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ORIGINAL ARTICLE

Ameliorative effect of Panax ginseng and/or Ginkgo biloba on reproductive and thyroid functions in mature albino rats

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ABSTRACT

The current study is an endeavor for profound exploration about the effect of two brain stimulant medicinal herbs (Panax ginseng and Ginkgo biloba) alone or in combination on the improvement of some physiological reproductive and thyroid activities in mature male rats. Forty mature male albino rats were classified into four equal groups (n=10). Group I control; received distilled water (1ml/day). Group II; administered with Panax ginseng only (200 mg/kg BW) Group III; administered with G. biloba only (150 mg/kg BW). Group IV administered with Panax ginseng + G. biloba (100 and 75 mg/kg BW) respectively. After 60 days of administration, serum level of reproductive (testosterone, LH and FSH) as well as thyroid (T3 and T4) hormones, some semen parameters, and histology of testicular tissue were evaluated. Serum level of testosterone hormone decreased significantly ($P<0.001$) in all groups while, there was no change in the level of LH and FSH. Moreover, T3 and T4 increased significantly ($P<0.001$) in all groups except in mixed group which showed non-significant increase in level of T3. Rats administered both herbs individually showed significant increase ($P<0.001$) in epididymal sperm count, abnormal sperm percent decreased significantly ($P<0.001$) in all treated groups however, sperm motility significantly elevated ($P<0.05$) only in Ginseng received group. Histologically, testicular tissue of all rats disclosed enhancement in testes by stimulating the activity of seminiferous tubules with mature spermatozoa. In conclusion Panax ginseng and G. biloba have improvement effect on semen parameters however, serum testosterone hormone consumed in spermatogenesis process. Besides, both herbs have progressive power to synergize thyroid function ability by improvement level of thyroid hormones.

Keywords: Panax ginseng, Ginkgo biloba, Male reproduction, Thyroid

INTRODUCTION

Medicinal plants are widely used in both developing and developed countries to maintain their health or treat diseases (Smith-Hall et al. 2012). Herbal products reach about 25 % of medications in developed and about 80 % in developing countries (Scott et al. 1998). WHO reports that about 80 % of the world´s population use herbal medicine or vegetable extracts in therapy (Bezerra et al. 2013). People are heading to the use of medicinal herbs because they have many deep-seated beliefs about the importance of medicinal herbs (Omidbigi, 2011). In terms of People' beliefs about medicinal herbs, healthiness, lower side effects, and more affordable price were the most important factors influencing the demand for medicinal herbs (Sajedipoor and Mashayekhi, 2015). Moreover, 56 % of currently prescribed synthetic drugs are classified as follow 24 % are derivatives from plant species, 9 % are synthetic products modeled from natural products, 6 % are extracted directly from the plant species, and 5 % are of animal origin and 20 % of natural have been investigated for medicinal potential (Wink 2000; Kushiro et al. 2003).

Ginseng (Panax ginseng (P. ginseng)), family Araliaceae) is one of the most widely known and used oriental medicinal plants (Yun 2001). Ginseng is a shade plant that prefers a cool and dry climate, like that of Korea (Lee et al. 2011). The main species of ginseng are P. ginseng (Korea ginseng), P. quinquefolius (American ginseng), P. notoginseng (Tienchi ginseng), and P. japonicus (Japanese ginseng) (Baeg and So, 2013). Several studies have recently shed the light on the beneficial effects of ginseng on diseases such as cancer; immune disorders; diabetes; and liver, neuronal, cardiovascular, infectious diseases, as antibacterial and antiviral agent (Lee et al. 2014; Im et al. 2016). Besides, ginseng is involved in improvement of testicular dysfunction and sexual impairment (Leung and Wong, 2013; Won et al. 2014). Ginseng consists mainly of ginseng saponins (or ginsenosides) which are the main active ingredients of ginseng and responsible for its functions. Ginseng saponins are glycoside saponins and derivatives of triterpenoid dammarane, which consists of 30 carbon atoms (Lee and kim, 2014).

Ginkgo biloba (G. biloba) is one of the most ancient seed plants, it is originally native to China and now it is cultivated worldwide. Extract from G. biloba leaves has been used in traditional Chinese medicine for centuries to treat circulatory disorders, asthma, tinnitus, vertigo, and cognitive problems (Kleijnen et al. 1992). Today, G. biloba extracts are one of the most taken phytomedicines globally (Ernst 2002) and are often prescribed in Europe as a nootropic agent in old age and dementia (Kennedy et al. 2011) and antioxidant agent as it inhibits monoamine oxidase A abs B directly scavenges ROS (Trompezinski et al. 2010). G. biloba extract contains mainly terpenoids, flavonol glycosides, and proanthocyanidins. The most prevalent of these three groups are the flavonol glycosides (quercetin, catechin). The terpenoids include ginkgolides and bilobalides, which represent unique components of G. biloba. Terpenoids, flavonoids and proanthocyanidins are thought to be responsible for the pharmacological properties of Gb (Kleijnen et al. 1992).

