Research Article

Evaluation of the Protective Effect of *Panax Ginseng* Nanoparticles against Nicotine-induced Reproductive Disorders in Male Rats

Sabah A. A. Linjawi*
Department of Biology, College of Science, King Abdulaziz University, Jeddah, Saudi Arabia.
Cell Biology Department, National Research Center, Dokki, Giza, Egypt.
***Corresponding author's E-mail:** slinjawi@kau.edu.sa

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ABSTRACT

One of the serious health problems is remaining of use the nicotine through smoking. This problem has been associated with fertility weakness, although the mechanism responsible of nicotine-induced fertility disorders is still unclear. *Panax ginseng* taking orally may enhance male fertility. Nanoparticles performed from plants known to contain high content of flavonoids. These nanoparticles provide a biologically new way to designing therapeutic agents and consequently a means of reducing the toxicity of metal nanoparticles. Therefore, the main objective of the present study is to increase the efficiency of the *Panax ginseng* through using their nanoparticles against reproductive disorders induced by nicotine. Adult male albino rats (n=100) were assigned to several groups (n=10) to study the effect of *P. ginseng* (20 mg/kg) or *P. ginseng* nanoparticles (PGNPs: 4, 10 and 20 mg/kg) against nicotine (1.5 mg/kg) induced reproductive toxicity. The results revealed that nicotine treatment decreased the secretion of serum free testosterone, LH, and FSH and the expression levels of the genes (CYP19, LH and FSH) encoding these hormones, increased the sperm abnormalities due to increase the DNA damage in the testicular tissues. However, treatment of male rats with *P. ginseng* or PGNPs prevented the reproductive toxicity induced by nicotine. Moreover, the protective efficiency of this plant against nicotine increased in PGNPs-treated rats. The results suggested that ginsenosides increased its reproductive function on the hypothalamus pituitary-testis axis when the *Panax ginseng* formulated into nanoparticles form.

Keywords: Nicotine, *Panax ginseng,* Nanoparticles, Reproductive hormones Gene expression, DNA damage

INTRODUCTION

Exposure of nicotine through cigarette smoking is a major public health problem not only in the Arabic countries but worldwide. It has been reported that morbidity and mortality related to cigarette smoking is detected to account for approximately 430,000 deaths. Such deaths due to nicotine smoking have been reported to be resulted from lungs cancer, chronic obstructive pulmonary diseases and ischemic heart diseases. It expected that by 2030, if current trends continue, tobacco use was the mean reason to kill more than 8 million people worldwide each year[1]. It has been reported that the highest spread of smoking is showed in young male adults (between 20 and 39 years) during their reproductive period[2].

Most of literature demonstrated that cigarette smoking is associated with decline in semen quality including sperm concentration, motility and morphology[3], volume of semen and acidity[4]; and could induce specific lesions during the development of spermatozoon, which it may the reason either directly or indirectly harmful to spermatogenesis[5]. In comparison to non-smokers, smokers have a significantly higher percentage of abnormal forms of sperm morphology[6]. Mixed results have been reported from numerous studies regarding assessment the association between cigarette smoking and sperm quality (density, motility and morphology)[7,8]. Many of chemical compounds (more than 3000) produced from the cigarette smoke in which most of them believed to be genotoxic. The action mechanism of these genotoxic compounds is that they are entering the blood circulation of the testes and consequently having a direct cytotoxic impact on the spermatozoa by damaging DNA[9].

Normally the smoke is divided into several phases: (a) gaseous phase and (b) particles phase. The nicotine is the main component of the particle phase[10].

Smoking a single cigarette could produce and absorb as much as 1 mg of nicotine[11], in which about 80 - 90 % of the nicotine is metabolized by the organism[12].

The main compound produced from nicotine degradation is cotinine[13]. It is more stable than nicotine because their specificity and detectable concentration in human body fluids (including serum, urine, follicular fluid[13] and seminal plasma[14]), are longer half life of ~ 20 h compared with 2 h for nicotine[15]. In addition, several experimental studies indicated that serum levels of nicotine and cotinine in male rats exposed to smoking increased which influenced adversely the epididymal sperm content, spermatogenesis, motility and fertilizing potential[16].

