < 0.05$) decreased malondialdehyde level and non-significantly decreased it in rats received Gin 50 mg/kg BW relative to control rats. Moreover, it is cleared non-significant elevation in catalase level in all treated groups compared with control group.

Table 4. Effect of ginseng bulk and its nanoparticles on testicular oxidative/antioxidants status.

Group	Malondialdehyde (MDA) (nmol/g tissue)	Catalase (CAT) (U/g tissue)
Control	4.82±0.49	0.28±0.03
Gin 50	3.43±0.23	0.35±0.04
Gin100	2.27±0.28*	0.41±0.06
Gin NPs 30	2.51±0.41*	0.32±0.04

Data are expressed as means ± standard error. Significant differences vs. the control group are marked by different asterisks: * $p \leq 0.05$ vs. control group.

Histological findings in control and treated rats

There was an increase in the size of seminiferous tubules and the number of spermatozoa filling its lumen with improvement in the spermatogenic cycle from spermatogonia till spermatids in all treated groups when compared with control group Fig. 2. Testicle tissue section in control group showing normal structured seminiferous tubule, lined by basement membrane rested on it myoid cells, germ cells rest on basement membrane in order from basal lamina to center as spermatogonia (Sg), spermatocytes and spermatids. The Sertoli cells were found throughout the entire thickness of the germinal epithelium from the basal lamina to the spermatids. Spermatozoa flagella fills the lumen of tubule. The interstitial tissue (ICT) consisted of sparse loose connective tissue, capillaries, arterioles, venules, lymphatics, nerve-cells and Leydig cells (Fig. 2. A& b).

The testicle tissue section from the Gin 50 treated group exhibited normal structured seminiferous tubules (ST) with spermatozoa filled lumen, and normal interstitial cells (Fig. 2. C). The normal structured seminiferous tubule lined by germ cells in order from basal lamina to center as spermatogonia, spermatocytes and flagellated spermatozoa fill the lumen of seminiferous tubule. The Sertoli cells were found throughout the entire thickness of the germinal epithelium from the basal lamina to the spermatids (Fig. 2. D).

Microscopic examination of testicle tissues from Gin 100 treated group displayed normal structured seminiferous tubule lined by basement membrane rested on spermatogenic epithelium as spermatogonia, spermatocytes and flagellated spermatozoa fill the lumen of tubule. The Sertoli cells were found throughout the entire thickness of the germinal epithelium from the basal lamina to the spermatids (Sertoli). The interstitial tissue consisted of sparse loose connective tissue. Capillaries, arterioles, venules, lymphatics, nerve-cells and Leydig cells (Fig. 2. E&F).