The accurate treatment of male subfertility is of major economic importance in the livestock breeding industry, especially in milk and meat producing animals. In addition, using low-fertile males leads to colossal loss to the farming community in terms of conception

failure and reduced life-time reproductive efficiency of females. Nowadays, using chemical and inorganic materials in treatment of such problems reflects deleterious effects on both animals and human. Therefore, the male reproduction care and improvement must be primary concern by finding new strategies of ameliorative substances with natural sources without side effects.

The scope of the current research topic is to investigate the effect of P. ginseng and G. biloba extract separately or in combination on the improvement of some physiological male reproductive and thyroid activities in mature male rats. Reproductive and thyroid pattern will be assessed by complete semen analysis (Epididymal sperm count, percent abnormality and motility) in addition, estimation of serum level of testosterone, LH, FSH, T3 and T4 hormones as well as histological examination of the testes was investigated.

MATERIALS AND METHOD

The experimental protocol followed institutional animal care and was approved by the Ethics Committee in Animal Experimentation of the Sohag University, Egypt. The experiment was executed by Physiology department and Veterinary Services Center, Faculty of Veterinary Medicine, Sohag University, Egypt.

Animals. Forty mature male albino rats (250±10 g) were housed in a specific clean pathogen free plastic cages in the animal house at Physiology department, Faculty of Medicine, Sohag University, Sohag with a 12 h light/dark cycle and at temperature of 23 ± 2 °C with ad libitum access to standard rodent pellets food and water for one week to be acclimatized laboratory environment. Mature male rats which were randomly distributed into four groups (10 rats for each); Group I (control); received distilled water (1ml/day). Group II; administered with P. ginseng (200 mg/kg BW). Group III; administered with G. biloba (150 mg/kg BW). Group IV; administered with P. ginseng + G. biloba (100 and 75 mg/kg BW respectively). All rats received distilled water and drug solution orally using drenching tube for 60 successive days.

Reagents. P. ginseng were purchased in form of Ginsana capsules 100 mg (E.I.P.CO Egyptian, 10th of Ramadan city, Egypt). G. biloba were purchased in the form of capsules 260 mg (EMA pharm pharmaceuticals, Nasr City, Cairo, Egypt). Solution of each drug were daily freshly prepared by dissolving each capsule in distilled water to obtained required concentration in 1ml.

Samples collection. Rats were anesthetized by diethyl ether; individual blood samples were collected from retro-orbital venous plexus from all treated groups after the end of experimental period in plain vacutainer tubes. Blood was centrifuged at 3000 rpm for 15 minutes then sera were collected separately in Eppendorf tubes. Sera were kept at -80°C until hormonal assay. Rats were euthanized by overdose of sodium thiopental (50 mg/kg BW, ip), epididymal semen samples and testes were collected. Testes were kept in neutral buffered paraformaldehyde 4% for histological processing.

Epididymal semen analysis. Epididymal semen was collected in prewarmed physiological saline at 37°C according to D'souza (2003). Immediately after semen collection and dilution, a drop of semen was placed on a prewarmed, dry, and clean slide, then sperm motility was assessed according to Seed et al. (1996). At the same time, one drop of semen was placed on a prewarmed dry and clean slide, then mixed with one drop of Eosin-Nigrosine

stain for evaluation of sperm vitality (dead and live percent) according to Esteso et al. (2006). Sperm abnormalities were monitored by mixing one drop of diluted semen with Eosin stain according to Wyrobek and Bruce (1978). Finally, epididymal sperm concentration was evaluated by dilution with sodium bicarbonate solution and formalin then sperms were counted by Neuber's hemocytometer as described by Srinivasulu, and Changamma (2017).

Hormonal Assay. Serum level of testosterone, LH, FSH, T3 and T4 were measured by enzyme-linked immunosorbent assay (ELISA) kits according to Chen et al. (1991); Ulloa-Aguirre et al. (1998); Maes et al. (1997); T3 and T4 Agharanya (1990) respectively, following manufacturer's instructions (Bioactiva diagnostica Gmbh, Homburg, Germany) by using microplate reader (Infinite 50, Männedorf, Switzerland) at wavelength 450 nm.

