ISSN 2618-1584 Vol. 3, Issue 5: 24-29, DOI: 10.25236/FMSR.2021.030504

Protective Effect of Panax ginseng on osteoporosis in Ovariectomized Female Guinea-pigs

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Abstract: Objective: *To investigate the protective effect of Panax ginseng on osteoporosis in guinea-pigs induced by ovariectomy.* ***Methods:*** *Six-month-old 36 SPF non-pregnant female guinea-pigs, were divided into four groups randomly: sham-operated group (Sham), blank model group (Model), recommended dose of Panax ginseng group (RD) and Maximum dose of Panax ginseng group (MD), 9 guinea-pigs in each group. Guinea-pigs models of osteoporosis were prepared by ovariectomized (OVX), except Sham group. 2 weeks later, OVX guinea-pigs were administered gavage with Panax ginseng powder twice daily for 100 days, RD group with 2.5mg/100g·BW, MD group with 5mg/100 g·BW. Sham and Model group were given with the same dose of saline, all the gavage dose were 3mL. The serum of guinea-pigs were obtained to measure the electrolytes ions, serum Ca^{2+}, Mg^{2+}, P^{3+}, to measure serum bone metabolism indexes BALP, OC, TRACP-5b, BMD and BMC.* ***Results:*** *Compared with Model group, the level of serum Ca^{2+}, P^{3+} decreased in the Panax ginseng groups, but the level of Mg^{2+}increased ($P<0.05$), the statistics difference in MD group was significant ($P<0.01$). The influence of serum BALP, OC, and TRACP-5b: compared with Model group, the Panax ginseng groups had statistical differences ($P<0.05$), with the dose correlated positively. Compared with Model group, the index values of BMD and BMC of the Panax ginseng groups were significantly Maximum ($P<0.05$), there was a dose-dependent difference with drug intervention.* ***Conclusion:*** *Panax ginseng can inhibit bone loss, decrease bone turnover rate, and improve the osteoporosis with dose-dependent effect.*

Keywords: *Panax ginseng, osteoporosis, guinea-pigs, ovariectomy.*

1. Introduction

Osteoporosis (Osteoporosis, OP) is a metabolic bone disease characterized by bone mass decreases and bone microstructure degenerates, leading decreased bone strength and increased fracture risk [1-2]. Estrogen can regulate the balance of bone metabolism and maintain bone mass and strength [3]. The function of ovarian declined with the estrogen reduced sharply, bone metabolism impaired [4-5], postmenopausal women were induced osteoporosis. Among clinical osteoporosis, postmenopausal osteoporosis (PMOP) has the Maximum incidence accompanying by Maximum disability rate. Statistics show that the incidence of osteoporosis in postmenopausal women is as Maximum as 60% [6]. Symptom-targeted therapy is a major treatment for PMOP, which has great toxic side effect and unsatisfactory adverse reactions.

Panax ginseng (Panax ginseng C.A.Mey.) is a perennial herb of araliaceae with wide range of biological activities, nourishing vitality, nourishing blood and nourishing yin, etc. *Shen Nong Ben Cao Jing* records: "Ginseng, sweet, slightly cold. The main complement the organs, calm the spirit, calm the soul, and stop palpitations, in addition to evil, bright eyes, happy puzzle. Long service, light longevity." Panax ginseng can effectively improve human immunity and regulate human metabolic function [7-9], and effectively correct bone metabolism disorders caused by estrogen deficiency, inhibit bone resorption, and reduce bone loss. Panax ginseng is well known to exert a wide range of pharmacological effects, but few studied the effects of Panax ginseng for on osteoporosis. This experiment studied the healing effect of Panax ginseng on osteoporosis in OVX female guinea-pigs and provided experimental evidence for the clinical application of Panax ginseng in the treatment of female PMOP.

