Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org
Original Research

Can *Panax Ginseng* protect against fertility disorders in hypothyroid female albino rats?

Amany H. Mahmoud Elgoly[1], Lobna F. Wahman[1,2], Magda HM Yousef [2*]

[1] National Organization for Drug Control and Research (NODCAR), Egypt
[2] Faculty of Medicine, Taibah university, KSA

Correspondence to: mhm.youssef@gmail.com

Received 19, 2018; **Accepted** October 25, 2018; **Published** October 30, 2018

Doi: http://dx.doi.org/10.14715/cmb/2018.64.13.18

Abstract: Hypothyroidism is an endocrine disorder due to decreased thyroid hormone production. This endocrine disorder significantly affects the menstrual cycle and fertility. The aim of this present study was to assess the efficacy of *Panax ginseng*, one of traditional Chinese medicine, in ameliorating the gonadal hormonal dysfunction and lowering oxidative stress accompanied with hypothyroidism in adult female albino rats. After confirming regularity of the oestrus cycle in the female rats in this study, hypothyroidism was induced by using daily 5.0 mg kg-1 oral dose of Neo-mercazole. The hypothyroid rats were randomly grouped into two groups; hypothyroid group (H): did not received any treatment, group II (H+G) was treated with *Panax ginseng* extract for one months after hypothyroidism induction. Another two groups were included in the study, a negative control group (Euthyroid group) and a positive control group; received *Panax ginseng* extract only. Hypothyroidism resulted in irregularity of oestrus cycle accompanied with decrease in luteinizing hormone (LH), follicular stimulating hormone (FSH) and estradiol (E_2), while prolactin (PRL), progesterone (P) and testosterone (T) hormone were significantly elevated. Hypothyroidism elevated capsae-3 and 8OH-deoxy guanosine expression and increased secretion of corticosterone and ERK1/2. This study showed that *Panax ginseng* improved hypothyroid-induced deterioration in trophic and gonadal hormones through free radicals' scavenger.

Key words: Ginseng; ROS; Gonadal hormones; Cortisol; ERK1/2.

Introduction

Thyroid hormones are essential for normal mammalian development and play fundamental roles in the cardiovascular, nervous, immune and reproductive systems. Thyroxin (T_4), the metabolically active hormone, represents 97 % of the thyroidal secretion while 3 % is triiodothyronine (T_3). The majority of T_3 is produced in the peripheral tissues by conversion of T_4 to T_3 by a selenium-dependent enzyme. (1,2)

Hypothyroidism is a public health problem characterized by low basal metabolic rate caused by insufficient synthesis, secretion or biologically dysfunctional thyroid hormones. Decreased T_3, T_4, TRH are accompanied with increasing in TSH level. These changes are considered the assessment criteria of hypothyroidism along with the clinical manifestations including; low basal metabolic rate, fatigue, sluggishness, chills, and hypothermia. (3) Shahrani *et al.* reported that prevalence of goitre in some Arabic countries including Egypt, Algeria and Bahrain were 25.25, 86 and 1.7%, respectively. (4) Female gender, dietary and iodine deficiency are the most common risk factors associated with hypothyroidism. Consequently, prevalence of hypothyroidism is 0.2 and 2% in male and female populations respectively (5). Hypothyroid dysfunction in female has been associated with menstrual abnormalities, anovulation and hyperprolactinemia which cause ovulation disorders with increased risk of miscarriage, premature delivery, placental abruption and poor perinatal outcome with low birth weight. (5)