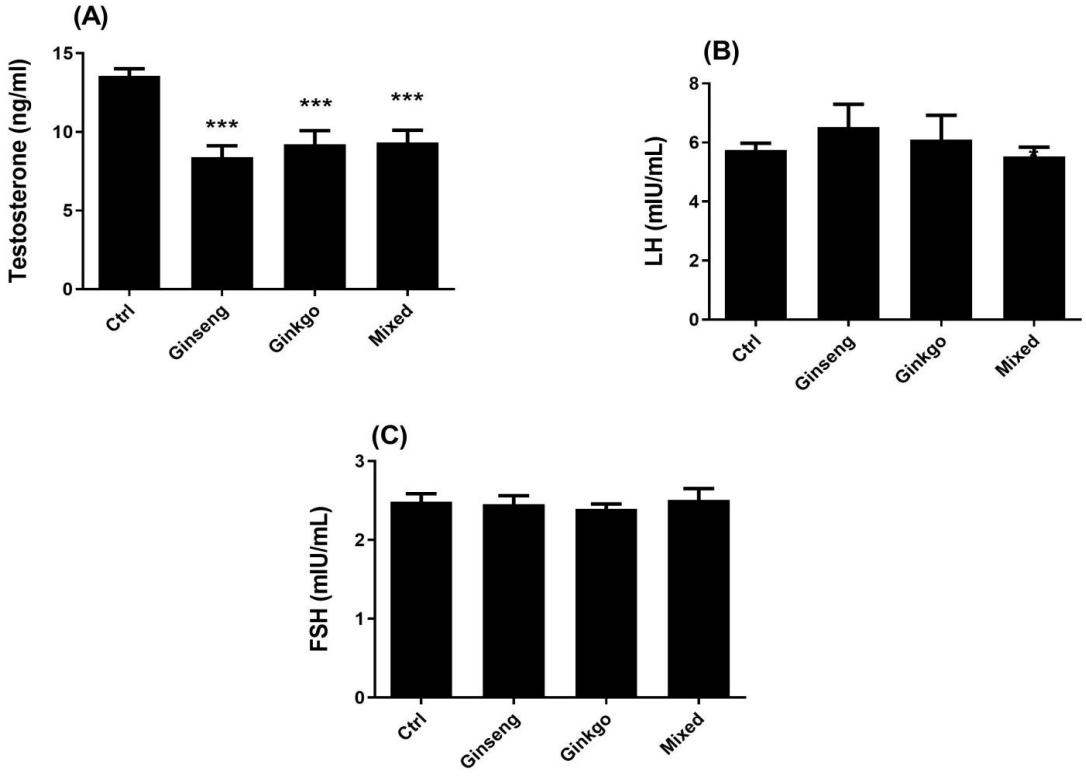

Figure 1: **Serum level of reproductive hormones in different treated and control rats after 60 days of herbal administration**. n= 10, values are expressed in Mean ± S.E.M. ***: P< 0.001. Testosterone (ng/ml), FSH (mIU/mL), LH (mIU/mL).

Histological examination. After testicular excision, they were sliced and fixed in 10% neutral-buffered formalin for at least 24 h. The specimens were then immersed in tap water and dehydrated in ascending dilutions of ethanol (70-100%), cleared in xylene, and embedded in paraffin wax at 56°C in a hot air oven for 24 h. Serial sections of 5 µm thick were cut using a rotary microtome then were processed for hematoxylin and eosin (H&E) staining (Bancroft and Gamble, 2002).

Statistical analysis. Results were analyzed statistically by Graph pad prism 5 (GraphPad

Software, San Diego, California USA) and Excel 2016 software. Data were expressed as (mean ± standard error of the mean (SEM)) and differences between groups were analyzed 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

Reproductive hormones. The effect of P. ginseng and G. biloba was variable among different treated groups along experimental period. Their effect was clear on testosterone level (ng/ml) as shown in figure 1A; testosterone significantly (P<0.001) decreased in P. ginseng, (8.39±0.72), G. biloba (9.22±0.86) and their mixture (9.33±0.78) treated rats compared with control (13.57±0.45). However, level of LH and FSH showed no change in treated groups compared with corresponding control. Figure 1B showed serum level of LH (mIU/mL) in control (5.72±0.15), P. ginseng, (6.15±0.56), G. biloba (6.07±0.65) and their mixture (5.53±0.21) treated rats. Also, figure 1C disclosed serum level of FSH (mIU/mL) in different groups; control (2.51±0.06), rats administrated P. ginseng (2.44±0.08), G. biloba (2.40±0.05) and their mixture (2.51±0.09).