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2. Experimental materials

2.1. Experimental drugs

Panax ginseng slice with 5 year-old sun-dried (Tonghua Baiquan Ginseng Industry Group Co., Ltd.). Grind the Panax ginseng slices into powder, and put them in a brown medicine sealed tank in a refrigerator at 4℃ standby.

2.2. Experimental animals

36 non-pregnant female guinea-pigs of 6 months old, SPF (Specific pathogen Free, SPF) grade, average body weight (655.97±5.57) g, purchased from National Institutes for Food and Drug Control , animal quality certificate number SCXK (京) 2017-0005.Guinea-pigs are kept in a large plastic box in the animal room. The animal room is kept at a constant temperature 23℃, relative humidity is 35%~40%, 12 h/d normal light, clean and quiet environment (SD<50 dB), adaptive breeding 1 week. Throughout the experiment, high-quality and high-protein (18.5%) formula feed, VC supplements and purified water were provided. Guinea-pigs were free to drink and eat.

2.3. Experimental reagents

Serum osteocalcin (OC) ELISA test kit (Beijing Union Bioengineering Research Institute Co., Ltd.); Serum tartrate resistant acid phosphatase 5b (TRACP-5b) ELISA test kit (Shanghai Jianglai Biotechnology Co., Ltd.) Technology Co., Ltd.); other chemical reagents are commercially available.

2.4. Main instruments

JY3006 electronic balance (Shanghai Precision Scientific Instrument Company); A702 ultra-low temperature freezer (Themo, USA); TGL-16B Maximum-speed centrifuge (Hunan Xingke Scientific Instrument Company); AU480 automatic biochemical analyzer (Beckman Coulter, USA); QDR- 4000 Dual Energy X-ray Bone Densitometer Analyzer (Hologic, USA), etc.

3. Experimental methods

3.1. Grouping and model preparation

36 guinea-pigs were divided into 4 groups according to the Random Number Table: sham operation group (Sham), blank model group (Model), Panax ginseng recommended dose group (recommended dose for human, RD) and Panax ginseng Maximum-dose group (Maximum tolerable dose for human dose, MD), 9 guinea-pigs in each group, Model group, RD group and MD group were excised the bilateral ovaries to prepare osteoporosis models.

Guinea-pigs were anesthetized by intraperitoneal injection of 10% chloral hydrate (0.35 mL/100 g). After successful anesthesia, the guinea-pigs were placed in a prone position, limbs fixed, skin preparations were performed locally, and jaw-spine angle approach was taken after iodophor disinfection. Of Model, RD and MD groups, the fallopian were ligated and the bilateral ovaries were excised. Sham group only excised the same quantities of fat near bilateral ovaries. After the operation, the penicillin sodium 200,000 U/ml (0.3ml each) was used prophylactically and injected into the lateral muscle to prevent infection for 3 days; returned to the original environment for restorative feeding and observed the guinea-pigs daily.

3.2. Drug intervention

Drug intervention was started two weeks following ovariectomy. According to the human recommended dose of Panax ginseng powder 3g/(60kg·d) and the human Maximum tolerable dose 6g/(60kg·d). The experimental dosage is based on the human standard weight of 60 kg and the 6 month-old female guinea-pigs average weight is 656g. RD group were given the recommended dose with 2.5mg/100g·BW, MD group was given the Maximum tolerable does with 5mg/100 g·BW, twice a day. Panax ginseng powder was mixed thoroughly with physiological saline, warmed by water bath, and then gavage by mouth; Sham and Model group were given equivalent physiological saline warmed by gavage,

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all the gavage dose were 3mL, fed at a fixed time (8:00 am, 6:00 pm) and completed within 5 minutes. 15 minutes later, gave free formula feed and water for 100 days.

3.3. Measure serum bone-related ion content and bone tissue metabolism biochemical indicators

Following 100 days drug intervention, the last gavage finished, the guinea-pigs were anesthetized with 10% chloral hydrate (0.35 mL/100 g) and collected blood from the abdominal aorta. Coagulated at 23℃ for 20 minutes, the serum was centrifuged at 3000 r/min for 20 minutes, then placed into the refrigerator with - 80℃ for test ready.