Traditional Asian practitioners discovered Korean ginseng (*Panax ginseng*). It belongs to the *Panax* genus of the Araliaceae family. (6) Ginseng grows in the coolest climate regions of the Northern Hemisphere, it is effective as a traditional complementary and alternative medicine. (7) P*anax ginseng* can be classified into white ginseng (air-dried), red ginseng (steamed), and sun ginseng. Notably, red ginseng, have an elevated content of ginsenosides, which are bioactive compounds. It though that red one exhibits various biological activities against chronic diseases, such as diabetes mellitus, cancer, and cardiovascular disease , pulmonary disease , improving the immune system and atherosclerosis (8, 9). chemical analysis of ginseng revealed the presence of many ingredients, including organic acids, vitamins, sugars, inorganic salts, sterols, oligopeptides, polysaccharides, volatile oils, and 112 saponins. The class of saponins called ginsenosides which the most important bioactive compound in Korean ginseng. (10) Ginsenosides have antimicrobial, antifungal effect and many therapeutic activities including vasorelaxation, antioxidation, anti-inflammation and anticancer. (9) Ginseng is used safely and the incidence of adverse effects seems to be low. Ginseng has benefits for all the endocrine system and therefore thyroid gland which support its function. (11) There are some evidence confirm that Korean ginseng not only helping in decreasing the onset of menopause symptoms but also boosting sexual arousal in menopausal woman. (12) Ginsenosides are lipophilic so it can pass through the cell membrane by simple diffusion and bind to its intracellular target proteins in the cytoplasm and nucleus. Ginseng characterized by a complex activity profile that includes antioxidant, anti-inflammatory,

anti-apoptotic, and immune- stimulatory properties and has the effects of stabilizing and balancing the entire physiology. (13) So for the importance of this herbal medicinal plant and referring to previous scientific researches, the present study conducted to assess the effect of Panax Ginseng extract administration on gonadal disorders associated with hypothyroidism in female albino rats.

Materials and Methods

Korean Panax ginseng

Dried roots of the Korean Panax ginseng obtained from Pharco Pharmaceuticals Company; the dose was 1.8 mg/ 200 gm Body weight dissolved in distilled water.

Neo-mercazole

Carbimazole was obtained as a commercial tablet (Neo-Mercazole) from AFT Pharmaceuticals. Each tablet contained 5 mg of the active principle, carbimazole, to prepare a suspension of drug, 10 tablets were grinded and suspended in 100 ml distilled water to obtain a dose of 5 mg carbimazole/Kg Body weight.

Animals

Thirty-two Wister adult female albino rats weighing 180-200 gm, were used in this study. The animals were obtained from the animal house of National Organization for Drug Control and Research (NODCAR). The animals' acclimatization took two weeks before the experimentation. Animals were housed under controlled temperature of 25 ± 2 °C and 12-hour light/12 hour dark cycle throughout the experiment. The animals were fed on standard rodent pelleted diet and water *ad labium* and examined for the regularity of estrus cycle before the start of experiment. Animals were maintained in accordance with the Guide for the Care and Use of Laboratory Animals and all applicable institutional guidelines were followed.

The reproductive cycle of female rats is called estrus cycle which last between 4-5 days and is characterized by four stages; proestrus, estrus, metestrus and diestrus**.** During the estrus cycle, prolactin, LH and FSH remain low and increase during proestrus phase (Pre-Ovulatory day). Estradiol (E_2) levels begin to increase at metestrus, reaching peak levels during proestrus and returning to baseline at estrus. Before the starting of the present experiment, the rats were examined of their estrous cycle regularity; for one month, every morning between 8:00 and 9:00 a.m.; the vaginal of each rat was examined with plastic pipette filled with 10 mL of normal saline (NaCl 0.9%). Theses vaginal smears were then stained with hematoxylin and eosin according to the method of Marcondes, et al., (14) and Caligioni (15). Figure (1).

Experimental design

The animals were randomly divided into 4 groups (n=8). Euthyroid group (-ve Control) rats which received saline; the positive control group (+ve control) which received *ginseng extract* (1.8 mg/200gm b.wt) orally for two months, the whole experimental period. For induction of hypothyroidism, rats in the other two groups were orally administered a daily dose of 5.0 $mg.kg^{-1}$ NeoMercazole® for 30 days. Hypothyroidism was confirmed by measurement of fT_3 & fT_4, TSH, TBG and TTR in serum by ELIZA kits. These hypothyroid rats were then dived into two main groups; hypothyroid group (H) left without any treatment and the other hypothyroid group was orally treated with ginseng (H+G) for one months. The protocol of the study was approved by the Institutional Animal Care and Utilization Committee (IACUC) at the National Herpetology and Tropical Medicine Research Institute, Egypt.