Moreover, nicotine molecules have toxic effects on testicular functions by making nicotine-induced biochemical alterations in testes tissues[17]. In addition, nicotine particles cause histopathological alterations in the sertoli cells and the germ cells in which they may be degenerated resulting in excess cytoplasm of the spermatids[18]. In addition nicotine causes reduction in

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sperm quality including sperm count, motility and viability[19].

The impact of nicotine on reproduction is much greater in male than in female. Several studies showed that male rodents have a less tolerance to nicotine compared to the female[20]. In addition, analysis of the cotinine concentration in several samples showed that its concentration was similar in blood and seminal plasma. Therefore this finding indicates that active transfer may be suggested between the testicular cells into blood vessels[21].

Several drugs such as phosphodiesterase-5 inhibitors[22], dopamine agonists[23], synthetic prostaglandins[24], and α-adrenergic receptor antagonists[25] are used to treat sexual disorders. However, these drugs have several side effects, including flushing, headache, dyspepsia, impaired vision and nasal congestion and other[26]. Therefore, drugs and agents having fewer side effects to improve sexual function are desirable.

Korean red ginseng (KRG) usually is taking orally to improve physical strength by people in East Asia for more than many years ago. Many studies indicated that KRG is using for therapeutic purposes such as diabetes[27], atherosclerosis[28], erectile disorder[29], immune disorders[30], cancer disease[31], and stress physiology[32] and other dysfunctions. In addition, it has been reported that the supplementation of KRG extract is effective to enhance the testicular function[33] and sperm viability as well as increase the quality of sperm in guinea pigs[34]. Moreover, several reasons are taking in consideration for using KRG for therapeutic purposes such as its lower price, availability and safety. However, no published data were found about using the nanoparticles of ginseng in therapeutic purposes to increase the effectively of testicular disorders.

In the very recent years, nanotechnology has been used in biomedicine as a basic science technology[35,36] in therapy purposes. Plant nanoparticles are helping as less toxic and efficient carriers for delivery and enhancing the drug bioactivity within the cells and tissues. An effort was made in the present study to explore one of the modern ways of pharmaceutical interference to formulate nanoencapsulation of the *Panax ginseng* root extract against nicotine exposure.

Although the growing knowledge of opposite fertility impacts of smoking on reproduction due to influence of many compounds such as nicotine, the smokers are increasing continuously year by year. Therefore, the main aim of the present project is to increase the efficiency of the *Panax ginseng* nanoparticles against reproductive disorders induced by nicotine. Moreover, the present study is designing to investigate the protective effect of *Panax ginseng* nanoparticles against nicotine-induced infertility using several parameters such as alteration of male reproductive hormones, sperm quality and abnormalities, DNA fragmentation in sperm and gene expression of reproductive related genes in male albino rats.

MATERIALS AND METHODS

Chemicals

Nicotine and *Panax ginseng* were purchased from the Sigma–Aldrich Company (St. Louis, MO, USA). Trizol was bought from Invitrogen (Carlsbad, CA, USA). The reverse transcription and PCR kits were obtained from Fermentas (Glen Burnie, MD, USA). SYBR Green Mix was purchased from Stratagene (La Jolla, CA, USA).

Formation of *P. ginseng* Loaded Nanoparticles (PGNPs)

To prepare the poly-lactic-co-glycolic acid (PLGA) encapsulation of *P. ginseng*, solvent displacement technique of Samadder[37] deployed under optimal conditions. To 20 mL of an aqueous solution of F68; w/v stabilizer (1% polyoxyethylene-polyoxypropylene), an organic phase mixture containing 10 mg of dried *P. ginseng* dissolved in 3 mL acetone along with 50 mg PLGA in a dropwise manner (0.5 mL/min) was added. Stirring the mixture continuously was performed at room temperature until complete evaporation of the organic solvent; the redundant stabilizer was removed by centrifugation at 2500 g at 4°C for 30 minutes. The pellet was re-suspended in Milli-Q water and washed three times and the nanoparticles obtained were stored in suspensions at 4°C until further use.

