International Journal of Comprehensive Veterinary Research

Article:

Pilot study to know the best doses of ginseng bulk and its nanoparticles in normal testicular function of rats

Hanan Hassan [1]*, Eid A. Mabrouk[2], Nermeen Atef [2], Hassan Ahmed [3], Fatma Abo Zakaib Ali [4].

[1]Department of Physiology, Faculty of Veterinary Medicine, Sohag University, Sohag, [2]Department of Physiology, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, [3] Department of Physiology, Faculty of Veterinary Medicine, South Valley University, Qena, [4]Department of Pathology and clinical pathology, Faculty of Veterinary Medicine, Sohag University, Sohag.

Received: 21 October 2023; Accepted: 21 November 2023; Published: 05 May 2024

Abstract

The present study is a trial to investigate the effective doses of ginseng bulk (Gin) and ginseng nanoparticles (Gin NPs) on male rat reproduction. One-hundred and twenty adult male rats were randomly distributed into three groups; control:10 rats received distilled water (1 ml). Gin group: subdivided into 6 subgroups (10 rats per each) were administered with ginseng at a dose of (20, 50, 100, 200, 400 and 600) mg/ kg BW. Gin NPs group: subdivided into 5 subgroups (10 rats per each) were administered Gin NPs (30, 60, 150, 300 and 450) mg/ kg BW. All rats received distilled water and drug solution orally/once a day/8 weeks. Hormonal assay, semen analysis, body weight gain, testis weight, gonadosomatic index and histological examination were carried out. Surprisingly, the higher doses of ginseng bulk (200, 400 and 600) and its nanoparticles (150, 300 and 450) were toxic, where rats died during the experimental period. All surviving rats showed no change in testosterone and FSH levels compared with control. However, rats treated with Gin 100 showed significant elevation in LH level compared to control, Gin 20 and Gin 50. In addition, rats treated with Gin NPs 30 and 60 showed significant increase in LH level compared with Gin 100 and 50, respectively. Rats treated with Gin 100 and Gin NPs 30 showed significant increase in sperm count. Besides, Gin NPs 30 showed significant increase in sperm motility compared with control. The histological examination of animals revealed normal testicular architecture, sperm stages, Sertoli and Leydig cells. However, in Gin 100, Gin NPs 30 groups showed significant number of spermatozoa fill the lumen of seminiferous tubules. In conclusion, the most effective doses of ginseng bulk and its nanoparticles were (20, 50 and 100) and (30 and 60), respectively, specifically Gin 100, Gin NPs 30 and 60 showed more improvement in sperm analysis and hormonal level.

Keywords: Ginseng bulk, Nanoparticles, Reproductive hormones, Spermatogenesis.

Introduction

Ginseng is a medicinal herbal plant that has been used for thousands of years as an adaptogen to increase physical energy and enhance fertility [1]. It has been reported to have positive effects on sexual dysfunction and sperm abnormalities [2,3]. Korean red ginseng (KRG) usually is taken orally to improve physical strength by people in East Asia for more than many years ago. Many studies indicated that KRG is used for therapeutic purposes such as diabetes [4], immune disorders [5] and stress and other dysfunctions [6]. In addition, it has been reported that the supplementation of KRG extract is effective to enhance the testicular function [7] and sperm viability as well as increase the quality of sperm in guinea pigs [8]. Moreover, several reasons are considered for using KRG for therapeutic purposes such as its lower price, availability, and safety.

The ginseng oral bioavailability is low due to low absorption rate of ginseng saponins [9]. Oral bioavailability of ginseng can be enhanced by increasing the dose to saturate its metabolism or by changing its pharmaceutical formulation. Furthermore, the reduction of particle size of

IJCVR *Corresponding author: Hanan Hassan, *Email: Hanan.hassan@vet.sohag.edu.eg, Address: [1]Department of Physiology, Faculty of Veterinary Medicine, Sohag University, Sohag, Egypt*

plant extracts increases the surface area, thus improving absorption, bioavailability, and release of functional ingredients [10]; such as nanoparticles formulation [11].

It has been reported that using ginseng nanoparticles was more effective than ginseng extract in enhancing male rat's fertility. The formulation of the ginseng particles to nanoform increased the ability of its active ingredients to reach the target cells of the hypothalamus-pituitary-testis axis and enhance the fertility of male rats [12]. Looking throughout the available literature; there is a limited and insufficient studies concerning the effect of ginseng bulk or ginseng bulk nanoparticles on animal reproduction. So, the present study is aimed to evaluate the effect of ginseng bulk and nano ginseng bulk on male reproduction.

Materials and Methods

Approval and registration

The ethical research committee of the Faculty of Veterinary Medicine at Sohag University approved the study's protocol (Approval number: Soh.un.vetm / 00039), and the animals were handled in accordance with the Egypt National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Reagents

Ginseng was purchased in 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).

Gin NPs were prepared by ball milling technique with 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 [13,14]. Characterization was carried out by using X-ray diffraction (XRD) and the morphology of the prepared nanoparticles was investigated by using transmission electron microscopy (TEM) was performed on JEOL JEM-2100 high resolution transmission electron microscope at an accelerating voltage of 200 KV, respectively and their elemental quantification was performed by energy-dispersive spectroscopy (EDS) attached to SEM. Optical properties were studied by using UV / VIS spectroscopy [15].

Animals

One hundred twenty mature male Wistar albino rats of average body weight 250 ± 10 g and of age 8 weeks [16] were purchased from Lab animal house, faculty of Medicine, Sohag university. 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 for one weeks to be acclimatized laboratory environment [17].

Experimental Design

After the end of acclimatization period, rats were randomly distributed into three main groups:

Group I (control): 10 rats received distilled water (1 ml / day) .