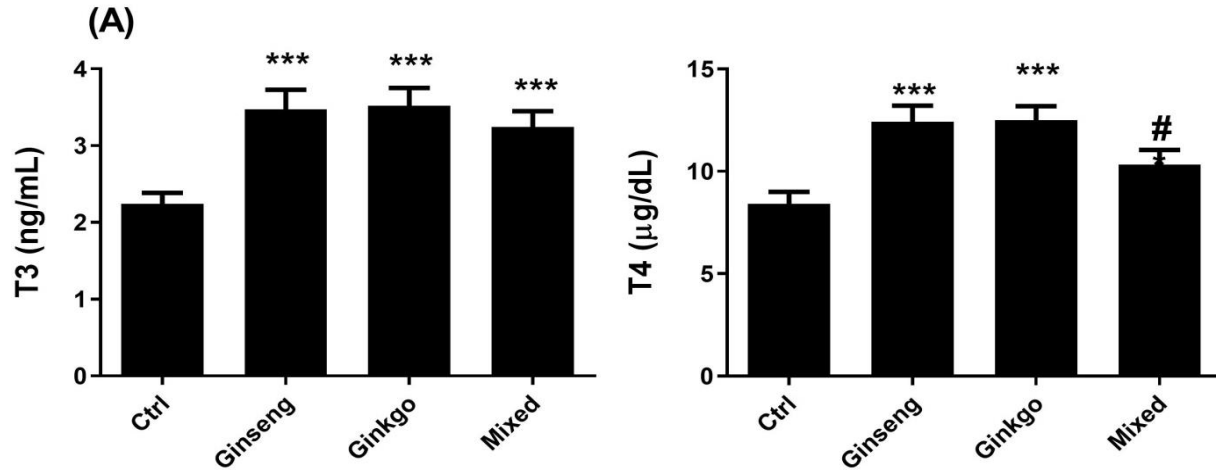

Figure 2: Serum level of thyroid hormones in different treated and control rats after 60 days administration of P. ginseng and/or G. biloba. n= 10, values are expressed in Mean ± S.E.M. #p<0.05 compared with Ginkgo-reated rats, ***p<0.001 compared with control group. T3 (ng/ml), T4 (µg/dL). (n=10, ***: P < 0.001, #: P<0.05).

Thyroid hormones. Unlike reproductive hormones, T3 and T4 markedly affected with P. ginseng and/or G. biloba. Serum T3 level (ng/mL) markedly increased significantly (P<0.001) in all treated groups (3.47±0.26), (3.52±0.23), (3.24±0.20) in P. Ginseng, G. biloba and their combination treated rats, respectively) (Figure 2A). Figure 2B Showed serum level of T4 (µg/dL) with significant increase (P<0.001) in P. ginseng (12.41±0.79) and G. biloba (12.50±0.68) compared with control rats (8.40±0.59). However, rats received mixture of two herbs showed non-significant elevation of T4 level (10.32±0.77 compared with control and significant (P<0.05) decrease compared with G. biloba treated rats.