Experimental Animals

Adult Albino male rats (n=100, 110.5 ± 4.7 g, ranged from 100–120 g), purchased from the Animal provider, were maintained on standard laboratory diet (protein, 16.04%; fat, 3.63%; fiber, 4.1%; and metabolic energy, 0.012 MJ) and water *ad libitum* at the Animal House Laboratory, King Fahad Medical Research Center, King Abdulaziz University. After an acclimation period of 1 week, animals were divided into several groups (10 rats/group) and housed individually in filter-top polycarbonate cages, housed in a temperature-controlled (23 ± 1°C) and artificially illuminated (12 h dark/light cycle) room free from any source of chemical contamination. All animals received humane care in compliance with the guidelines of the Animal Care and Use Committee of King Fahad Medical Research Center, King Abdulaziz University.

Experimental Design

The animals were assigned to 10 groups (n=10) as follows: Group 1: Normal healthy animals served as the control group. Group 2: Animals were intragastrically administered with 20 mg/kg *P. ginseng* for 4 weeks. Groups 3, 4, 5: Animals were intragastrically administered with 4, 10 and 20 mg/kg *P. ginseng* nanoparticles for 4 weeks, respectively. Groups 6: Animals were intragastrically administered with 1.5 mg/kg of nicotine for 4 weeks. Group 7: Animals were intragastrically administered with 1.5 mg/kg of nicotine plus 20 mg/kg *P. ginseng* for 4 weeks. Groups 8, 9 and 10: Animals were

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intragastrically administered with 1.5 mg/kg of nicotine plus 4, 10 and 20 mg/kg *P. ginseng* nanoparticles for 4 weeks, respectively.

Biochemical Analyses

Alteration of Male Reproductive Hormones

Quantitative estimation of free testosterone was carried out in the samples of rat's serum using enzyme linked immune sorbent assay (ELISA) according to McCann and Kirkish[38]. Quantitative estimation of follicle stimulating hormone (FSH) was conducted in the samples of rat's serum using enzyme linked immune sorbent assay (ELISA) according to Knobil[39].

Quantitative estimation of luteinizing hormone (LH) in the sample of rat's serum was carried out using enzyme linked immune sorbent assay (ELISA) according to Wakabayashi[40]. The kits used for determination of (free testosterone, follicle stimulating hormone and luteinizing hormone) in rat serum were obtained from Gloryscience. Co., Ltd.

Sperm Quality and Abnormalities

After experiment termination, 35 days were calculated (duration of spermatogenesis) to investigate the sperm quality and abnormalities[41]. Half number of the rat animals of each treated group was sacrificed. The epididymides and testes from each rat were removed and weighed. Sperms were collected quickly when each rat was dissected. To release sperms, the cauda epididymides were cut in a pre-warmed Petri dish having saline solution at 37 °C. After mincing with scalpels, the suspension was stirred and dropped on grease-free clean slide to investigate the motility of sperms using microscope. Spermatozoa were counted using heamocytometer and on a cleaned slide.

DNA Fragmentation in sperms of Rats

Apoptotic DNA fragmentation was qualitatively analyzed by detecting the laddering pattern of nuclear DNA as described according to Lu[42]. Testis tissues were homogenized, washed in PBS, and lysed in 0.5 ml of DNA extraction buffer for overnight at 37 °C.

The lysate was then incubated with DNase-free RNase for 2 h, followed by three extractions of an equal volume of phenol/chloroform and a subsequent reextraction with chloroform by centrifuging for 5 min.

The isolated DNA was precipitated in two volume of ethanol with sodium acetate for 1h, followed by centrifuging at for 15 min. After washing with 70% ethanol, the DNA pellet was air dried and dissolved in tris-HCl/EDTA.