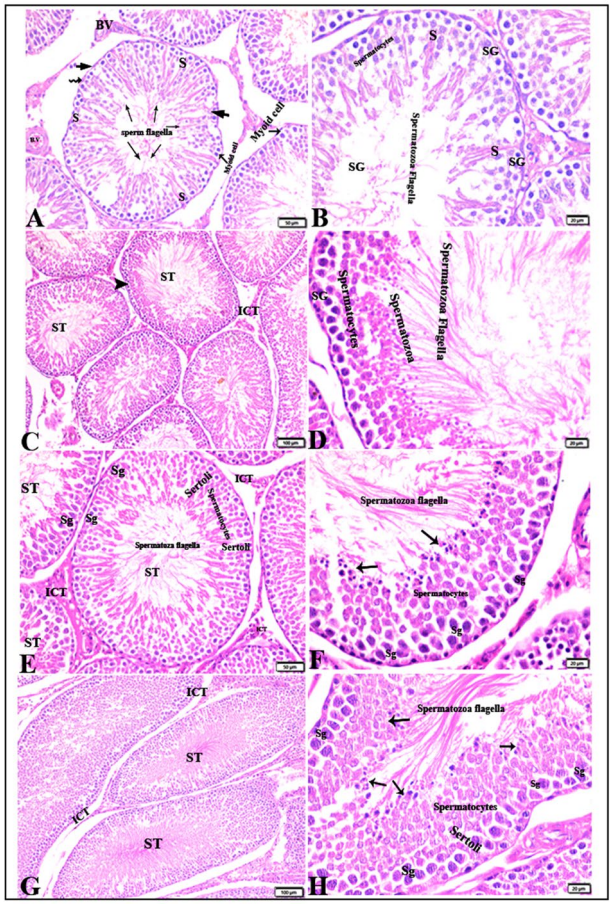

Fig. 2. Photomicrograph of testicle tissue section stained with HE stains, from the experimental groups. (A&B): Testis of control group showing: (A): normal structured seminiferous tubule (ST) and interstitial cells (ICT). Normal blood vessel (B.V). (B): The seminiferous tubule, lined by basement membrane rested on it myoid cells (arrowheads), germ cells rest on basement membrane in order from basal lamina to center as spermatogonia (Sg), spermatocytes and spermatids. The Sertoli cells were found throughout the entire thickness of the germinal epithelium from the basal lamina to the spermatids (arrows). Spermatozoa flagella fills the lumen of tubule. The interstitial tissue consisted of sparse loose connective tissue, capillaries, arterioles, venules, lymphatics, nerve-cells and Leydig cells. (C&D): The testicle tissue section from the Gin 50 mg/kg BW treated group showing: (C): normal structured seminiferous tubules with spermatozoa filled lumen, and normal interstitial cells. (D): Seminiferous tubule lined by germ cells in order from basal lamina to center as spermatogonia, spermatocytes and flagellated spermatozoa fill the lumen of seminiferous tubule. The Sertoli cells were found throughout the entire thickness of the germinal epithelium from the basal lamina to the spermatids. (E&F): Testis of Gin 100 mg/kg BW treated group showing: normal structured seminiferous tubule lined by basement membrane rested on spermatogenic epithelium as spermatogonia, spermatocytes and flagellated spermatozoa fill the lumen of tubule. The Sertoli cells were found throughout the entire thickness of the germinal epithelium from the basal lamina to the spermatids (Sertoli). The interstitial tissue consisted of sparse loose connective tissue (ICT). Capillaries, arterioles, venules, lymphatics, nerve-cells and Leydig cells.(G&H): Testicle tissue section from the Gin NPs 30 mg/kg BW treated group showing: (G): normal structured large sized seminiferous tubule with tuft of flagellated spermatozoa filled lumen. Normal interstitial cells (ICT). (H): Large sized seminiferous tubule lined by intact basal lamina (arrowheads) rest on it spermatogenic epithelium arranged in normal order as spermatogonia, Spermatids (arrows) and flagellated spermatozoa fill the lumen of seminiferous tubule. The Sertoli cells were found throughout the entire thickness of the germinal epithelium from the basal lamina to the spermatids. Interstitial cells contain (ICT) normal Leydig cells (The scale bar size A&E=50µm, C&G, =100µm, B, D, F&H=20µm).

Histological structure of testicles from Gin NPs 30 treated group showing normal structured large sized seminiferous tubule with tuft of flagellated spermatozoa filled lumen. Normal interstitial cells (Fig. 2. G).

Seminiferous tubules were large in size, lined by intact basal lamina rest on it spermatogenic epithelium arranged in normal order as spermatogonia, Spermatids and flagellated spermatozoa fill the lumen of seminiferous tubule. The Sertoli cells were found throughout the entire thickness of the germinal epithelium from the basal lamina to the spermatids. Interstitial cells contain normal Leydig cells (Fig. 2. H).

Diameter and hight of seminiferous tubules

There was significant increase in diameter of seminiferous tubules in Gin NPs30 mg/kg BW group and is non-significant change between all other treated groups compared with control group (Table 5).

Height of spermatogenic cells (SGs) showed a- no significant change in seminiferous tubules of all treated groups and their control one (Table 5).

Table 5. Diameter of seminiferous tubules (µm) in control and treated rats.

Group	Diameter of seminiferous tubules (µm)	Height of seminiferous epithelium (µm)
Control	329.8± 6.45	68.24 ± 3.115
Gin 50	349.0±21.37	62.89 ± 3.079
Gin100	421.2± 41.32	59.52 ± 2.683
Gin NPs 30	527.7± 115.70*	64.66 ± 2.266

Data are expressed as means ± standard error. Significant differences vs. the control group are marked by different asterisks: * $p \leq 0.05$ vs. control group.

Johnsen-like scores

Quantification of Johnsen like scores showed there is no significant change in spermatogenesis mean values between all treated groups (Gin 50, Gin 100, and Gin NPs 30) (9.700±0.15, 9.600±0.16, 9.400±0.26 respectively) and their control group (9.400±0.22).

Immunohistochemical assessment of androgen receptor protein in control and treated rats.

Androgen receptor protein was expressed normally and intensely in spermatogenic epithelial cells from spermatogonia till sperms, Sertoli cell nuclei and Leydig cells in all treated groups as well as control group (Fig. .3).