Group II (Gin group): 60 rats that subdivided into 6 equal subgroups (10 rats per each) administered with Korean red ginseng roots powder extract (20, 50, 100, 200, 400 and 600) mg / kg BW dissolved in 1 ml / day distilled water .

Group III (Gin NPs group): 50 albino male rats divided into 5 equal subgroups (10 rats per each) were gavages with Gin NPs powder extract (30, 60, 150, 300, and 450) mg / kg BW dissolved in 1 ml/ day distilled water.

Dry powder of Gin NPs was suspended in deionized water, then sonicated at room temperature for 10 min to form a homogeneous suspension. The time interval from preparation to oral gavage was limited to 20 min to ensure that the NPs will not be aggregated before administration. All rats received distilled water and drug solution orally using drenching tube for eight weeks.

Recording of the body and reproductive organs weights:

The initial body weights (I.W) of all groups in the experiment were measured just before starting the treatment in the experimental period and weighing of rats was done continuously weekly throughout the experimental period. At the same time, the final body weights of all experimental rats were recorded at sacrificing time. Moreover, the testes and epididymis from each rat were excised out quickly, weighed to calculate the gonadosomatic index by using the following formula according to Kumari and Singh [18]. Testes weight (g) / Final body weight (g) x 100.

Blood samples collection

At the end of experimental period, rats were anesthetized by diethyl ether; individual blood samples were collected from retro-orbital venous plexus from each group in test tubes without anticoagulant. Blood was left to be coagulated then centrifuged at 3000 rpm for 15 minutes. The sera were collected separately in Eppendorf tubes and kept at -80°C until hormonal assay.

Hormonal Assay

The frozen serum was thawed for measuring serum testosterone Catalogue NO. (TE187S), Follicle stimulating hormone (FSH) Catalogue NO. (FS232F) and Luteinizing hormone (LH) Catalogue NO. (LH231F) (Calbiotech, El Cajan, CA, USA). The assay was performed by enzyme-linked immunosorbent assay (ELISA) kits according to manufacturer's instructions [19, 20, 21] respectively, using microplate reader (Infinite 50, Männedorf, Switzerland) at wavelength 450 nm.

Testes samples collection

At the end of the experimental periodrats were sedated with an intraperitoneal dose of sodium thiopental (50 mg / kg BW, IP), then sacrificed and the testes were excised out quickly for epididymal semen samples collection and histological examination.

Semen analysis

Semen was collected from the epididymis and diluted in warmed physiological saline at 37°C according to D'Souza [22]**.** Sperm motility and vitality (dead and live percent) were evaluated by placing one drop of semen was applied simultaneously on a dry, clean and warm slide then the percent of dead and live sperms were calculatedaccording to Esteso et al. [23]. According to Wyrobek and Bruce [24] 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 [25]**.**

Histological examination

Testes tissue samples from the experimental groups (Control, Gin 100, Gin 50, Gin 20, Gin NPs 30 and 60) were dissected from epididymis, weighed, and quickly fixed in neutral buffered paraformaldehyde (PFA) 4 %, processed through the conventional paraffin embedding technique [26], sectioned at 5 µm thick and stained with Hematoxylin and Eosin (H & E) for histological examination.

Statistical analysis

Collected data were analyzed statistically by GraphPad prism 8 (GraphPad Software, San Diego, California USA). Data were expressed as mean ± standard error of mean (SEM) and differences between groups were analyzed by using one-way analysis of variance (ANOVA). Means were compared by Tukey's test when a significant difference was detected. The P value less than 0.05 was considered to be statistically significant compared with control [27].

Results

1- Physical characteristics of Gin NPs

The produced Gin NPs are pale brown colored powder that dispersed in H_2O, it has average size (TEM): 60 10 nm (L), 25 ± 5 nm (D) and rod- like shape **Figure 1 A and B.**

2- Animal survivability

Few days from the beginning of the experiment, most of rats of group II received 200, 400 and 600 mg/ kg Gin bulk extract and those of group III received 150, 300 and 450 mg/ kg Gin NPs showed severe loss of body weight and emaciation, and then died before the end of the experimental time. However, the survived numbers of rats in both groups till the end of the experiment were slaughtered to collect blood, semen and testes samples.

3- Effect of ginseng bulk form and its nanoparticles on sperm parameters

Rats treated with Gin 100 and Gin NPs 30 showed a significant ($p \leq 0.05$) increase in sperm concentration (106/ µl) compared with the corresponding control. Meanwhile, sperm concentration decreased significantly ($p \leq 0.05$) in the Gin 60 subgroup compared with Gin 100. Rats received Gin 100 and Gin NPs 60 denoted improvement of semen quality by decreasing abnormal sperm percent non-significantly compared with the control and other treated groups. Similarly, all treated groups showed no change in dead sperm percent except that treated with Gin NPs 60 which denoted non-significant change in dead sperm percent compared with the control and other treated groups. Additionally, Gin NPs 30-treated rats denoted a significant ($p \leq 0.05$) elevation in sperm motility percent compared with the control group **Table 1.**

4- Effect of ginseng bulk form and nanoparticles on male reproductive hormones

In survived treated rats, testosterone and FSH level denoted a non-significant change compared with the corresponding control as disclosed in Table2. However, rats treated with 100 mg / kg ginseng extract showed significant ($p \leq 0.05$) increase of LH level compared with the control, Gin 20 and Gin 50 subgroups. Further, rats administered 30 mg/kg Gin NPs denoted a significant ($p \leq 0.05$) decrease in LH level compared with Gin 100 subgroup. Moreover, rats received 60 mg/ kg Gin NPs disclosed a non-significant change in LH level compared with the corresponding control and a significant ($p \leq 0.05$) increase compared with Gin 50 group **Table 2.**

5- Effect of ginseng bulk form and nanoparticles on Body weight gain (g), testis weight (g) and the gonadosomatic index (GSI) in control and treated rats.