The DNA was then electrophoresed on agarose gel and stained with ethidium bromide in TAE buffer. DNA ladder (Invitrogen, USA) was included as a molecular size marker and DNA fragments was visualized and photographed by exposing the gels to ultraviolet transillumination.

Expression of Aromatase, LH and FSH genes

Isolation of Total RNA

Total RNA was isolated from testis and brain tissues of male rats by the standard TRIzol® Reagent extraction method (cat#15596-026, Invitrogen, Germany). After isolation, the RNA was dissolved in diethylpyrocarbonate (DEPC)-treated water by passing solution a few times through a pipette tip.

Total RNA was treated with RQ1 RNAse-free DNAse (Invitrogen, Germany) to digest DNA residues and then the RNA was re-suspended in DEPC-treated water. The purity of total RNA was assessed. In addition, integrity of RNA was assured with ethidium bromide-stain analysis of 28S and 18S bands by formaldehyde-containing agarose gel electrophoresis. The RNA was allocated in several aliquots and was used immediately for reverse transcription (RT).

Reverse Transcription Reaction

The complete Poly(A)$^+$ RNA isolated from male rats tissues was reverse transcribed into cDNA using RevertAidTM First Strand cDNA Synthesis Kit (MBI Fermentas, Germany). An amount of total RNA (5µg) were used with a reaction mixture, termed as master mix (MM). The RT reaction was carried out and the reaction was stopped by heating at 99 °C. Afterwards the reaction tubes containing RT products were flash-cooled on ice chamber until being used for amplification through quantitative real time-polymerase chain reaction (qRT-PCR).

Quantitative Real Time-Polymerase Chain Reaction

PCR reactions were set up in reaction mixtures containing SYBR® Premix Ex TaqTM (TaKaRa, Biotech. Co. Ltd., Germany), sense primers, antisense primer, distilled water, and cDNA template. The reaction program was allocated to 3 steps. At the end of each qRT-PCR a melting curve analysis was performed to check the quality of the used primers. Each experiment was included a distilled water control.

The quantitative values of RT-PCR (qRT-PCR) of Aromatase (Cyp19-F: 5′-ATA CCA GGT CCT GGC TAC TG-3′, Cyp19-R: 5′-TTG TTG TTA AAT ATG ATG CC-3′), LH (LH -F: 5′-TCT CAC CACCAC CGT CTG TA-3′, LH-R: 5′-TGC AGT CGC TGT AGT CCA TC-3′), and FSH (FSH-F: 5′-GGG CCA GGA ACT GTG AAA TA-3′, FSH-R: 5′-TCT CAG AAC TGC CGA GGT TT-3′) genes were normalized on the bases of ß-actin (ß-actin-F: 5′-TTG CCG ACA GGA TGC AGA A-3′, ß-actin-R: 5′-GCC GAT CCA CAC GGA GTA CT-3′) expression.

At the end of each qRT-PCR a melting curve analysis was performed at 95.0 °C to check the quality of the used primers.

Calculation of Gene Expression

First the amplification efficiency (Ef) was calculated from the slope of the standard curve using the following formulae[43]:

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$$Ef = 10^{-1/slope}$$

$$Efficiency\ (\%) = (Ef - 1) \times 100$$

The relative quantification of the target to the reference was determined by using the ΔCT method if E for the target (Cyp19, LH and FSH) and the reference primers (β-Actin) are the same[43]:

$$Ratio_{\ (reference/\ target\ gene)} = Ef^{\ CT(reference) - CT(target)}$$

Statistical Analysis

All data were analyzed using the General Liner Models (GLM) procedure of Statistical Analysis System[44] followed by Scheffé-test to assess significant differences between groups. The values were expressed as mean ± SEM. All statements of significance were based on probability of $P< 0.05$.