Automatic Biochemical Analyzer was used to measure the serum bone specific alkaline phosphatase (BALP), the level of bone electrolytes ions, serum calcium Ca^{2+}, serum magnesium Mg^{2+} and serum phosphorus P^{3+}. Enzyme-linked immunosorbent assay with detection kit was used to measure serum OC and TRACP-5b.

3.4. Measure bone mineral density (BMD) and bone mineral content (BMC)

The bone mineral density (BMD) and bone mineral content (BMC) of the guinea-pigs lumbar vertebrae L2-3 and bilateral femurs were measured by bone mineral density absorption method through Dual Energy X-ray Bone Densitometer Analyzer (DXA), averaged the results.

After the last gavage, the guinea-pigs were fasted with water for 6 hours, anesthetized with 10% chloral hydrate (0.35 mL/100 g). Lumbar vertebrae L2-3 were scanned in a supine position with a scan width of 6 cm, adjust the length with lumbar of the guinea-pigs, the scanning speed is 31.1s/cm; the femur scan took the prone position, the scanning width is 5cm, adjust the length with the guinea-pigs' femur, the scanning speed is 31.1s/cm, use DXA's small animal measurement software to analyze and determine BMD and BMC.

3.5. Statistical processing

SPSS 22.0 software was used for statistical analysis of the data. Measurement data conforming to the normal distribution were expressed as mean ± standard deviation ($\bar{x} \pm s$), and comparisons between groups were performed by one-way analysis of variance or Krusal-Waillis H test. $P<0.05$ means the difference was statistics significance.

4. Results and Comprehensive Analysis

4.1. The effect of Panax ginseng on serum bone ion levels and bone metabolism biochemical indicators of guinea-pigs

4.1.1. Serum Ca^{2+}, Mg^{2+} and P^{3+} content measure results

Compared with Sham group, the serum Ca^{2+} and serum P^{3+} level of the OVX Guinea-pigs Model group increased, but the serum Mg^{2+} decreased, the difference was statistically significant ($P<0.05$); compared with Model group, the level of serum Ca^{2+} and P^{3+} in RD group of Panax ginseng decreased, and the level of serum Mg^{2+} increased ($P<0.05$); The changes in serum ion levels in MD group were more obvious, with statistical differences Significant ($P<0.01$, Table 1).

Table 1: Comparison of cavies serum bone ion concentration between groups ($\bar{x} \pm s$)

Group	n	Ca^{2+} (mmol/L)	Mg^{2+} (mmol/L)	P^{3+} (mmol/L)
Sham	9	2.13±0.06	0.91±0.08	1.16±0.09
Model	9	2.82±0.24$^{\#\#}$	0.65±0.16$^{\#}$	1.95±0.27$^{\#\#}$
RD	9	2.49±0.19*	0.80±0.05*	1.55±0.23*
HD	9	2.17±0.09**	0.87±0.03*	1.21±0.04**

$^{\#}P<0.05$, $^{\#\#}P<0.01$ VS(Sham group); *P<0.05, **P<0.01 VS(Modle group)

Serum Ca^{2+}, Mg^{2+} and P^{3+} are essential elements for bone growth. OVX female guinea-pigs have a

ISSN 2618-1584 Vol. 3, Issue 5: 24-29, DOI: 10.25236/FMSR.2021.030504

sharp decrease in estrogen led to abnormal metabolism of serum bone salts, resulting in Ca^{2+} and P^{3+} concentration increases, Mg^{2+} concentration decreases due to the antagonistic effect of Ca^{2+}, and the biochemical indicators are abnormal. After Panax ginseng intervention treatment, RD group has a certain relief, and the serum bone ion level tends to normal; after the intervention of MD group, the serum Ca^{2+}, Mg^{2+} and P^{3+} index levels all return to normal.