Blood samples collection

At the end of the experiment, blood samples were withdrawn from the animals, after that the animals were dissected, and the organs were kept for further wok. The collected blood from retro-orbital plexuses left to coagulate at room temperature. The samples were centrifuged at 5000 r.p.m for 10 min to separate the sera which stored at for -20°C for biochemical investigations.

Biochemical parameters

fT_3, fT_4 and TSH were assessed in serum using immunoassay technique (ELISA) kits according to manufacturer's instruction (WKEA, WKEA medical supplier co. China). Thyroid binding protein (TBG), transthyretin (TTR), serum follicular stimulating hormone (FSH), Prolactin (PRL), Luteinizing Hormone (LH) as well as Extracellular Signal Regulated Kinase 1/2 (ERK1/2), 8-hydroxy-guanosine (8-OHdG) and Caspase 3 (Caspase-3) were examined in serum using Rat ELIZA test according to the manufacture instruction (GSCIENCE, Glory Science Co., Ltd. USA). Serum corticosterone (cortisol) was determined using ELISA kit according to the manufacture instruction (AccuBind- Monobind Inc, USA). Serum testosterone (T), progesterone (P) and Estrogen (E2) were determined using Rat ELISA kit according the manufacture instruction of (BioCheck,

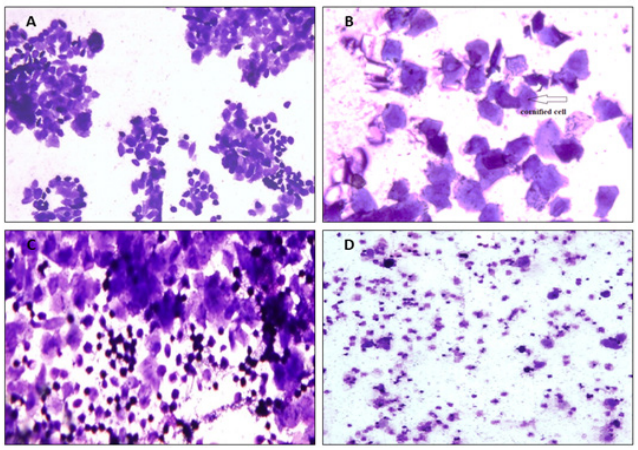

Figure 1. photomicrograph of vaginal smears showing the different phases of estrous cycle. Pro-estrus (A), consists mainly from nucleated epithelial cell, Estrus (B), characterized by cornified cells; Metestrus (C), consisting of the three types of cell, leukocytes, cornified, and nucleated epithelial cells; and Diestrus (D) consisting predominantly of leucocytes. Figure (1): photomicrograph of vaginal smears showing the different phases of estrous cycle. Pro-estrus (A), consists mainly from nucleated epithelial cell, Estrus (B), characterized by cornified cells; Metestrus (C), consisting of the three types of cell, leukocytes, cornified, and nucleated epithelial cells; and Diestrus (D) consisting predominantly of leucocytes.

BioCheck Co., Ltd. USA) , (CUSABIO Co., Baltimore, USA) and (Immnunospec, CO., USA), respectively .

Statistical analysis

The statistical analysis of the biochemical data was performed using SPSS version 22. Data were expressed as mean ± S.D. Statistical differences between groups were performed using ANOVA test with significance level P<0.05, the *post Hoc* if groups have an equal assumed variance carried out using Bonferroni test.

Results

Serum Thyroid hormones

Induction of hypothyroidism state with Neo-Mercazol caused significant elevation of TSH along with significant decrease in fT_4 and non-significant decrease in fT_3 as compared with the control group. Regarding the TTR, it was significantly increased while and the TBG was non-significantly decreased. Treatment with ginseng significantly lowering the level TSH as compared with hypothyroid group, also the levels of fT_3 and fT_4 were increased as compared to hypothyroid group. The transporters proteins TTR and TBG were improved as compared with hypothyroid and control group. (Table 1).