Quantified androgen receptors protein expression

The percentage area of androgen receptor protein expression in Gin 50, Gin 100 and Gin NPs 30 were (3.834±0.18, 4.265±0.79 and 5.688±0.26) respectively and non-significantly differ from control group (4.451±0.68) (Fig 3).

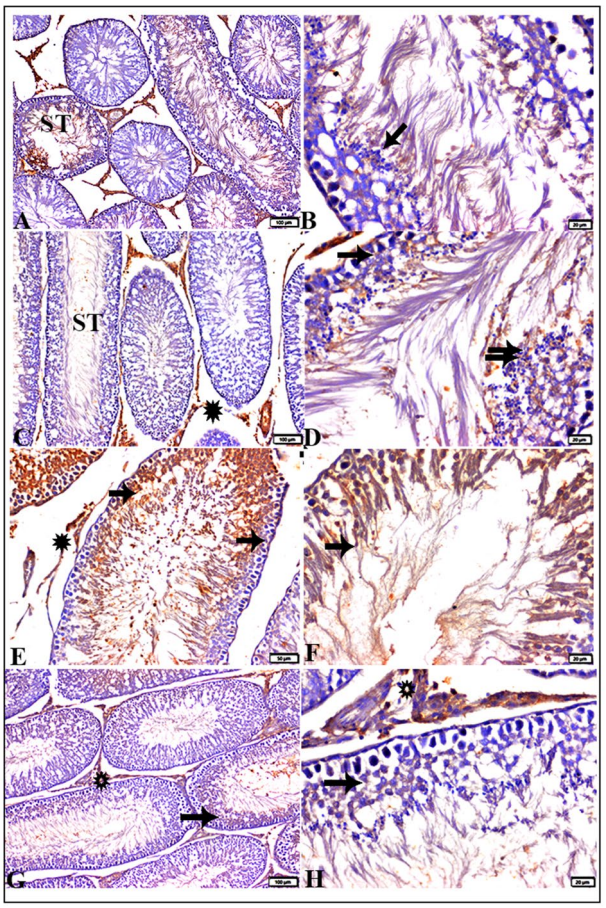

Fig. 3. Immunohistochemical staining for androgen receptor protein in testes of control (A&B), Gin 50 (C&D), Gin 100 (E&F) and Gin NPs 30 (G&H) treated rats respectively. Spermatogenic epithelial cells are marked by arrows, spermatids are marked by double arrows, and interstitial Leydig cells are marked by stars. (The scale bar size A, C, &G=100µm, E=50µm, B, D, F&H=20µm).

Discussion

The obtained data indicated a non- significant change in body weight gain, testis weight and GSI in Gin 50, Gin 100 and ginseng bulk nanoparticles (30 mg/kg BW) groups. Previous literatures on *P. ginseng* extract were agree with our results on ginseng bulk; Lee *et al.* (2007); Hwang *et al.* (2010); Jung *et al.* (2015); Kopalli *et al.* (2015) reported that *P. ginseng* extract didn't affect testis weight in rats. Additionally, Ku *et al.* (2020) mentioned that administration of *P. ginseng* extract showed non- significant changes in body weight and testis weight in both young and aged mice. However, our obtained results are in contrast with Karu *et al.* (2007); Lin *et al.* (2014) and Li *et al.* (2018) who reported significant decrease in weight gain in mice treated with *P. ginseng* extract, as *P. ginseng* has anti-obesity and anti-diabetic effect, due to the inhibition of pancreatic lipase by the saponin fraction (ginsenoside Rb1) of *P. ginseng*. The obtained results of the current study showed non- significant changes in body weight gain, testis weight and GSI in Gin NPs 30 group. There are no available literatures that examined the effect of nano-ginseng extract on body weight gain, testis weight and gonadosomatic index and support our results.

The result of the present study showed a significant increase in sperm concentration in Gin100, and a non-significant increase in Gin50, non-significant changes in abnormal sperm % and dead sperm % and non-significant increase in motility % in Gin50 and Gin100 groups compared with control group. These results are in line with the results of Hassan *et al.* (2024) who found that administration of 50 and 100 mg/kg BW of ginseng bulk for 60 days resulted in significant increase in sperm concentration in rats administrated Gin 100 and non-significant increase in Gin 50 treated groups. There are many previous studies obtained by using ginseng extract and in agreement with our findings, Yang *et al.* (2011) and Hassan *et al.* (2020) reported significant increases in sperm concentration and sperm motility % with decrease in abnormal sperm % after *P. ginseng* extract administration in treated human and rats. Also, Hwang *et al.* (2004) reported the same results in guinea pigs.