RESULTS

Hormonal Assessment

The results depicted in Table 1 showed the effect of *Panax Ginseng*, PGNPs and/or nicotine on serum levels of free testosterone, LH, and FSH of adult male rats. The results indicated that there were significant decreases in serum free testosterone LH, and FSH in male rats treated with nicotine compared with control group (Table 1). In contrary, the levels of free testosterone, LH, and FSH in rats treated with *Panax Ginseng* and *Panax Ginseng* nanoparticles (PGNPs) increased compared with control or nicotine treated rats, however, these levels were significantly different with the highest dose of PGNPs. Furthermore, treatment of male rats with *Panax Ginseng*, medium and high doses of PGNPs increased significantly the secretion of free testosterone, LH, and FSH levels in nicotine-treated rats compared with those treated with nicotine alone (Table 1).

Sperm Abnormalities Assessment

The mean values of different types of the abnormalities of sperm morphology were shown in Table 2. The most frequently observed abnormality was head without hock, Banana head and coiled tail sperm.

The results revealed that *Panax Ginseng* and *Panax Ginseng* nanoparticles (PGNPs) treatment did not increase the frequencies of the morphologically abnormal sperm compared with control group (Table 2). However, the frequency of the sperm abnormalities increased significantly with the nicotine treatment compared with control group (Table 2).

On the other hand, treatment of male rats with *Panax Ginseng* and PGNPs decreased the frequencies of the sperm abnormalities in nicotine-treated rats especially with the medium and high doses of PGNPs.

DNA Fragmentation

Quantitative DNA fragmentation was determined in rats exposed to nicotine alone or in combination with *Panax Ginseng* and PGNPs. The DNA damage was examined in testis tissues collected from nicotine-treated groups using gel electrophoresis laddering assay (Figure 1). The results of this assay revealed that nicotine treatment increased the DNA damage compared with control group (Figure 1). Where, the DNA damage was observed as different bands of the DNA.

In contrary, the DNA was remained without damage when the male rats were treated with *Panax Ginseng* and PGNPs. Furthermore, treatment of nicotine-treated rats with *Panax Ginseng* and PGNPs inhibited the DNA damage compared with rats treated with nicotine alone (Figure 1).

Expression Analysis of CYP19, LH and FSH genes

The effect of toxicity effect of nicotine and protective impact of *Panax Ginseng* and PGNPs on the expression of CYP19 in the testis tissues and LH as well as FSH genes in pituitary tissues of adult male rats is summarized in Figures 2-4.

The expression levels of CYP19, LH and FSH genes were increased with *Panax Ginseng* and PGNPs compared with control group, in which, the increase of the expression levels was significantly in CYP19, LH and FSH genes (Figures 2-4).

On the other hand, treatment of male rats with nicotine decreased significantly the levels of CYP19, LH and FSH genes compared with control group.

Administration of nicotine-treated rats with *Panax Ginseng* and PGNPs increased the expression levels of CYP19, LH and FSH genes. Where, the expression levels in nicotine-treated rats exposed to *Panax Ginseng* and low dose of PGNPs were relatively similar to control group (Figures 2-4). In contrary, the expression levels of CYP19, LH and FSH genes were increased significantly with medium and high doses of GNPs treatment compared with control and nicotine alone (Figures 2-4).

DISCUSSION

The present study was conducted to investigate the protective effect of *Panax ginseng* against nicotine-induced reproductive toxicity in male rats. In addition, to increase the efficiency of the *Panax ginseng*, its particles were formulated into nanoparticles to enhance its pharmaceutical effect against reproductive disorders induced by nicotine.

The results indicated that nicotine treatment decreased the fertility of male rats which it decreased the secretion of serum free testosterone LH, and FSH and increased the sperm abnormalities.

In addition, the current study indicated that treatment of nicotine induced DNA fragmentation and down-regulated the expression of reproductive genes. In deep agreement with our results several studies have reported that nicotine treatment was accompanied with reduction in fertilization rates[45], semen volume[46], concentration, motility[47], sperm density, viability, forward progression[48],

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linearity[49], and higher incidence of oligospermia[50] and germ cell aneuploidy[51]. A worsening of sperm morphology has been shown as well as a reduced capacity of spermatozoa to undergo the acrosome reaction[52]. In addition, mutagenic effects on germ cells[53] have been observed; and mutations in spermatozoa have been noted that could lead to cancer and genetic diseases[54].