The body weight gain, testis weight and the gonadosomatic index after administration of ginseng powder 20, 50 and 100 mg/kg and ginseng NPs 30 and 60 mg / kg for 8 weeks were assessed. All treated rats showed non-significant changes compared with the control group **Table 3**.

Table (1): Epididymal semen analysis in control and treated rats after administration with Gin (20, 50 and 100) and Gin NPs (30 and 60) mg / kg BW for 60 days.

Group	Concentration (10^6 / ml)	Abnormal sperms %	Dead sperms %	Motility %
Control	217.64 ± 41.87	3.20 ± 0.73	3.60 ± 0.68	78 ± 4.06
Gin 20	292.24 ± 45.70	3.40 ± 0.51	3 ± 0.84	88.6 ± 1.86
Gin 50	272.90 ± 78.68	3.80 ± 0.73	3.20 ± 0.73	86 ± 1.87
Gin 100	496.10 ± 44.14*	2.40 ± 0.60	2.20 ± 0.37	89 ± 4
Gin NPs 30	362.40 ± 53.17*	3.80 ± 0.80	3.40 ± 0.60	93.6 ± 2.23*
Gin NPs 60	237.50 ± 60.69&	2.60 ± 0.24	4.80 ± 1.46	90 ± 3.87

*Values are expressed in mean ± S.E.M. * $p \leq 0.05$ compared with control, & $p \leq 0.05$) compared with Gin 100.*

Table 1 Serum level of reproductive hormones in control and Gin (20, 50 and 100) and Gin NPs (30 and 60) mg /kg BW treated rats for 60 days.

Group	Testosterone	FSH	LH
Control	10.00 ± 1.10	17.80 ± 1.05	6.05 ± 00.64
Gin 20	8.57 ± 00.66	16.57 ± 1.10	6.31 ± 00.80
Gin 50	9.80 ± 1.48	18.41 ± 1.62	5.33 ± 00.89
Gin 100	8.80 ± 00.62	15.13 ± 00.70	11.61 ± 00.84*Δ$
Gin NPs 30	11.96 ± 00.83	14.79 ± 00.60	7.69 ± 1.15&
Gin NPs 60	9.17 ± 00.78	15.47 ± 1.53	9.21 ± 00.53$

*Values are expressed in mean ± S.E.M. * $p \leq 0.05$ compared with control, Δ $p \leq 0.05$ compared with Gin 20, $ $p \leq 0.05$ compared with Gin50 and & $p \leq 0.05$ compared with Gin 100.Testosterone (ng / ml), Follicle stimulating hormone (FSH) (MIU / mL) and Luteinizing hormone (LH) (MIU / mL).*

Table 2 Body weight gain (g), testis weight (g) and the gonadosomatic index (GSI) in control and Gin (20, 50 and 100) and Gin NPs (30 and 60) mg / kg BW treated rats for 60 days.

Group	Body weight gain (g)	Testis weight (g)	GSI
Control	115.60 ± 26.09	2.65 ± 0.13	0.89 ± 0.05
Gin 20	58 ± 11.56	2.53 ± 0.07	1 ± 0.09
Gin 50	86.80 ± 19.31	2.56 ± 0.12	0.84 ± 0.06
Gin 100	74.20 ± 10.79	2.33 ± 0.06	0.84 ± 0.03
Gin NPs 30	63 ± 13.19	2.39 ± 0.16	0.87 ± 0.05
Gin NPs 60	83.20 ± 20.23	2.45 ± 0.07	0.85 ± 0.08

values are expressed in mean ± S.E.M.

6- Histological assessment

The testicle tissue section from the control group showed normal structured seminiferous tubule and interstitial cells lined by basement membrane. The Sertoli cells were found with prominent nucleus throughout the entire thickness of the germinal epithelium from the basal lamina to the spermatids, normal myoid cells rest on basement membrane. Lumen of seminiferous tubules filled with flagellated sperms and normal blood vessels. The Sertoli cells were found throughout the entire thickness of the germinal epithelium from the basal lamina to the spermatids and spermatozoa flagella fill the lumen of tubule **(Figure 2 A)**. Besides, the testicle tissue section from all treated groups showing normal structured large sized seminiferous tubule with improvement in the spermatogenic cycle as the tubules appear lined by germ cells in order from basal lamina to center as spermatogonia, spermatocytes and flagellated spermatozoa fill the lumen of seminiferous tubule, the highest concentration of spermatozoa is found in Gin 100 and Gin NPs 30 treated subgroups. The Sertoli cells were found throughout the entire thickness of the germinal epithelium from the basal lamina to the spermatids. Moreover, normal interstitial cells contain normal Leydig cells and normal blood vessels except inGin 20 treated group that showed congestion in blood vessels **(Figure 2 B-F).**