Additionally, many studies reported that *P. ginseng* has a protective and preventive effect against testicular damage induced by many disorders, as the testicular damage induced by using anticancer drugs (Akram *et al.*, 2012; Jung *et al.*, 2015), radiation therapy in treatment of cancers (Gosselin and Mautner, 2002), streptozotocin-induced diabetes in rats (Sawiress *et al.*, 2011), toxins (Lee *et al.*, 2007; Wang *et al.*, 2012) and aging produced testicular damage (Hwang *et al.*, 2010). Millsop *et al.* (2013) explained the effect of ginseng extract in the enhancement of testicular function and the improvement of male rat's fertility by decreasing abnormal sperm% through inhibition of DNA damage, increasing the secretion of male reproductive hormones (testosterone, FSH and LH) and increasing expression levels of reproductive genes in the testis and pituitary. Also, Kim *et al.* (1999) reported that ginsenosides of *P. ginseng* are structurally resemble steroid hormones such as androgens, which is necessary for maintenance of male sexual characteristics and regulation of spermatogenesis. Solakidi *et al.* (2005) and Park *et al.* (2017) reported that ginsenosides stimulate the steroid receptors that found in the male genital tissues and spermatozoa, so it improves spermatogenesis. While Wang *et al.* (2012) reported that *P. ginseng* has effect on testis or pituitary due to its antioxidative effect.

The result of the present study showed a non-significant increase in sperm concentration, non-significant changes in abnormal sperm % and dead sperm % and non-significant increase in motility % in Gin NPs 30 group compared with control group. These results agree with Hassan

et al. (2024) who reported that rats treated with 30 mg/kg BW of ginseng bulk nanoparticles for 60 days showed significant increase in sperm concentration, non-significant changes in abnormal sperm % and dead sperm % and significant improvement in motility %. Linjawi (2015) reported that *P. ginseng* and *P. ginseng* nanoparticles ameliorated the testicular destruction induced by nicotine as it improved the male fertility, elevated serum free testosterone, LH, and FSH secretion with decreased abnormal sperm% by inhibition of DNA damage and increasing the expression levels of the fertility genes. Also, Ali *et al*. (2015) reported that ginseng and ginseng nanoparticles prevented nicotine-induced sperm abnormalities by inhibiting testicular apoptotic DNA damage caused by nicotine in rats. These findings are in line with Millsop *et al*. (2013) who reported that P. ginseng and *P. ginseng* nanoparticles ameliorated the testicular destruction induced by receiving Methotrexate (MTX) and the case of oligospermia, sexual dysfunction and reversible sterility.

Our results cleared that there was a non-significant increase in testosterone level in Gin100, non-significant decrease in Gin50 and non-significant differences in FSH and LH levels in Gin100 & Gin50 groups compared with control group. Furthermore, there was a non-significant change in testosterone and LH levels and non-significant differences in FSH in Gin NPs 30 group compared with control group. These results are in consistent with those of Hassan *et al*. (2024) who observed that oral intake of 50 and100 mg/kg BW of ginseng bulk resulted in non-significant changes in testosterone and FSH levels in both groups compared with control rats. However, there were significant increase in LH level in rats received Gin 100 mg/kg BW compared with Gin 50 and control rats and non-significant change at in LH level in Gin 50 compared with control rats. The obtained findings agree with Hassan *et al*. (2020) who recorded significant decrease in testosterone, non-significant changes in levels of both FSH and LH and increased sperm count, improved abnormal morphology %, motility and spermatogenesis in rats administered *P. ginseng* for 60 days compared with control, this effect of *P. ginseng* on sex hormones can be attributed to increased utilization of hormones by the testicle. This increased utilization of hormones can be explained by the finding of Kim *et al*. (2017) who mentioned that male rats showed significant increase in sex hormones receptors (androgen receptor, luteinizing hormone receptor and follicle stimulating hormone receptor) in *P. ginseng* treated groups. On the other hand, Yang *et al*., (2011) and Linjawi, (2015) reported that there was increase in serum testosterone level following *P. ginseng* extract administration. Also, Fahim *et al*. (1982) found that rats treated with 5% *P. ginseng* in the diet for 60 days significantly increased serum testosterone level, whereas treatment with 1% *P. ginseng* had no effect. It was also reported by Yoshimura *et al*. (1998) that rats administrated with American ginseng (10-100 mg/kg) For 28 days orally showed no significant change in testosterone and LH levels. These differences in the results may be attributed to the variations in dose and duration of administration of *P. ginseng*. Tsai *et al*. (2003) and Wang *et al*. (2010) concluded that ginsenoside Rb1 is the main active ingredient in *P. ginseng*, which is responsible for the increase in testosterone, FSH and LH levels by direct stimulation of anterior pituitary. Also, the ginsenoside of *P. ginseng* are needed for distribution of receptors of sex hormones which are needed for the action of these hormones on testis (park *et al*., 2017).