One of the most abundant organic particles in cigarette smoke is nicotine, which is responsible for some positive or negative effects on the various organs. Nicotine is a very toxic alkaloid[55]. Inhaled nicotine is quickly oxidized to its major metabolite cotinine[56]. Cotinine has a longer half-life than NIC, which is about 20 hours versus 2 hours for NIC; for this reason COT is a better marker of the smoke absorbed[57]. Therefore, the reproductive toxicity of nicotine on male rats may attributed to oxidized form "cotinine".

The present study reported that *Panax Ginseng* and PGNPs treatment enhanced the fertility of male rats which it increased the secretion of serum free testosterone LH, and FSH and decreased the sperm abnormalities. Moreover, *Panax Ginseng* treatment inhibited the DNA damage and increased the expression levels of the fertility genes.

These findings are in line with several studies which found that supplementation of *Panax Ginseng* extract is effective to enhance the testicular function[33], and sperm viability as well as increase the quality of sperm in guinea pigs[34].

To date, some drugs have been reported to improve sexual dysfunction in males, however, most of them are synthetic compounds which cause health side effects. *Panax ginseng* is one of the oldest and best-known medicinal plants used to prevent sexual dysfunction. Ginseng has several pharmacological properties and potential therapeutic applications, and some studies suggest that the antioxidant and organ protective actions of ginseng are associated with enhanced nitric oxide (NO) synthesis in the endothelium of the lung, heart, kidney, and corpus cavernosum[58]. Enhanced NO synthesis causes vasodilatation and might be responsible for the aphrodisiac properties of ginseng. Although many researchers have recognized that *Panax ginseng* reverses erectile dysfunction[59,60], few attempts have been made to determine its effects on age-associated sexual function. Moreover, no reported trial has compared the efficacy of the whole PGNPS which contains the main active ingredients of ginseng in effective form to be delivered to the target cells.

Recently, nanotechnology has been used in biomedicine as a basic science technology[35,36] in therapy purposes. Plant nanoparticles are helping as less toxic and efficient carriers for delivery and enhancing the drug bioactivity within the cells and tissues. An effort was made in the present study to explore one of the modern ways of pharmaceutical interference to formulate nanoencapsulation of the *Panax ginseng* root extract against nicotine exposure.

The current study indicated that PGNPs was more effective than the *Panax ginseng* extract in inhibition the toxicity of nicotine and to enhance the fertility symptoms in male rats.

It is known that *Panax ginseng* enhance 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.

These nanoparticles provide a biologically new way to designing therapeutic agents and consequently a means of reducing the toxicity of metal nanoparticles.

Therefore, the formulation of the particles of *Panax ginseng* to nanoparticles in the current study enhanced the active ingredients such as flavonoids to protect the testicular cells from the toxicity effect of nicotine and enhance the function of the testis.

In conclusion: the use of PGNPs showed an increase in serum testosterone, FSH and LH levels, increase in the genes encoding these hormones, decrease sperm abnormalities due to protect the DNA of the testis tissues from the 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 nanoparticles forms.

Table 1: Concentration of testosterone, LH, and FSH in male rats treated with *Panax Ginseng*, PGNPs and/or nicotine.

Groups	Free testosterone (ng/L)	LH (mlu/ml)	FSH (mlu/ml)
Control	5.2 ± 1.2^{bc}	0.82 ± 0.04^{b}	0.67 ± 0.04^{ab}
P. ginseng	6.2 ± 1.1^{b}	0.96 ± 0.02^{ab}	0.75 ± 0.03^{ab}
PGNPs4	5.9 ± 1.3^{bc}	0.83 ± 0.03^{b}	0.68 ± 0.02^{ab}
PGNPs10	6.7 ± 1.4^{b}	0.97 ± 0.03^{ab}	0.74 ± 0.01^{ab}
PGNPs20	8.5 ± 1.5^{a}	1.06 ± 0.10^{a}	0.87 ± 0.02^{a}
Nicotine	2.8 ± 0.4^{d}	0.61 ± 0.04^{c}	0.37 ± 0.04^{c}
***P. ginseng*+nicotine**	4.6 ± 1.3^{c}	0.73 ± 0.03^{bc}	0.58 ± 0.09^{b}
PGNPs4+nicotine	4.2 ± 1.4^{c}	0.69 ± 0.02^{bc}	0.51 ± 0.02^{b}
PGNPs10+nicotine	5.1 ± 1.1^{bc}	0.82 ± 0.04^{b}	0.64 ± 0.03^{b}
PGNPs20+nicotine	5.7 ± 1.2^{bc}	0.94 ± 0.03^{ab}	0.72 ± 0.02^{ab}