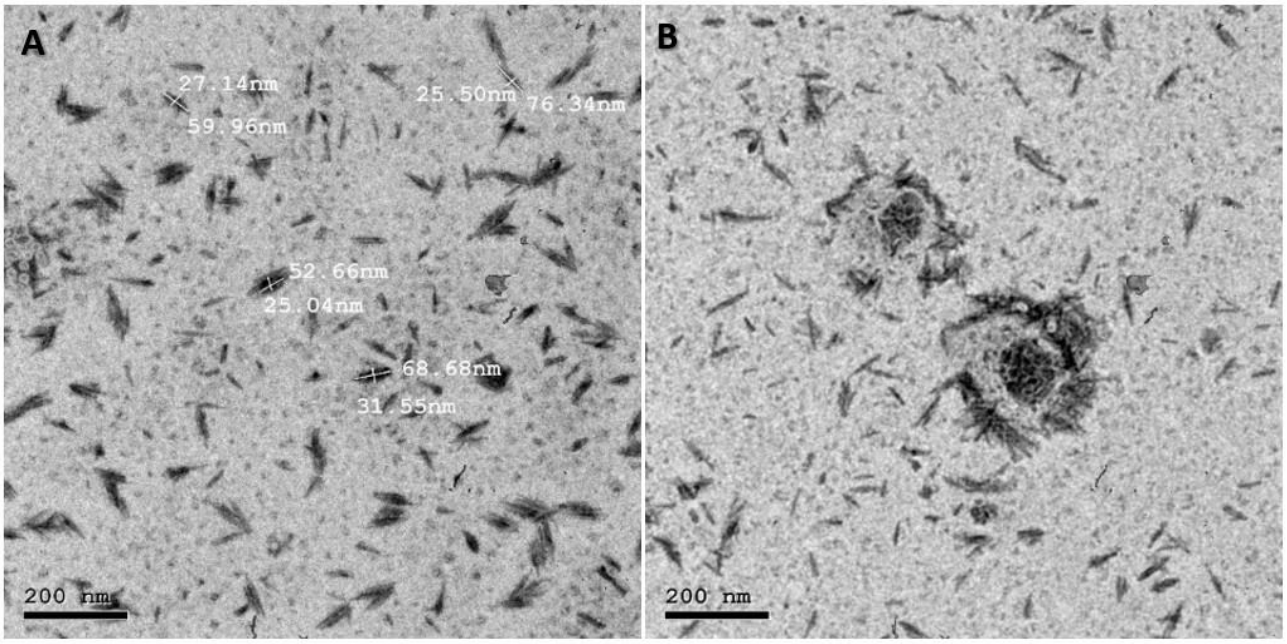

Figure 1. Transmission electron microscopy (TEM) images of cross-sections of powder particles produced after different milling times.