Serum Fertility Hormones

In the present study, hypothyroidism was resulted in significant decrease in serum LH, FSH and E_2 while PRL, P and T hormones were significantly increased comparing to negative and positive control. In hypothyroid rats treated with ginseng, the levels of gonadal and trophic hormones were restored to the control level. (Table 2).

Serum ERK 1/2 level and E2/T ratio after hypothyroidism induction

There was significant elevation in extracellular signal-regulated protein kinases 1/2 (ERK1/2) along with the decreased E2/T ratio in hypothyroidism as compared with the both control groups. Treatment of hypothyroid rats with ginseng ameliorate the level of ERK1/2 as well as estradiol/ testosterone ratio to near the normal levels Table (3).

The effect of hypothyroidism induction on serum corticosterone

It was observed that corticosterone, stress hormone, was significantly elevated in hypothyroid rats comparing with negative control. Treatment with ginseng ameliorated this effect as shown in Table (3).

Serum oxidative stress markers after hypothyroidism induction

In this study, serum level of oxidative marker (8-OH-Guanosine) and cell apoptotic marker (Caspase-3) were elevated in hypothyroid rats as compared to negative

Table 1. Thyroid hormones and their carrying proteins levels of in the studied groups.

Parameter	-ve control	+ve control	H	H+G
fT_3(ng/ml)	1.99 ±0.06	1.54 ± 0.05	1.59 ± 0.02	1.80 ± 0.04 (b*)
fT_4(µg/ml)	6.61± 0.14	7.32±0.36	4.07±0.12 (a*)	6.78±0.34 (b**)
TSH(mIU/ml)	12.31 ±0.62	12.81 ±0.69	14.09 ±0.42 (a*)	10.75 ±0.26 (b**)
TTR(ng/ml)	32.56 ±2.04	36.93 ±2.95	44.19 ±3.34 (a*)	27.41 ±1.96 (b*)
TBG(pg/ml)	2.54 ±0.19	2.49 ±0.06	2.26 ±0.07	2.37 ±0.13

H: Hypothyroid group. H+G: Hypothyroid group treated with ginseng. Data represented as mean±SD, n=8 animals. *, **, ***: represent p<0.05, p<0.01, p<0.001 respectively. a: represent the mean significance difference from the control group. b: represent the mean significance difference from the hypothyroid group.

Table 2. Serum fertility hormones levels in hypothyroid and treated adult female albino rats

parameter	-ve control	+ve control	H	H+G
FSH(mIU/L)	7.11±0.16	7.98±0.14	5.99±0.18 (a*)	7.03±0.12
LH(ng/L)	6.47±0.29	7.38±0.38	4.35±0.19 (a**)	6.73±0.28 (b**)
E_2(Pg/ml)	20.76±0.64	20.3±0.39	14.7±0.8 (a*)	18.00±0.77 (a*,b**)
P (ng/ml)	23.24±1.66	20.66 ±1.51	30.41±1.18 (a*)	20.56±1.67 (b*)
T (ng/ml)	0.32±0.06	0.35±0.03	0.39±0.07 (a*)	0.35±0.04
PRL (ng/ml)	94.06±4.89	104.12±4.41	149.11±11.33 (a**)	101.02±6.58 (b*)

H: Hypothyroid group. H+G: Hypothyroid group treated with ginseng. Data represented as mean±SD, n=8 animals. *, **, ***: represent p<0.05, p<0.01, p<0.001 respectively. a: represent the mean significance difference from the negative control group. b: represent the mean significance difference from the hypothyroid group.