Data are presented as mean ± SEM. ^{a,b,c}Mean values within columns with unlike superscript letters were significantly different (P<0.05).

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Table 2: Mean values of different types of sperm abnormalities induced by *Panax Ginseng*, PGNPs and/or nicotine in male rats.

Treatment	Sperm abnormalities*				
	Head without hock	Amorphous head	Banana head	Coiled tail	Total
Control	0.3 ± 0.1^{b}	0.0 ± 0.0	0.2 ± 0.0^{b}	0.4 ± 0.1^{c}	0.9 ± 0.1^{c}
P. ginseng	0.4 ± 0.0^{b}	0.1 ± 0.0	0.3 ± 0.1^{b}	0.3 ± 0.1^{c}	1.1 ± 0.1^{bc}
PGNPs4	0.3 ± 0.1^{b}	0.0 ± 0.0	0.3 ± 0.1^{b}	0.3 ± 0.1^{c}	0.9 ± 0.2^{c}
PGNPs10	0.2 ± 0.0^{b}	0.1 ± 0.0	0.4 ± 0.1^{b}	0.1 ± 0.1^{c}	0.8 ± 0.1^{c}
PGNPs20	0.4 ± 0.0^{b}	0.0 ± 0.0	0.3 ± 0.0^{b}	0.2 ± 0.0^{c}	0.9 ± 0.0^{c}
Nicotine	1.6 ± 0.2^{a}	1.4 ± 0.1^{a}	0.7 ± 0.1^{a}	1.2 ± 0.1^{a}	4.9 ± 0.2^{a}
***P. ginseng*+nicotine**	0.4 ± 0.1^{b}	0.3 ± 0.0^{b}	0.4 ± 0.1^{b}	0.7 ± 0.1^{b}	1.8 ± 0.2^{b}
PGNPs4+nicotine	0.4 ± 0.0^{b}	0.6 ± 0.1^{b}	0.7 ± 0.1^{b}	0.5 ± 0.1^{bc}	2.2 ± 0.1^{b}
PGNPs10+nicotine	0.4 ± 0.2^{b}	0.3 ± 0.1^{b}	0.2 ± 0.1^{b}	0.6 ± 0.1^{b}	1.5 ± 0.1^{bc}
PGNPs20+nicotine	0.3 ± 0.2^{b}	0.2 ± 0.1^{b}	0.4 ± 0.1^{b}	0.3 ± 0.2^{c}	1.2 ± 0.3^{ba}

*After 35 days of the treatment animals of each group were used to assess the sperm morphology. ^{a,b,c}Mean values within columns with unlike superscript letters were significantly different (P < 0.05).