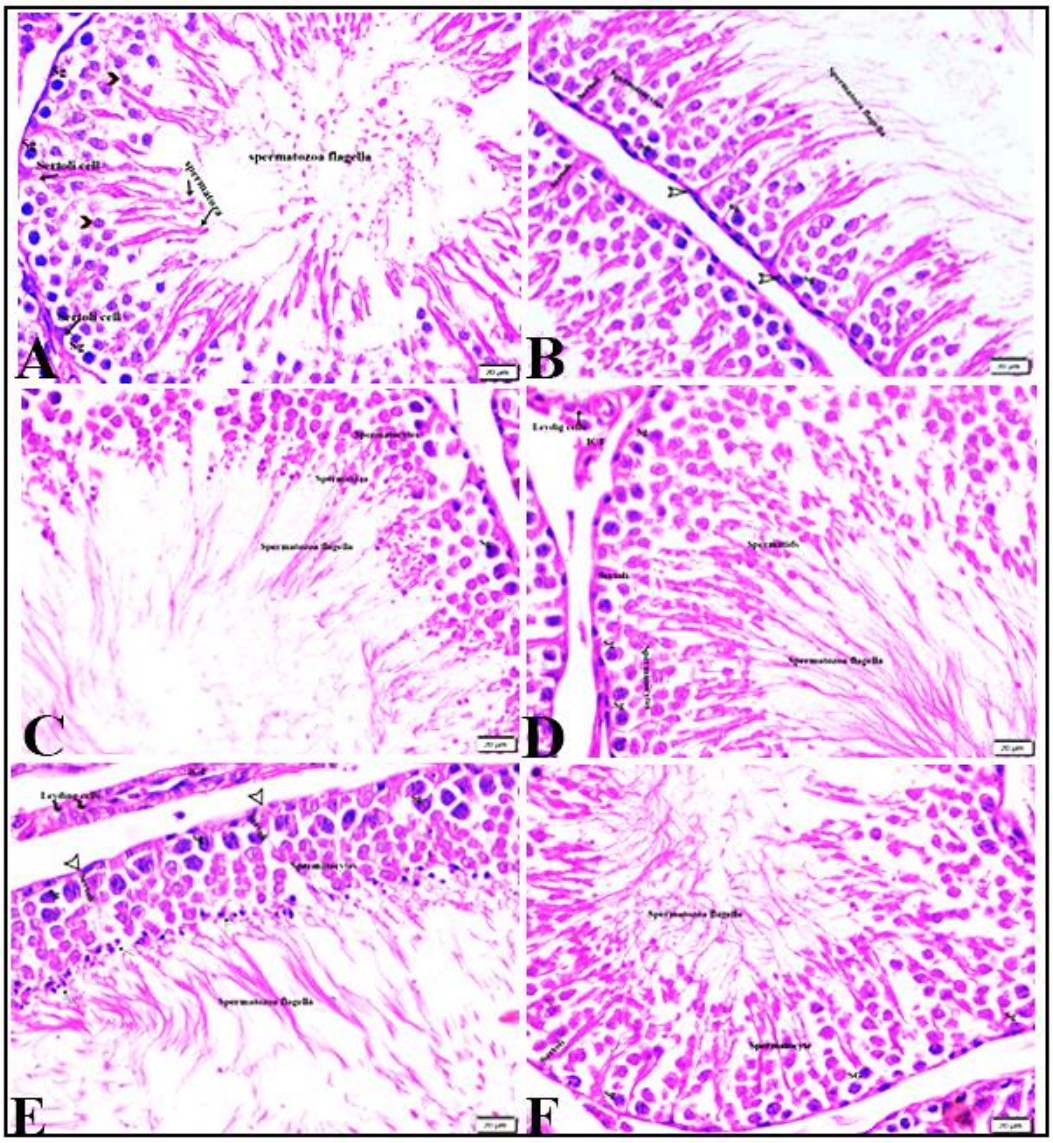

Figure (2): Photomicrograph of testis in control and treated rats. *control group showing normal structured seminiferous tubule and interstitial cells, lined by basement membrane (arrowheads), the Sertoli cells were found with prominent nucleus (zigzag arrow) throughout the entire thickness of the germinal epithelium, normal myoid cell. Lumen of seminiferous tubules filled with flagellated sperms. Normal blood vessels (a). Testicular tissue section from the Gin 20 mg/ kg BW treated group showing normal structured large sized seminiferous tubule lined by germ cells in order from basal lamina (arrowheads) with spermatozoa filled lumen, congested blood vessels (arrowheads)The Sertoli cells were found throughout the entire thickness of the germinal epithelium from the basal lamina to the spermatids (b). Testis of rats administrated the Gin 50 mg / kg BW showing normal structured seminiferous tubule lined by germ cells as spermatogonia (Sg), spermatocytes and flagellated spermatozoa fill the lumen of seminiferous tubule, normal interstitial cells contain normal blood vessels (arrowheads) (c). Testis of rats from the Gin 100 mg / kg BW treated group showing normal structured large sized seminiferous tubule lined by germ cells 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. Interstitial cells contain normal Leydig cells (d).Testicular tissue section from the Gin NPs 30 mg / kg BW treated group showing normal structured large sized seminiferous tubule with lined by intact basal lamina (arrowheads) rest on it spermatogenic epithelium arranged in normal order as spermatogonia (Sg), 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 normal Leydig cells (e). Testis of rats administrated with Gin NPs 60 mg / kg BW showing normal structured large sized seminiferous tubule lined spermatogenic epithelium arranged in normal order as spermatogonia, 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, Normal interstitial cells (f). (H & E bar size = 20 µm).*

Discussion

Our results showed that majority of rats given Gin at doses of (200, 400 and 600) mg / kg BW and rats given Gin NPs at doses of (150, 300 and 450) mg/ kg BW are died before the end of the experiment, and this indicates that these doses are toxic to the animals. While animals were given Gin at doses of (20, 50 and 100) mg/kg BW and rats given Gin NPs at doses of (30 and 60) mg / kg BW showed accepted results.

The current obtained results showed significant increase in sperm concentration in rats administrated Gin 100 and non-significant increase in Gin 20 and Gin 50 treated groups compared with control group. Also showed a non-significant decrease in the abnormal sperm % and dead sperm % in Gin 100 treated groups and non-significant change in Gin 20 and Gin 50 treated groups compared with control group. Moreover, the data showed non-significant change in motility % in Gin 20, Gin 50 and Gin 100 treated groups compared with control group.

There are many previous studies performed by using ginseng extract and support our results that obtained by using ginseng bulk. Hassan et al. [3] who showed that administration of Panax ginseng extract on mature rats for 60 days significantly increased sperm concentration with decrease in abnormal sperm % and improvement of motility % compared with control. Park et al. [28] found that adult male rats treated with P. ginseng (100, 500, 1000 mg / kg) for 5 days per week for 5 weeks showed significant improvement in sperm motility % from control. As, they found that P. ginseng caused induction of CatSper expression which is a protein stimulate the motility of sperm. The same results were obtained by Eskandari et al. [29] who found that P. ginseng improved the normal sperm %, mass motility % and individual motility %.

Kim et al. [7] reported that treatment with KRG extract is effective to enhance the testicular function. The effect of P. ginseng on testicular function is produced through the ginsenosides of P. ginseng. Ginsenosides structurally resemble steroid hormones such as, androgens which are important for improvement and maintenance of male sexual characteristics and regulate spermatogenesis [30]. Ginsenosides affect male sexual functions and spermatogenesis by stimulation of steroid receptors which found in male genital tissues and spermatozoa [2, 31]. The effect of P. ginseng on testis or pituitary is because it has antioxidative effect [32].

The obtained results of the current study also, showed significant increase in sperm concentration in rats administrated Gin NPs 30 and non-significant increase in rats administrated Gin NPs 60 compared with their control and significant decrease in Gin NPs 60 group compared with Gin 100. Also, show non-significant changes in abnormal sperm % and dead sperm % in Gin NPs 30 and Gin NPs 60 treated groups compared with control group.In addition, showed that there is significant improvement in motility % in Gin NPs 30 treated group and non-significant improvement in motility % in Gin NPs 60 treated group compared with control group.

There are many previous studies obtained by using nano-ginseng extract and in agree with our results that obtained by using nano-ginseng bulk For example, Linjawi,. who reported thatginseng and ginseng nanoparticles treatment ameliorated the testicular destruction induced by nicotine and improved the male fertility with decreased abnormal sperm %. Moreover, Panax Ginseng (P. ginseng) treatment inhibited the DNA damage, it elevated the serum free testosterone LH, and FSH secretion and increased the expression levels of the fertility genes [33]. P. ginseng and P. ginseng nanoparticles treatment ameliorate the testicular destruction induced by receiving Methotrexate (MTX)and the case of oligospermia, sexual dysfunction and reversible sterility [34].

The results of the present study showed that there are non-significant changes in testosterone and FSH levels in rats treated with Gin (20, 50 and 100) compared with control group. However, there is significant increase in LH level in rats received Gin100 mg/kg BW compared with control, Gin 20 and Gin 50 groups and there is non-significant increase in LH level in Gin 20 group. While, there is non-significant decrease at in LH level in Gin 50 compared with control rats.