The results of the present study showed significant decrease in MDA level in Gin 100 group and non-significant decrease in Gin 50 group and showed non-significant increase in CAT level in Gin 50 and Gin 100 groups compared with control group. There was a significant decrease in MDA level in Gin NPs 30 group and a non-significant increase in CAT level in Gin NPs 30 compared with control group. The increased level of CAT and the decreased level of MDA after ginseng and nano-ginseng treatment in our study is due to the antioxidant activity of ginseng extract and its components so, its use decreased the oxidative stress marker like MDA and increased the antioxidants like CAT and these results are agreeing with Karadeniz *et al*. (2009); Zhao *et al*.(2009); Wang *et al*. (2012) and Linjawi (2015).

Our histological findings of the testicle tissue section from Gin 50 and Gin 100 mg/kg BW treated groups showed normal structured large sized seminiferous tubules lined by basement membrane rested on spermatogenic epithelium as spermatogonia, spermatocytes and flagellated spermatozoa fill the lumen of tubule with increase in sperms concentration, and normal interstitial cells, normal interstitial tissue and Leydig cell. The Sertoli cells were found throughout the entire thickness of the germinal epithelium from the basal lamina to the spermatids. The obtained findings agree with the results of Hassan *et al*. (2024) who reported that administration of ginseng bulk 50 and100 mg/kg BW to adult male rats showed normal structured large sized seminiferous tubule with spermatozoa filled lumen, normal interstitial cells, normal Leydig cells and increased number of Sertoli cells throughout the entire thickness of the germinal epithelium. There are many results of the previous studies obtained by using ginseng extract and in line with our results that obtained by using ginseng bulk. For example, Yang *et al*. (2011) and Hassan *et al*. (2020) who showed that administration of *P. ginseng* for mature rats for 60 days caused significant improvement in testicular histological findings and spermatogenesis. Also, Lee *et al*., (2007) reported that the histological examination of testicle of *P. ginseng* extract treated group showed normal testes with usual arrangement of tubular cells. Spermatogonia and Sertoli cells were found on the basement membrane surrounded by a concentric myofibroblast layer, and Leydig cells were scattered within the interstitial.

There are several studies mentioned that administration of *P. ginseng* has a protective effect against testicular damage induced by many disorders, Cho *et al*., (2011) reported that pre- and co-treatment with ginseng ameliorate zearalenone-induced testicular degenerative changes and impaired spermatogenesis by decreasing apoptotic DNA damage of germ cells. Kopalli *et al*. (2015) reported that ginseng treatment improved the histopathological disturbances on aged rat testis that induced by oxidative stress during aging so korean red ginseng treatment to aged rats reversed this morphology showing intact tubules, normal lumen and germ cells. Wang *et al*. (2009) demonstrated that ginsenosides were found to protect Sertoli cells in vitro against the cytotoxic damage of bisphenol A. Ginsenosides significantly inhibited bisphenol A-induced decrease in Sertoli cell viability and increase in apoptosis via inhibiting extracellular signal-regulated kinase (ERK 1/2) phosphorylation, modulation of Bcl- 2 and Bax protein expression in Sertoli cells. Our histological findings of the testicle tissue section from Gin NPs 30 mg/kg BW treated group showed normal structured large sized seminiferous tubule lined by intact basal lamina rest on it spermatogenic epithelium arranged in normal order as spermatogonia, with tuft of flagellated spermatozoa filled lumen. Normal interstitial cells contain normal Leydig cells. The Sertoli cells were found throughout the entire thickness of the germinal epithelium. our study is in accordance with Hassan *et al*. (2024) who reported that administration of ginseng bulk nanoparticles 30 mg/kg BW to adult male rats showed, normal structured large sized seminiferous tubule with tuft of flagellated spermatozoa filled lumen. Normal interstitial cells. The Sertoli cells were found throughout the entire thickness of the germinal epithelium in these treated group compared with control group. There are many results of the previous studies obtained by using ginseng extract nano- particle and in agreement with our results that obtained by using ginseng bulk nano- particle. Kamel *et al*. (2019) who reported that ginseng and ginseng nanoparticles pre-treatment of methotrexate in rats markedly recovered the testicular histopathological effects and impaired spermatogenesis induced by MTX via significantly increasing the Johnsen's tubular biopsy score (JTBS) due to its antiapoptotic and antioxidant actions.