Table 3. ERK1/2 , E2/T ratio and cortisol level in serum of hypothyroid and treated female albino rats**.**

Parameter	-ve control	+ve control	H	H+G
ERK 1 /2 (pg/ml)	47.38±1.93	50.17±2.57	63.17±3.13 (a*)	42.26±1.7 (a*b**)
E2/T ratio	64.87±10.3	58.0±13.0	37.6±11.4(a***)	51.4±19.25(b*)
Corticosterone(µg/dl)	1.8±0.06	1.7±0.11	2.82±0.21 (a**)	1.73±0.10 (b*)

H: Hypothyroid group. H+G: Hypothyroid group treated with ginseng. Data represented as mean±SD, n=8 animals. *, **, ***: represent p<0.05, p<0.01, p<0.001 respectively. a: represent the mean significance difference from the negative control group. b: represent the mean significance difference from the hypothyroid group.

Table 4. Oxidative stress markers in serum of hypothyroid and ginseng -treated female albino rats.

Parameter	-ve control	+ve control	H	H+G
8-OH Guanosine (ng/L)	48.46±2.21	32.79±0.05	57.99±0.02 (a*)	48.63±0.08 (b*)
Caspases (ng/ml)	5.26±0.20	5.94±0.15	12.44± 0.67 (a***)	5.48±0.14 (b***)

H: Hypothyroid group. H+G: Hypothyroid group treated with ginseng. Data represented as mean±SD, n=8 animals. *, **, ***: represent $p<0.05$, $p<0.01$, $p<0.001$ respectively. a: represent the mean significance difference from the negative control group. b: represent the mean significance difference from the hypothyroid group.

and positive control. Ginseng treatment succeeded to reduce the levels of these oxidative and apoptotic markers to near the control levels Table (4).

Discussion

The present study was carried out to investigate the ameliorating effect of Korean ginseng root on the gonadal hormonal dysfunction and lowering oxidative stress accompanied hypothyroidism in female albino rats. The study revealed a significant elevation in serum TTR, decrease in fT_3, fT_4 and TBG in hypothyroid rats. Administration of *Panax ginseng* resulted in noticeable increase in the THs and this could attributed to the thyromimetric effect of ginseng. (16). Ginseng is aromatic herb containing high level of iodine which is main component of the thyroid hormones. (17) Ginsenosides which is the main components of ginseng considered one of the adaptogenic substances that shares in re-stabilization of many physiological processes and promotes cellular homeostasis by decreasing the sensitivity of the cell to any stress-like hypothyroidism as stated in European Medicines Agency (13) . So, adaptogenic ginsenosides in the present study raised the circulating level of fT_4 and fT_3 by activating the enzyme responsible for converting fT_4 to active fT_3 as well as reducing the inhibitory thyroid-hormone-blocking reverse T_3 (rT3), which is bound to normally functioning T_3 receptors, subsequently inhibited active T_3 and decreased its vital metabolic rule. (10, 14)

The hypothyroid pituitary adrenal gonadal axis was clarified in present study. Hypothyroidism in present work deteriorated the pituitary trophic hormones (LH& FSH), as compared to negative control group. These reproductive hormonal changes was accompanied with irregularity of estrous cycle of the rats after initiation of hypothyroidism induction by about one week and this was confirmed the importance of THs in the regulation of reproductive hormone and estrus cycle (18). The mechanisms by which the trophic pituitary hormones regulate steroid hormone biosynthesis include, the transcription of steroidogenic genes, regulating cholesterol uptake and transport, as well as modulating steroidogenic enzyme activity. The reduction in trophic hormone (FSH &LH) associated with hypothyroidism in present model, led to decreasing the E_2 hormone level, whereas the concentration of P and T were significantly increase. The inhibition in gonadal activity in hypothyroid rats in present model was confirmed by the lowering of E_2/T ratio which is a marker of the aromatase enzyme activity (estrogen synthase, CYP19A1), this is a key enzyme converted testosterone into estrogen in granulosa cell. (19) Hyperprolactinemia , as recorded in present study, negatively affected the activity of aromatase enzyme and this lead to hypoestrogenism. Moreover, hyperprolactinamia inhibited the release of gonadotrophic hormones (LH & FSH) from pituitary gland and potentiated the inhibitory action of inhibin hormone that stimulated a negative feedback and lowered estradiol level. (20)