There are many previous studies concerning the effect of ginseng extract on the male reproductive hormones and similar to our results that obtained by using ginseng bulk. Hassan et al. [3] who showed that there was significant decrease in testosterone level in rats administered P. ginseng for 60 days compared with control. But there were non-significant changes in levels of both FSH and LH compared with control with increased sperm count, improved abnormal morphology %, motility and spermatogenesis, so 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. [35] 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, there are some previous studies used ginseng extract reported different results from our study that used ginseng bulk whole particle. For example, yang et al. [36] who reported that there was increase in serum testosterone level following P. ginseng extract administration. Also, Linjawi [33] mentioned significant

elevation in serum testosterone, LH and FSH levels in rats treated with P. ginseng.

It was detected that the main active ingredient in P. ginseng is ginsenoside Rb1 and is responsible for the increase in testosterone, FSH and LH levels by direct stimulation of anterior pituitary [37,38]. 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 [31].

The results of the present study showed that there are non-significant changes in testosterone, FSH and LH levels in Gin NPs 30 and Gin NPs 60 groups compared with control group. While rats administered Gin NPs 30 mg/kg denoted a significant decrease in LH level compared with Gin 100 group. Moreover, rats received 60 mg/ kg Gin NPs disclosed significant increase in LH level compared with Gin 50 group.

There are no, available literature that detect the effect of nano-ginseng extract or nano- ginseng bulk on male reproductive hormones.

The obtained data clears no significant changes in the body weight gain, testis weight and the gonadosomatic index after administration of ginseng powder 20, 50 and 100 mg / kg for 8 weeks compared with the control group.

There are many previous studies obtained by using ginseng extract and in line with our results that obtained by using ginseng bulk. Yang et al. [36] reported that there was a little difference in body weight of rats administrated with P. ginseng extract (1.0 gm / kg/ day) for 56 days compared with control group. Also, Hwang et al. [39] and Kopalli et al.[40]said that there was no significant change in testis weight after treatment with p. ginseng extract in old rats. Mice treated with P. ginseng extract (50 mg / kg daily) orally for 4 weeks showed non- significant change in body weight and testis weight in both young and aged rats compared with control [41]. There was significant decrease of weight gain in mice treated with P. ginseng extract. P. ginseng has anti-obesity and anti-diabetic effect, these effects produced due to inhibition of pancreatic lipase produced by P. ginseng [42,43]. The inhibition of pancreatic lipase is due to p. ginseng contain saponin fraction (ginsenoside Rb1) which is responsible for inhibition of pancreatic lipase, and which produced decrease in body weight gain [44,45,46].

The results of the present study showed non-significant decrease in body weight gain, testis weight and gonadosomatic index in NPs 30 group and Gin NPs 60 group compared with control group.

There are no available literatures studied the effect of nano-ginseng extract or nano- ginseng bulk on body weight gain, testis weight and gonadosomatic index. In the present study the histological examination of the testicle tissue in Gin 20, Gin 50 and Gin 100 treated groups showed that, the testicle tissue sections from all treated groups showing normal structured large sized seminiferous tubule with spermatozoa filled lumen, but there are congested blood vessels in Gin 20 treated group, normal interstitial cells contain normal blood vessels in Gin 50 group and seminiferous tubule lined by germ cells as spermatogonia in Gin 100 treated group.

The Sertoli cells were found with prominent nucleus throughout the entire thickness of the germinal epithelium from the basal lamina to the spermatids in Gin 20 and Gin 100 treated groups with normal myoid cell rest on basement membrane in Gin 20 treated group and interstitial cell contain normal Leydig cells in Gin 100 treated group compared with control group.

There are many previous studies obtained by using ginseng extract and in line with our results that obtained by using ginseng bulk. Yang et al. [36] Hassan et al. [3] who showed that administration of P. ginseng for mature rats for 60 days caused significant improvement in histological findings and spermatogenesis.

In the present study the histological findings of the testicle tissue in Gin NPs 30 and Gin NPs 60 groups showed that, normal structured large sized seminiferous tubule with tuft of flagellated spermatozoa filled lumen. Normal interstitial cells. the seminiferous tubule lined by spermatogenic epithelium arranged in normal order as spermatogonia, 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 in these treated groups compared with control group.

The current study also showed that using ginseng bulk nanoparticles is more effective than using ginseng bulk whole particles in the improvement of reproduction in normal conditions. There are many previous studies obtained by using nano ginseng extract and similar to our results that obtained by using nano ginseng bulk. Millsop et al. [34] found that using ginseng extract nanoparticles was more effective than ginseng extract in enhancing male rat's fertility by decreasing sperm abnormalities through inhibition of DNA damage, increasing secretion testosterone, FSH and LH with increasing expression levels of reproductive genes in the testis and pituitary studies in case of oligospermia, sexual dysfunction and reversible sterility induced by MTX intake.

Conclusion

We concluded that the doses of ginseng bulk whole particles (20, 50 and 100) mg / kg BW and the doses of ginseng bulk nanoparticles (30 and 60) mg/kg BW are recommended and can be used to improve male reproduction.

Authors' contribution

The work was equally distributed between authors. All authors have read and approved the final version of the manuscript.

Conflict of interest

There is no conflict of interest.

References

1. **Gray SL, Lackey BR& Boone WR.** Effects of Panax ginseng, zearalenol, and estradiol on sperm function. Journal of Ginseng Research. 2016; 40 (3): 251-259.

2. **Leung KW and Wong AS.** Ginseng and male reproductive function. Journal of Spermatogenesis. 2014; 3(3): e26391.

3. **Hassan H, Mabrouk EA, Moghazy HM, Al-Amgad Z & Ahmed H.** Ameliorative effect of Panax ginseng and/or Ginkgo biloba on re-productive and thyroid functions in mature albino rats. Journal of Experimental and Applied Animal Sciences. 2020; 3 (2): 128-142.