The present study revealed a non-significant increase in the percentage area of androgen receptor protein expression in Gin 100 and non-significant decrease in Gin 50. there were non-significant increases in spermatogenesis and diameter of seminiferous tubules and non-significant changes in height of spermatogenic cells (SGs) of seminiferous tubules in both groups compared with control group. Many studies examined the effect of ginseng extract in androgen receptor protein expression, diameter of seminiferous tubules and height of spermatogenic cells and agree with our results. Kopalli *et al*. (2016) and Kim *et al*. (2017) mentioned that ginseng act directly on androgen receptors. Also, Lee *et al*. (2007) reported that treatment with *P. ginseng* extract in rats showed non-significant changes in seminiferous tubule diameters and Johnsen scores compared with control rats. The obtained result also, showed that there is a non-significant increase in the percentage area of androgen receptor protein expression and in spermatogenesis, significant increase in diameter of seminiferous tubules and non-significant changes in height of spermatogenic cells of seminiferous tubules in Gin NPs 30 treated group compared with the control group. There are no available literatures examined the effect of nano-ginseng extract or nano- ginseng bulk on androgen receptor protein expression, diameter of seminiferous tubules and height of spermatogenic cells.

The current study showed that using ginseng bulk nanoparticles is more effective than using ginseng bulk whole particles in the improvement of reproduction either in normal conditions or in the presence of reproductive disorders. Millsop *et al*. (2013) found that using ginseng extract nanoparticles was more effective than ginseng extract in enhancing male rat's fertility by inhibition of DNA damage, increasing secretion of reproductive hormones, and increasing expression levels of reproductive genes after MTX intake. Additionally, Karakoti *et al*. (2006); Bhattacharyya *et al*. (2010) and Linjawi *et al*. (2015) reported that ginseng extract nanoparticles were more effective than the *P. ginseng* extract in inhibition of nicotine-induced toxicity and enhancing the male fertility by acting directly on the pituitary hormones as it reduces prolactin production or on the central nervous system which increase dopaminergic actions.

Therefore, we could suggest that the nanoparticles of *Panax ginseng* increased the efficiency of the particles of this plant to arrive the target cells of the hypothalamus pituitary-testis axis to improve the fertility of male rats. Nanoparticles performed from plants known to contain high

content of flavonoids and considered as efficient carriers for the delivery and enhancing the drug bioactivity within the target cells and tissues. Therefore, the formulation of the particles of *P. ginseng* to nanoparticles enhanced the active ingredients such as flavonoids to reach testis and enhanced the function of the testis, which was reflected on increased serum testosterone, FSH and LH levels, increase in the genes encoding these hormones, and decrease sperm abnormalities. The latter effect may be attributed to the protection of the testicular tissue DNA from damage. It is suggested that the active ingredients ginsenosides may enhance its effect in different levels of the hypothalamus pituitary-testis axis when the *Panax ginseng* formulated in the nano-particles forms. In addition, Wen *et al*. (2009) reported that the reduction of particle size of plant extracts increases the surface area, thus improving absorption, bioavailability and release of functional ingredients. Oral bioavailability of ginseng can be enhanced by increasing the dose to saturate its metabolism or by changing its pharmaceutical formulation (Qi *et al*., 2011) such as nanoparticles formulation (Ali *et al*., 2015; Voruganti *et al*., 2015).

Conclusion

The use of ginseng bulk (50 and 100) mg/kg BW and ginseng bulk nanoparticle 30 mg/kg BW maintain the normal testicular function. Furthermore, Ginseng bulk and its nanoparticle considered the most suitable form of ginseng plant for medical use when compared with other forms of ginseng plant for its good effect. That is because it contains all the active ingredients of ginseng plant not only one or two ingredients like in ginseng extract, easy prepared, low costs.

Conflict of interest

The authors declare that they have no conflict of interest related.

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