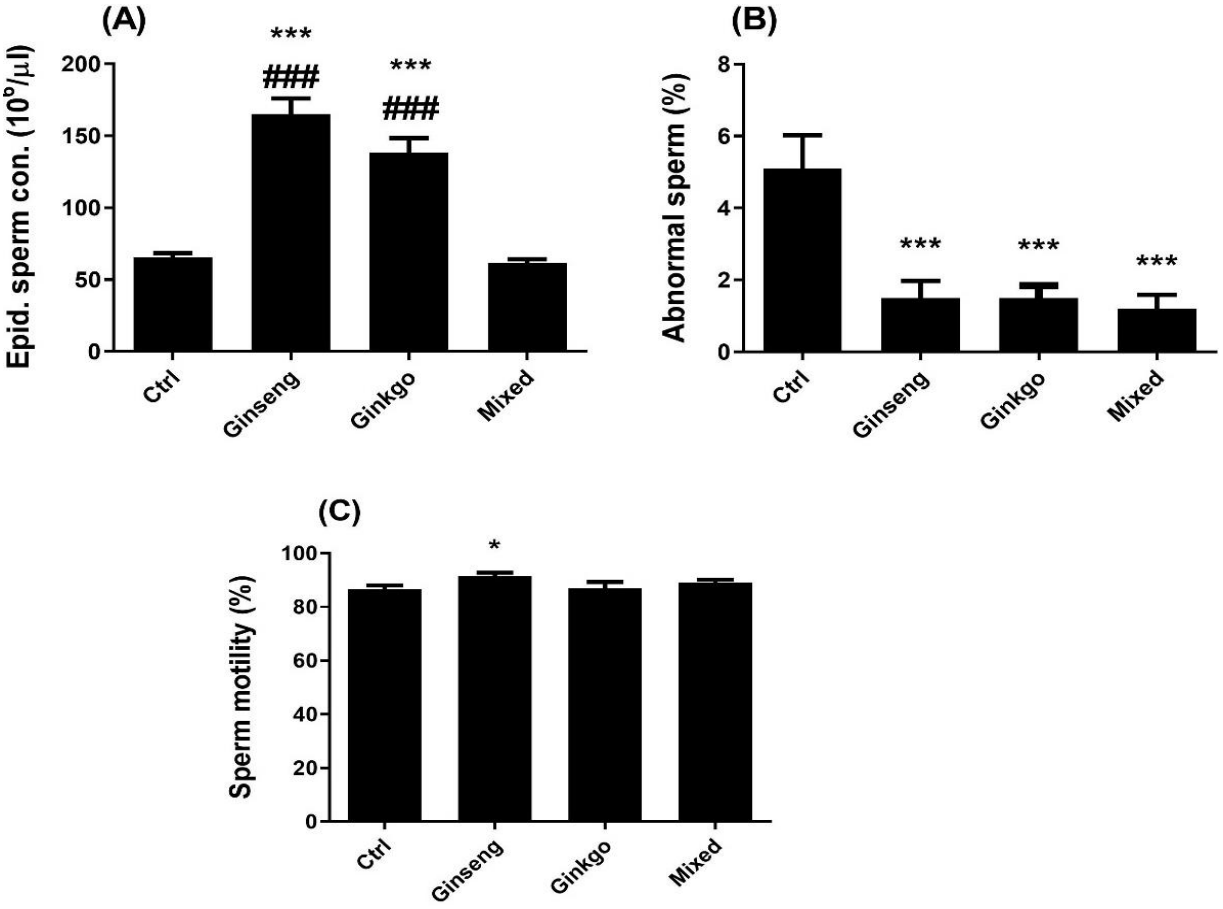

Figure 3: Epididymal semen analysis in treated and control rats after administrated of P. ginseng and/or G. biloba for 60 days. n= 10, values are expressed in Mean ± S.E.M. *p<0.05, **p<0.01, ***p<0.001 compared with control, ###p<0.001 compared with mixed.

Epididymal semen analysis. Figure 3 showed the effect of herbal treatment on some epididymal semen parameters in treated and control rats. Rats received P. ginseng and G. biloba showed significant (P<0.001) (165.24±10.46 and 138.26±10.09, respectively) increase in epididymal sperm count (10^6/µl) (165.24±10.46 and 138.26±10.09, respectively) compared with control (65.36±3.30). Whilst rats administered both P. ginseng and G. biloba showed no change (61.42±2.79) compared with control and significant decrease compared with P. ginseng and G. biloba treated rats (Figure 3A).

Expectedly, administration of P. ginseng and/or G. biloba improve semen quality by decreasing the percent of abnormal sperm as shown in figure 3B. The percent of abnormal sperm in different group was 5.10±0.92, 1.50±0.48, 1.50±0.34 and 1.20±0.39 in control, P. ginseng, G. biloba and mixture treated rats, respectively.

Regarding to the percent of sperm motility, only rats received P. ginseng showed significant (P<0.05) increase (91.50 ±1.30) compared with control (86.50± 1.50) whereas, G. biloba and mixture treated rats disclosed no change (87.00±2.26 and 89.00±1.25, respectively) compared with control.

Histological examination. The testicular tissue of control rats exhibited normal histological ar-

chitecture of the seminiferous tubules with normal arrangement of the germ cells including spermatogonia, spermatocytes and spermatids as well as spermatozoa with stimulating the activity of seminiferous tubules (Figure 4 a and b). Testes of the rats received P. ginseng revealed mature seminiferous tubules with adult spermatids and spermatozoa with different stages of spermatozoa and slight vacuolation (Figure 4 c and d). Moreover, testes of the rats received G. biloba showed mature spermatozoa inside the seminiferous tubules, in addition to edematous infiltration of red acidophilic homogenous materials in the interstitium (Figure 4 e and f). Finally, testicular tissue of rats received both P. ginseng and G. biloba showed activity of seminiferous tubules represented by mature spermatocytes, besides cytoplasmic vacuolation in some seminiferous tubules (Figure 4 g and h).