4. **Yuan HD, Shin EJ & Chung SH.** Anti-diabetic effect and mechanism of Korean red ginseng in C57BL/KsJ db/db mice. Journal of Ginseng Research. 2008; 32 (3): 187-193.

5. **Sung H, Kang SM, Lee MS, Kim TG & Cho YK.** Korean red ginseng slows depletion of CD4 T cells in human immunodeficiency virus type 1-infected patients. Clinical and Vaccine Immunology. 2005; 12 (4): 497-501.

6. **Kim HS, Chung MW, Jang CG, Park WK & Oh KW.** Effects of ginseng total saponin on stress-induced analgesia. Journal of Ginseng Research. 1993; 17 (1): 22-28.

7. **Kim W, Hwang S, Lee H, Song H & Kim S.** Panax ginseng protects the testis against 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin induced testicular damage in guinea pigs. BJU international. 1999; 83 (7): 842-849.

8. **Hwang SY, Kim WJ, Wee JJ, Choi JS, Kim SK.** Panax ginseng improves survival and sperm quality in guinea pigs exposed to 2,3,7,8-tetrachlorodibenzo- p-dioxin. BJU international. 2004; 94 (4): 663-668.

9. **Qi LW, Wang CZ, Du GJ, Zhang ZY, Calway T and Yuan CS.** Metabolism of ginseng and its interactions with drugs. Current Drug Metabolism. 2011; 12 (9): 818–822.

10. **Wen HW, Li WC, Chung RJ, Yin SY, Chou TH, Hsieh PC& Lin I.** Evaluation of nanofabricated ginseng extract powders. Journal of Nanoscience and Nanotechnology. 2009; 9 (7): 4108–4115.

11. **Ali E, Tousson E, Abdel Daim H.** Effect of short- and long-term growth promoter boldenoneundecylenate treatments on antioxidant enzyme activity and oxidative stress markers in rabbit muscles. Pharmacologia. 2015; 7: 576–581.

12. **Fawad A, Naila K, Muhammad A, Qurban A & Niazi NK.** Crop improvement through conventional and non-conventional breeding approaches for grain yield and quality traits in Zea mays. Life Science Journal. 2015; 12 (4s): 38-50.

13. **Wei X, Wang X, Gao B, Zou W, Dong L.** Facile ball-milling synthesis of CuO/ Biochar nanocomposites for efficient removal of reactive red 120. ACS Omega. 2020; 5 (11): 5748-5755.

14. **Amusat SO, Kebede TG, Dube S& Nindi MM.** Ball-milling synthesis of biochar and biochar–based nanocomposites and prospects for removal of emerging contaminants: A review. Journal of Water Process Engineering. 2021; 41: 101993.

15. **Shashanka R.** Investigation of optical and thermal properties of CuO and ZnO nanoparticles prepared by Crocus Sativus (Saffron) flower extract. Journal of the Iranian Chemical Society. 2021; 18 (2): 415-427.

16. **Sengupta P.** A scientific review of age determination for a laboratory rat: how old is it in comparison with human age. Biomedicine International. 2011; 2 (2): 81-89.

17. **Ma XM& Lightman SL.** The arginine vasopressin and corticotrophin releasing hormone gene transcription responses to varied frequencies of repeated stress in rats. The Journal of physiology. 1998; 510 (Pt 2): 605-614.

18. **Kumari M & Singh P.** Study on The Reproductive Organs and Fertility of The Male Mice following Administration of Metronidazole. International journal of fertility & sterility. 2013; 7 (3): 225–238.

19. **Chen A, Bookstein JJ, Meldrum DR.** Diagnosis of a testosterone-secreting adrenal adenoma by selective venous catheterization. Fertility and sterility. 1991; 55 (6): 1202-1203.

20. **Qiu Q, Kuo A, Todd H, Dias JA, Gould JE, Overstreet JW, Lasley BL**. Enzyme immunoassay method for total urinary follicle-stimulating hormone (FSH) beta subunit and its application for measurement of total urinary FSH. Journal of Fertility and Sterility. 1998; 69 (2): 278-285.

21. **Frank JE, Faix JE, Hermos RJ, Mullaney DM, Rojan DA, Mitchell ML, Klein RZ.** Thyroid function in very low birth weight infants: effects on neonatal hypothyroidism screening. The Journal of pediatrics. 1996; 128 (4): 548-54.

22. **D'Souza UJ.** Toxic Effects of 5-Fluorouracil on Sperm Count in Wistar Rats. The Malaysian journal of medical sciences. 2003; 10 (1): 43-45.

23. **Esteso MC, Soler AJ, Fernandez Santos MR, Quinter Moreno AA& Garde JJ.** Functional significance of the sperm head morphometric size and shape for determining freezability in Iberian red deer (Cervus elaphus hispanicus) epididymal sperm samples. Journal of Andrology. 2006; 27 (5): 662-670.

24. **Wyrobek AJ and Bruce WR.** The induction of sperm-shape abnormalities in mice and humans. Chemical mutagens. 1978; 5: 257-285.

25. **Srinivasulu K and Changamma CA**. Study on the Effect of Ocimum sanctum (Linn.) Leaf Extract and Ursolic Acid on Spermatogenesis in Male Rats. Indian Journal of Pharmacological Science. 2017; 79 (1): 158-163.

26. **Bancroft J& Gamble M.** Theory and practice of histological techniques, 5th Edition. London Edinburgh New York Philadelphia, St. Louis Sydney, Toronto, 2002; 53: 5143-5147.

27. **Brown AM.** A new software for carrying out one-way ANOVA post hoc tests. Computer methods and programs in biomedicine. 2005; 79 (1): 89-95.