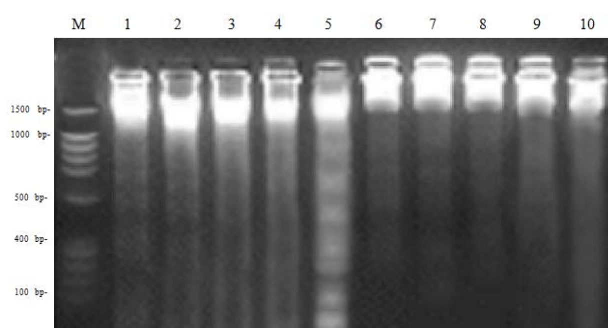

Figure 1: DNA fragmentation detected with agarose gel of DNA extracted from liver tissues of male rats by DNA gel electrophoresis laddering assay. Lane 10 represents control rats. Lane 9 represents rats treated with 20 mg/kg Panax Ginseng. Lanes 8-6 represent rats treated with 4, 10 and 20 mg/kg PGNPs, respectively. Lane 5 represents rats treated with 1.5 mg/kg nicotine. Lane 4 represents rats treated with 20 mg/kg Panax Ginseng plus 1.5 mg/kg nicotine nicotine. Lanes 3-1 represent rats treated with 4, 10 and 20 mg/kg PGNPs plus 1.5 mg/kg nicotine, respectively. Lane M: represents DNA ladder.

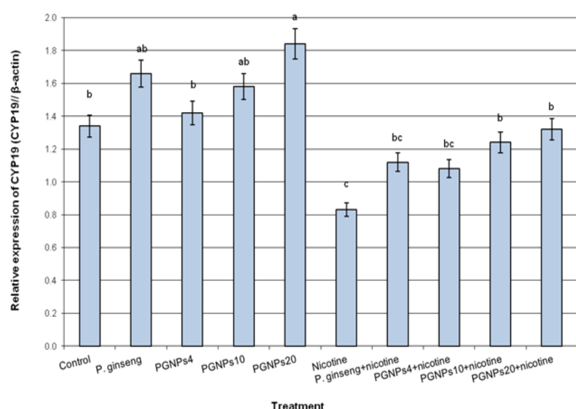

Figure 2: The alterations of CYP19-mRNA in testis tissues of male rats after treatment with *Panax Ginseng*, PGNPs and/or nicotine. Data are presented as mean ± SEM. ^{a,b,c}Mean values within tissue with unlike superscript letters were significantly different (P<0.05).

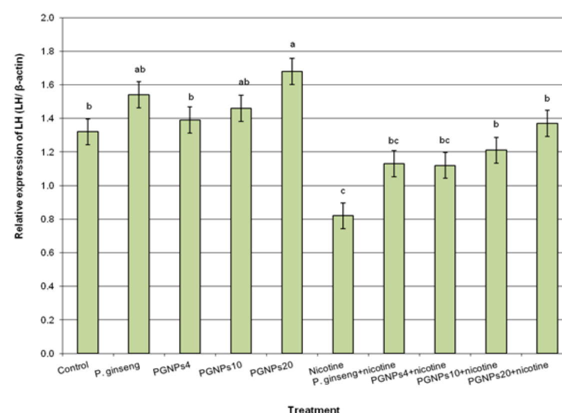

Figure 3: The alterations of LH-mRNA in brain tissues of male rats after treatment with *Panax Ginseng*, PGNPs and/or nicotine. Data are presented as mean ± SEM. ^{a,b,c}Mean values within tissue with unlike superscript letters were significantly different (P<0.05).

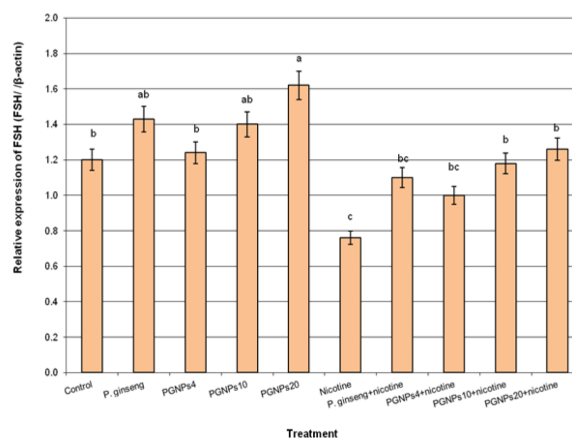

Figure 4: The alterations of FSH-mRNA in brain tissues of male rats after treatment with *Panax Ginseng*, PGNPs and/or nicotine. Data are presented as mean ± SEM.

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[a,b,c]Mean values within tissue with unlike superscript letters were significantly different (P<0.05).

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