DISCUSSION

The studies concerning the effect of this herbal stimulants in normal animal to increase the efficiency of male reproductive system are scarce. So, the aim of the current study is to know the effect of some herbal stimulants (P. ginseng and/or G. biloba on the normal animals which will reflect on their productivity. The result of the present study showed there was a significant decrease in testosterone level in rats administered panax ginseng for 60 days compared with control. But there was no significant change in level of both LH and FSH compared with control. The results of the previous studies as those carried out by Sabah and Linjawi (2015) different from ours; they found that three hormones have been increased in male rats after exposed to P. ginseng treatment. It is worth mention that serum level of reproductive hormones is dose dependent and time as reported by Yoshimura et al. (1998) who reported that rats fed with 5% P. ginseng in the diet for 60 days significantly increased blood testosterone level, whereas treatment with 1% had no effect. At the same time, rats treated with American ginseng (10-100 mg/kg) for 28 days orally showed no significant change in testosterone and LH levels. The variation of reproductive hormones owing to the is ginsenoside Rb1 which is the main active ingredient in ginseng; it is responsible for the elevation of serum testosterone, FSH and LH by direct stimulation of anterior Pituitary (Tsai et al. 2003; Wang et al. 2010). In addition to dose and time dependence, the effect of P. ginseng on reproductive hormones are age dependent as testosterone level marked elevated after oral administration of P. ginseng to aged rats with daily dose of 200 mg/kg body weight for 4 months compared with control whereas, there was decreased FSH and LH levels (Ford et al. 2000; Won et al. 2014).

Regarding our results, as mentioned before there was decreased level of testosterone and no changes in FSH and LH levels in rats treated with P. ginseng in same time, there were increased sperm count, decrease abnormal sperm, improve sperm motility percentage 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. (2017) who reported that male rats showed significant increase in sex hormones receptors (androgen, luteinizing hormone and follicle stimulating hormone receptors) in P. ginseng treated groups. Also, Park et al. (2017) mentioned that the ginsenoside of P. ginseng which are needed for distribution of receptors of sex hormones that are essential for the action of these hormones on testes.