28. **Park EH, Kim DR, Kim HY, Park SK& Chang MS.** Panax ginseng induces the expression of CatSper genes and sperm hyperactivation. Asian Journal of Andrology. 2014; 16 (6): 845-851.

29. **Eskandari M, Jani S, Kazemi M, Zeighami H, Yazdinezhad A, Mazloomi S& Shokri S.** Ameliorating Effect of Ginseng on Epididymo-Orchitis Inducing Alterations in Sperm Quality and Spermatogenic Cells Apoptosis following Infection by Uropathogenic Escherichia coli in Rats. Cell journal. 2016; 18 (3): 446-457.

30. **Solakidi S, Psarra AMG, Nikolaropoulos S& Sekeris CE.** Estrogen receptors α and β (ERalpha and ERbeta) and androgen receptor (AR) in human sperm: localization of ERbeta and AR in mitochondria of the midpiece. Human Reproduction. 2005; 20 (12): 3481-3488.

31. **Park J, Song H, Kim SK, Lee MS, Rhee DK& Lee Y.** Effects of ginseng on two main sex steroid hormone receptors: estrogen and androgen receptors. Journal of Ginseng Research. 2017; 41(2): 215- 221.

32. **Wang L, Hao J, Hu J, Pu J, Lu Z, Zhao L, Wang Q, Yu Q, Wang Y& Li G.** Protective Effects of Ginsenosides against Bisphenol A-Induced Cytotoxicity in 15P-1 Sertoli Cells via Extracellular Signal- Regulated Kinase 1/2 Signalling and Antioxidant Mechanisms. Basic & Clinical Pharmacology & Toxicology. 2012; 111(1): 42-49.

33. **Linjawi SA.** Evaluation of the protective effect of Panax ginseng nanoparticles against nicotine-induced reproductive disorders in male rats. Journal of Pharmacological Science Researches. 2015; 32 (1): 38-45.

34. **Millsop JW, Heller MM, Eliason MJ and Murase JE.** Dermatological medication effects on male fertility. Dermatologic Therapy. 2013; 26 (4): 337-346.

35. **Kim MK, Cha KM, Hwang SY, Park UK, Seo SK, Lee SH, Jeong MS, Cho S, Kopalli SR& Kim SK.** Pectinase-treated Panax ginseng protects heat stress-induced testicular damage in rats. Reproduction. 2017; 153 (6): 737-747.

36. **Yang WM, Park SY, Kim HM, Park EH, Park SK& Chang MS.** Effects of Panax ginseng on glial cellderived neurotrophic factor (GDNF) expression and spermatogenesis in rats. Phytotherapy research. 2011; 25 (2): 308-311.

37. **Tsai SC, Chiao YC, Lu CC& Wang PS.** Stimulation of the secretion of luteinizing hormone by ginsenoside-Rb1 in male rats. Chinese Journal of Physiology. 2003; 46 (1): 1-7.

38. **Wang X, Chu S, Qian T, Chen J& Zhang J.** Ginsenoside Rg1 improves male copulatory behavior via nitric oxide/cyclic guanosine monophosphate pathway. journal of sexual medicine. 2010; 7 (2 Part 1): 743-750.

39. **Hwang SY, Sohn SH, Wee JJ, Yang JB, Kyung JS, Kwak YS& Kim SK.** Panax ginseng improves senile testicular function in rats. Journal of Ginseng Research. 2010; 34 (4): 327-335.

40. **Kopalli SR, Hwang SY, Won YJ, Kim SW, Cha KM, Han CK& Kim S K.** Korean red ginseng extract rejuvenates testicular ineffectiveness and sperm maturation process in aged rats by regulating redox proteins and oxidative defense mechanisms. Experimental gerontology. 2015; 69: 94-102.

41. **Ku JY, Park MJ, Park HJ, Park NC& Joo BS.** Combination of Korean Red Ginseng Extract and Hydrogen-Rich Water Improves Spermatogenesis and Sperm Motility in Male Mice. Chinese Journal of Integrative Medicine. 2020; 26: 361-369.

42. **Kim HJ, Kang HJ, Seo JY, Lee CH, Kim YS& Kim JS.** Antiobesity effect of oil extract of ginseng. Journal of medicinal food. 2011; 14 (6): 573-583.

43. **Li Z, Kim HJ, Park MS& Ji GE.** Effects of fermented ginseng root and ginseng berry on obesity and lipid metabolism in mice fed a high-fat diet. Journal of ginseng research. 2018; 42 (3): 312-319.

44. **Kim JH, Hahm DH, Yang DC, Kim JH, Lee HJ& Shim I.** Effect of crude saponin of Korean red ginseng on high-fat diet-induced obesity in the rat. Journal of pharmacological sciences. 2005; 97 (1): 124-131.

45. **Karu N, Reifen R& Kerem Z.** Weight gain reduction in mice fed Panax ginseng saponin, a pancreatic lipase inhibitor. Journal of agricultural and food chemistry. 2007; 55 (8): 2824-2828.

46. **Lin N, Cai DL, Jin D, Chen Y, Shi JJ.** Ginseng panaxoside Rb1 reduces body weight in diet-induced obese mice. Journal of Cell biochemistry and biophysics. 2014; 68 (1): 189-194.

47. **Voruganti S, Qin JJ, Sarkar S, Nag S, Walbi IA, Wang S& Zhang R.** Oral nano-delivery of anticancer ginsenoside 25-OCH3-PPD, a natural inhibitorof the MDM2 oncogene: Nanoparticle preparation, characterization, in vitro and in vivo anti-prostate cancer activity, and mechanisms of action. Oncotarget. 2015; 6 (25): 21379- 21394.