The administration of *panax ginseng* to hypothyroid rats in the present work was ameliorated these changes in trophic and steroidal gonadal hormones due to the triterpenoid saponins which steroidal in nature and considered the precursors of steroidal hormones in both plants and animals. (21) Ginseng also have a positive effect on sexual function by altering the activity in the brain to facilitate hormonal behavior and secretion. (22) Korean Red Ginseng may have potent estrogenic properties on female rats either directly by binding to estrogen receptor or indirectly through up-regulating ERs in reproductive organs as uterus of both normal and ovariectomized mice as well as premenopausal woman. (23, 24) Another studies were potentiated our investigation, that the ginseng improved the sexual dysfunction in woman with hypothyroid dysfunction. (25). Abdalhafid stated that *Panax ginseng* has fertility property and androgenic activity in male rabbit by increasing testosterone level. (26) *Ginseng* also was normalized FSH, LH and T levels by a direct or an indirect effect on the HPG axis. The sexual dysfunction was reported in present work may be developed from psychological stress state exerted by hypothyroidism induction and this confirmed the role of pituitary adrenal gonadal axis (HPA) as a defense mechanism carried out by the organism against stress event. (27) The current study exhibited significant elevation of corticosterone hormone, serum 8-hydroxyguanosine, an oxidative stress marker, as well as caspase-3, an apoptotic marker. Elevation of serum TSH in present hypothyroid rat model augmented the synthesis of corticosteroid hormones together with the oxidative stress markers that in turn inhibited the release of pituitary gonadotrophic hormones (LH & FSH) affecting negatively the steroidogenesis process. (28)

In consistence with the present result, Xin et al. mentioned that TSH elevated with the hypothyroidism considered an apoptotic factor resulting in significant increase in serum 8-hydroxyguanosine and caspase-3. (29) The increasing in cortisol and stress oxidative markers in hypothyroid rats caused an elevation of reverse T_3 (rT3), which was interfered with beneficial activity of normal T_3. Ginseng by its adaptogenic capability action could destroy the (rT3) and restore the cellular homeostasis. (14). In addition, ginseng with its lipophilic nature and steroidal backbone can pass easily from the cell membrane by diffusion; bind to specific intracellular mineralocorticoid and glucocorticoid protein receptors in cytoplasm and nucleus , initiating its genomic pathway to regulate the cellular function. (30). So, the administration of *panax ginseng* to current hypothyroid rats was renormalized the cortisol and oxi-

dative stress markers because it increased the cellular resistance to stress and potentiated the role of immune system through triterpenes of ginsenosides. (27, 31) . Noteworthy, ERK1/2 was activated in response to the elevation of oxidative stress and cell death apoptotic markers as underlined in present study (32)

Based on all above investigations and correlation, the present work was provided a supporting mechanism by which the oral ginseng administration was improved the gonadal disturbance and stress markers in hypothyroid female rats.

In conclusion, the present study is pointed out to the pituitary-gonad-adrenal disturbances aroused from the hypothyroidism induction by Neo-mericazole and how ginseng, one of the most Asian medicinal traditional plant, significantly normalized the fertility disorders and acted as free radicals' scavenger in adult female rats.

Conflict of Interests

No potential conflict of interests relevant to this paper was reported.

References

1. Alkalby JMA, Alzerjawi SJS. Effect of propylthiouracil-induced hypothyroidism on reproductive effeciency of adult male rats. Bas J Vet Res. 2013 12(2): 113-123.

2. Kong, L. Wei Q, Sulieman J, Fangxiong F, Nagaoka K, Watanabe G. Effects of thyroid hormones on the antioxidative status in the uterus of young adult rats. J Reprod Dev. 2015 Jun; 61(3): 219–227.

3. Si Wu, Guangguo Tan, Xin Dong, Zhenyu Zhu, Wuhong Li, Ziyang Lou, Yifeng Chai. Metabolic Profiling Provides a System Understanding of Hypothyroidism in Rats and Its Application. PLoS One. 2013; 8(2): e55599.