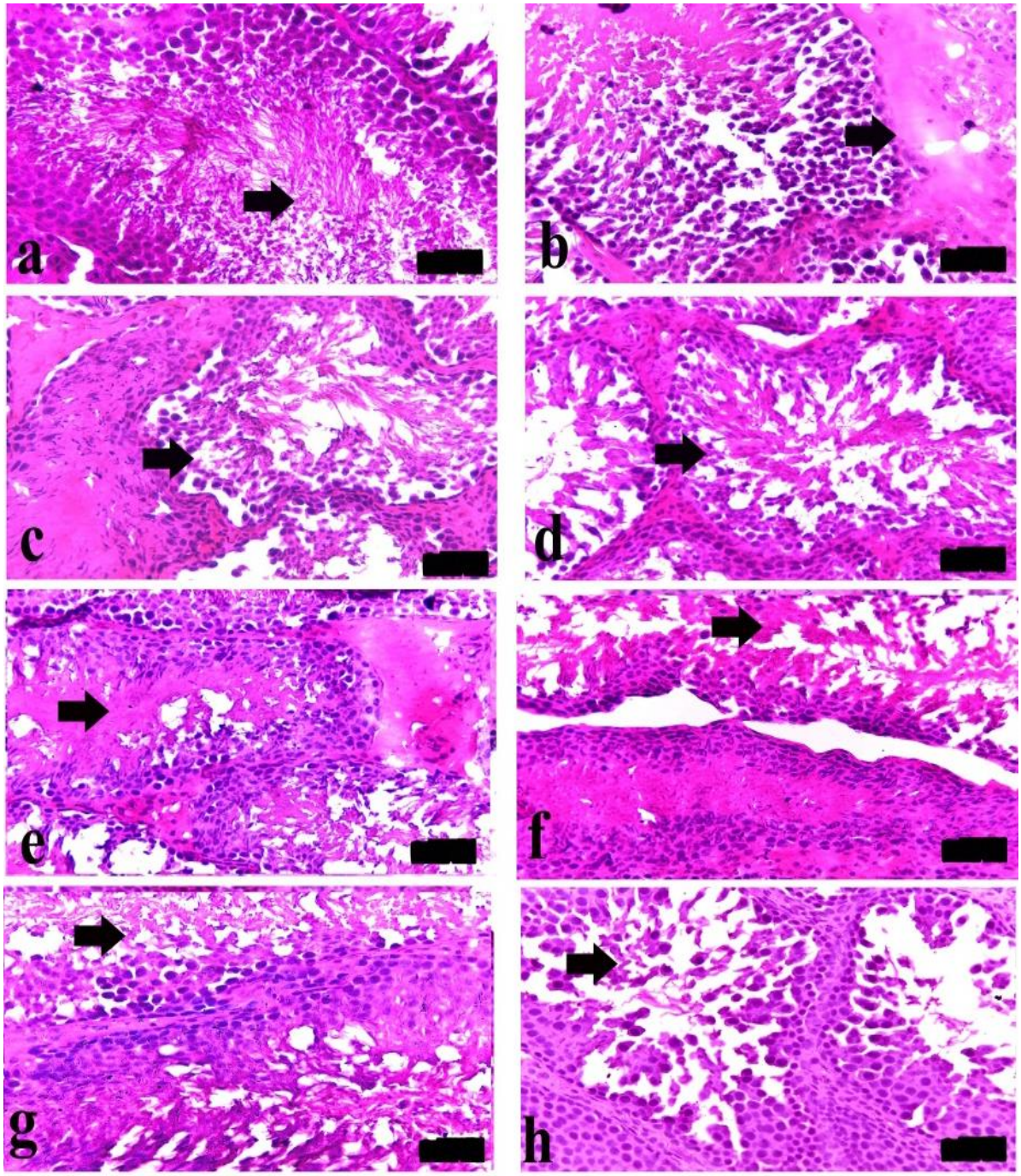

Figure 4: Photomicrograph of the control and treated rats after administration of P. ginseng and/or G. biloba. Control testicular tissue showing normal histological structure of the seminiferous tubules with normal arrangement (arrows) (a and b). Testes of the rats administered P. ginseng (200 mg/kg) for 60 days showing mature seminiferous tubules with adult spermatids and spermatozoa with slight cytoplasmic vacuolation in the seminiferous tubules (arrows) (c and d). Testes of the rats administered G. biloba (150 mg/kg) for 60 days mature spermatozoa inside the seminiferous tubules (arrows), in addition to edematous infiltration of red acidophilic homogenous materials in the interstitium (e and f). Testes of rats administered P. ginseng and G. biloba (100 and 75 mg/kg, respectively) for 60 days showing active seminiferous tubules with mature spermatocytes, besides cytoplasmic vacuolation in some seminiferous tubules (arrows) (g and h). (H&E, bar= 50 µm).

Our results revealed that there was a significant decrease in testosterone level without change in FSH and LH level in mature rats administered G. biloba extract for 60 days compared with the control. Looking throughout the available literatures there are few studies concerning the effect of G. biloba extract on testosterone level. These few studies have been confirmed there was no change in testosterone level in dose-independent manner as administration of G. biloba for 56 and 28 days failed to elevate serum testosterone (Yeh et al. 2008; Predes et al. 2011; Oshio et al. 2015). However, level of FSH increased markedly and improved of ischemia-induced testicular injury in adult male rats (Ahmed et al. 2016). Concerning our results, although there was a significant decrease in testosterone concentration in serum and no significant change on LH and FSH in rats treated with G. biloba extract for 60 days there were increased sperm concentration in compared with control and the histological examination cleared that there was improved spermatogenesis. These effects can be attributed to good utilization of hormones by the testis. There are no available studies conducted on a mixture of P. ginseng and gingko biloba extract. At the same time, our results showed no significant change in the sperm concentration compared with control as seen in discussion. So, it is concluded that the effect of each extract alone on male reproduction is better than their mixture in mature rats.

Most of the available studies conducted on the effect of P. ginseng on thyroid gland with hypothyroidism. Issa and El-Sherif (2017) mentioned that P. ginseng has a thyromimetic effect so, it reserved the deficiency of thyroid gland hormones in case of subclinical hypothyroidism. Also, Dai et al. (1999) P. ginseng injection increased T3 and T4 in case of hypothyroidism in patients with congestive heart failure. Whilst Kim (2010) and Xiao et al. (2017) found that P. ginseng able to recover the decrease in the thyroid gland weight, T3, T4 and triglycerides induced due to hypothyroidism produced by thiouracils. So, it is conducted that P. ginseng is beneficial on the thyroid gland hormone production with good effect on the hypothyroidism mediated by the modulator effects on the antioxidants defense system.

The obtained results showed that there was significant increase in the level of both T3 and T4 levels compared with control. However, the previous studies that manipulate the relationship between thyroid hormones and G. biloba are variable; for instance, Abdellah et al. (2017) reported that there was no change in T3 and T4 levels in rats treated with G. biloba in adult male rats. On the other hand, Rider et al. (2013) found that exposure to high dose for long periods induces toxicity and carcinogenicity in the thyroid gland of male and female mice and rats. Also, the study of (Dardano et al. 2012) recorded that the G. biloba extract may protect from possible oxidative and genotoxic damage associated with iodine-13 treatment in patients with thyroid cancer. We hypothesize the enhancement effect of G. biloba on thyroid activity results from activation of active transport of Iodine through biosynthesis of thyroid hormones in thyroid follicles besides, antioxidant properties of G. biloba gradients. Moreover, G. biloba is one of the most herbal brain stimulants so, it is expected to stimulate TSH secretion from pituitary gland under control of hypothalamus.

The obtained results showed that there were significant increase T3 level in rats administered the mixture compared with control. This can be attributed to synergetic action of both P. ginseng and G. biloba extracts to increase the thyroid hormones. Generally, it is

concluded that the effect of both P. ginseng and G. biloba extract on the thyroid gland need more studies and the available studies not enough to clear their effect on the physiology of thyroid gland and their hormones and our results can be attributed to the ant oxidative effect of both P. ginseng and G. biloba extract.

The obtained results showed that administration of P. ginseng for mature rats for 60 days significantly increased sperm concentration with decrease of abnormal sperm and motility percent compared with control. Further, the activity of spermatogenesis was obvious and as indicated by histological examination. Many studies were conducted on the effect of P. ginseng extract on testis function, also these studies support our results. For instant, administration of P. ginseng for 56 days has a potential role in spermatogenesis activity, increase sperm concentration and enhance sperm motility (Yang et al. 2011) as it leads to induction of Catsper expression which is a protein stimulates the motility of sperm (Park et al. 2014). The same results obtained by Eskandari et al. (2016) who found that P. ginseng improved the percentage of sperms with normal morphology, mass activity and progressive motility. A lot of studies mentioned that P. ginseng has a protective and preventive effect against anticancer drugs cause testicular damage (Akram et al. 2012; Jung et al. 2015). Furthermore, P. ginseng used in the protection of testis in case of use radiation therapy in treatment of cancers Gosselin et al. 2002) and protect the testis against the effect of toxins (Lee et al. 2007; Wang et al. 2012). Not only in normal rats, but also damaged fertility in diabetic model rats recovered after P. ginseng administration for 90 days improvement of induced testicular pathological signs (Sawiress et al. 2011). The effect of P. ginseng on testicular function is produced through the ginsenosides which structurally resemble steroid hormones such as, androgens which are important for development and maintenance of male sexual characteristics and regulate spermatogenesis (Solakidi et al. 2005). In addition, ginsenosides affect male sexual functions and spermatogenesis by stimulation of steroid receptors which found in male genital tissues and spermatozoa (Leung and Wong, 2013 and Park et al. 2017).

As mentioned before, G. biloba increased sperm concentration, motility and decrease abnormal sperm percent in addition, histological examination revealed mature spermatozoa in seminiferous tubules. Some previous reported effect of G. biloba supports the current results by amelioration of reproductive parameters such as sperm motility, vitality, and concentration due to the presence of quercetin which is one of the main components of G. biloba extract Taepongsorat et al. (2008). Further, G. biloba can protect the testis against some intoxication as that produced by cisplatin intoxication preventing the decrease in number and motility of sperms produced by this intoxication. This testicular protection is produced by the antioxidative effect of G. biloba extract as it increases the antioxidative enzymes such as Cat, SOD and MPO and decreased the content of malondialdehyde (Khafaga and Bayad 2016). Moreover, the recent results conducted by Gevrek et al. (2018) confirmed the essential role of G. biloba in protection of testicular injury and sperm morphology anomaly caused by artificial testicular torsion. This amelioration owing to increase of LH receptors subsequently, enhance Leydig cells and sperm activity as its antioxidant, anti-apoptotic and anti-inflammatory properties. In addition, G. biloba is considered one of the most broad-spectrum free radical scavenger due to its flavonoids scavenge free radicals, decrease lipid peroxidation

and increase antioxidant activities (Mohamed and El-Moneim, 2017) as well as it has superoxide radical anion scavenging capabilities and activities like superoxide dismutase and that it increased glutathione levels (Wang et al. 2008). While low dose administration of G. biloba elucidated no changes in sperm concentration, motility, and concentration (Oshio et al. 2015). However, other studies did not match our results and reported toxic effect of G. biloba in mice due to activation of GABA, glycine and glutamate (Al-Yahya et al. 2006).

CONCLUSION

In conclusion, P. ginseng and G. biloba have ameliorative effect on male reproductive performance as it enhanced and improved all semen parameters and elevated some reproductive hormones in mature and immature rats, P. ginseng and G. biloba have progressive power to synergize thyroid function ability by improvement level of thyroid hormones mature rats, P. ginseng and G. biloba administration for mature rats revealed enhancement of histological findings in testes by stimulation the activity of seminiferous tubules, the effect of each extract alone on male reproduction is more better than their mixture, The use of mixture for mature rats has no effect in sperm characters.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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