4. Shahrani A, El Metwally A, Al Surimi K, Bin Salih S, Saleh Y, Al-Shehri A, Ali A. The epidemiology of thyroid diseases in the Arab world: A systematic review. J Publ Health Epidemiol. 2016, 8(2): 7-26.

5. Hapon MB, Gamarra-luques C, Jahn GA. Short term hypothyroidism affects ovarian function in the cycling rat. Reprod Biol Endocrinol. 2010; 8: 14.

6. Rokot NT, Kairupan TS, Cheng K, Runtuwene J, Kapantow NH, Amitani M, Morinaga A, Amitani H, Asakawa A, Inui A. A Role of Ginseng and Its Constituents in the Treatment of Central Nervous System Disorders. Evid Based Complement Alternat Med. 2016; 2016: 2614742.

7. Wong, KWL, AS. Ginseng and male reproductive function. Spermatogenesis. 2013 Jul 1; 3(3): e26391.

8. Lee Y-M, Yoon H, Park H-M, Song BC, Yeum K-J. Implications of red *Panax ginseng* in oxidative stress associated chronic diseases. J Ginseng Res. 2017 Apr; 41(2): 113–119.

9. Park HJ, Choe S, Park NC. Effects of Korean red ginseng on semen parameters in male infertility patients: A randomized, placebo-controlled, double-blind clinical study. Chin J Integr Med. 2016 Jul;22(7):490-5.

10. Park KS, Park KI, Kim JW, Yun YJ, Kim SH, Lee CH, Park JW, Lee JM. Efficacy and safety of Korean red ginseng for cold hypersensitivity in the hands and feet: a randomized, double-blind, placebo-controlled trial. J Ethnopharmacol. 2014 Dec 2;158 Pt A:25-32.

11. Nm I, Nm E. Effect of Ginseng on the Testis of Subclinical Hypothyroidism Model in Adult Male Albino Rat. Austin J Anat. 2017; 4(2): 1065.

12. Lee HW, Young JC, Lee J, Kil K-J, Lee MS. Ginseng for managing menopausal woman's health: A systematic review of double-blind, randomized, placebo-controlled trials. Medicine (Baltimore) 2016 Sep; 95(38): e4914.

13. Yeo HB1, Yoon HK, Lee HJ, Kang SG, Jung KY, Kim L. Effects of Korean Red Ginseng on Cognitive and Motor Function: A Double-blind, Randomized, Placebo-controlled Trial. J Ginseng Res. 2012 Apr;36(2):190-7.

14. Dai X, Zhou Y, Yu X, Han M. Effect of ginseng injection on congestive heart failure and thyroid hormones Chinese Journal of Integrated Traditional and Western Medicine, 2000, 6(1): 29–31.

15. Caligioni C. NIH Public Access. Curr Protoc Neurosci, 2010: 1–11. doi: 10.1002/0471142301.nsa04is48.Assessing.

16. Paz-Filho G, Graf H, Ward LS. Comparative analysis of the new guidelines and consensuses for the management of hypothyroidism, thyroid nodules, and differentiated thyroid cancer. Arq Bras Endocrinol Metabol. 2013 Jun;57(4):233-9.

17. Park SJ, Lim KH, Noh JH, Jeong EJ, Kim YS, Han BC, Lee SH, Moon KS. Subacute oral toxicity study of korean red ginseng extract in sprague-dawley rats. Toxicol Res. 2013 Dec 31;29(4):285-92. doi: 10.5487/TR.2013.29.4.285.

18. Hatsuta M1, Abe K, Tamura K, Ryuno T, Watanabe G, Taya K, Kogo H. Effects of hypothyroidism on the estrous cycle and reproductive hormones in mature female rat. Eur J Pharmacol. 2004 Feb 23;486(3):343-8.

19. Wen L, Jiang X, Sun J, Li X, Li X, Tian L, Li Y, Bai W. Cyanidin-3-O-glucoside promotes the biosynthesis of progesterone through the protection of mitochondrial function in Pb-exposed rat leydig cells. Food Chem Toxicol. 2018 Feb;112:427-434. doi: 10.1016/j.fct.2017.10.008. Epub 2017 Oct 10.

20. Jeyaraj DA, Mani Maran RR, Aruldhas MM, Govindarajulu P. Progesterone induced modulations of serum hormonal profiles in adult male and female rats. Endocr Res. 2001 Feb-May;27(1-2):223-32.

21. Kuete V. Medicinal plant research in Africa : pharmacology and chemistry. 2013. 1st Edition. Elseiver. pp: 890.

22. Murphy LL, Lee TJ. Ginseng, sex behavior, and nitric oxide. Ann N Y Acad Sci. 2002 May;962:372-7.

23. Chung HS, Hwang I, Oh KJ, Lee MN, Park K. The Effect of Korean Red Ginseng on Sexual Function in Premenopausal Women: Placebo-Controlled, Double-Blind, Crossover Clinical Trial. Evid Based Complement Alternat Med. 2015;2015:913158. doi: 10.1155/2015/913158. Epub 2015 Dec 22.

24. Ding J, Xu Y, Ma X, An J, Yang X, Liu Z, Lin N. Estrogenic effect of the extract of Renshen (Radix Ginseng) on reproductive tissues in immaturemice. J Tradit Chin Med. 2015 Aug;35(4):460-7.

25. Oh KJ, Chae MJ, Lee HS, Hong HD, Park K. Effects of Korean red ginseng on sexual arousal in menopausal women: placebo-controlled, double-blind crossover clinical study. J Sex Med. 2010 Apr;7(4 Pt 1):1469-77. doi: 10.1111/j.1743-6109.2009.01700.x. Epub 2010 Feb 5.

26. Omar TY, Abdalhafid YKA. effect of panax ginseng root powder on fertility and antioxidant enzymes in male rabbits. EURO J PHARMA MED RES. 2016,3(5), 76-83.

27. Kim Y, Choi EH, Doo M, Kim JY, Kim CJ, Kim CT, Kim IH. Anti-stress effects of ginseng via down-regulation of tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH) gene expression in immobilization-stressed rats and PC12 cells. Nutr Res Pract. 2010 Aug;4(4):270-5. doi: 10.4162/nrp.2010.4.4.270. Epub 2010 Aug 31.

28. Sun Q, Liu A, Ma Y, Wang A, Guo X, Teng W, Jiang Y. Effects of forced swimming stress on thyroid function, pituitary thyroid-stimulating hormone and hypothalamus thyrotropin releasing hormone expression in adrenalectomy Wistar rats. Exp Ther Med. 2016 Nov;12(5):3167-3174. Epub 2016 Oct 11.

29. Xin W, Yu Y, Ma Y, Gao Y, Xu Y, Chen L, Wan Q. Thyroid-sti-

mulating hormone stimulation downregulates autophagy and promotes apoptosis in chondrocytes. Endocr J. 2017 Jul 28;64(7):749-757. doi: 10.1507/endocrj.EJ16-0534. Epub 2017 Jul 13.

30. 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. J Ginseng Res. 2017 Apr;41(2):215-221. doi: 10.1016/j.jgr.2016.08.005. Epub 2016 Aug 23.

31. Augustin JM, Kuzina V, Andersen SB, Bak S. Molecular activities, biosynthesis and evolution of triterpenoid saponins. Phytochemistry. 2011 Apr;72(6):435-57. doi: 10.1016/j.phytochem.2011.01.015. Epub 2011 Feb 16.

32. Yan L, Hu Q, Mak MS, Lou J, Xu SL, Bi CW, Zhu Y, Wang H, Dong TT, Tsim KW. A Chinese herbal decoction, reformulated from Kai-Xin-San, relieves the depression-likesymptoms in stressed rats and induces neurogenesis in cultured neurons. Sci Rep. 2016 Jul 22;6:30014. doi: 10.1038/srep30014.