4.1.2. Serum biochemical indicators of bone metabolism measure results

Compared with Sham group, the serum level of BALP, OC content and TRACP-5b activity in the OVX guinea-pigs, Model group were significantly increased (P<0.05); the Panax ginseng treatment group compared with the Model group, RD group serum BALP, OC and TRACP-5b all decreased (P<0.05); MD group decreased significantly (P<0.01), among which the biochemical values of serum TRACP-5b decreased more significantly (P<0.001) ,Table 2).

Table 2: Comparison of serim BALP, OC &TRACP-5b indices between groups ($\bar{x} \pm s$)

Group	n	BALP (μg/L)	OC (μg/L)	TRACP-5b (U/L)
Sham	9	2.58±0.06	0.68±0.04	3.36±0.05
Model	9	3.75±0.31##	1.12±0.20##	6.41±0.35##
RD	9	3.39±0.21*	0.92±0.08*	5.08±0.37**
HD	9	2.63±0.08**	0.73±0.06**	3.54±0.16**

#P<0.05, ##P<0.01 VS(Sham group); *P<0.05, **P<0.01 VS(Modle group)

Serum BALP is secreted by osteoblasts, promotes bone matrix calcification, can reflect the activity of osteoblasts accurately; OC is synthesized by osteoblasts and maintains normal bone mineralization, which is a sensitive indicator of bone turnover and bone formation. OVX guinea-pigs' estrogen is deficient, and bone metabolism presents a high conversion rate, which causes a compensatory increase in serum BALP and OC level. TRACP-5b is released by osteoclasts, regulates the activity of osteoclasts, intervenes in the process of bone resorption, and is a sensitive indicator of bone resorption. In this study, Panax ginseng intervention treatment made the index values of BALP, OC and TRACP-5b in RD group all decreased, and the three index values in MD group decreased significantly, reached the normal range.

4.2. The effect of Panax ginseng on guinea-pigs' BMD and BMC

The BMD measurement showed that compared with Sham group, BMD of Model group decreased significantly, the difference was statistically significant (P<0.01); compared with Model group, the Panax ginseng treatment group had a increase with statistical differences Significant (P<0.01), BMD is positively correlated with drug dose, showed a significant dose-dependent effect.

The BMC measurement showed that compared with the Sham group, the BMC of Model group decreased significantly, and the difference was statistically significant (P<0.05); compared with the Model group, the Panax ginseng treatment group had a certain increase with statistical differences (P<0.05), BMC increased significantly in MD group (P<0.01,Table 3).

Table 3: Comparison of bone mineral density and content between groups ($\bar{x} \pm s$)

Group	n	BMD (g/cm^2)	BMC (g)
Sham	9	0.28±0.04	0.64±0.03
Model	9	0.16±0.04##	0.38±0.06##
RD	9	0.22±0.03*	0.55±0.08*
HD	9	0.28±0.02**	0.65±0.04**

#P<0.05, ##P<0.01 VS(Sham group); *P<0.05, **P<0.01 VS(Modle group)

BMC represents bone mineral content, BMD represents the mineral content of bone units. The magnitude of the two is closely related, and it is "gold standard" for judging the efficacy of osteoporosis and anti-osteoporosis drugs [10]. After ovariectomized in female guinea-pigs, BMD decreased significantly, which proved that the experimental model of osteoporosis in this study was successfully

ISSN 2618-1584 Vol. 3, Issue 5: 24-29, DOI: 10.25236/FMSR.2021.030504

prepared. After Panax ginseng treatment, the BMD and BMC index values of RD group have been significantly improved, and the BMD and BMC of MD group have reached or exceeded the index values of the Sham group.

5. Discussion

The level of estrogen in postmenopausal women decreases sharply, the incidence of PMOP is Maximumer, and the risk of fractures increases. Osteoporotic fractures are often accompanied by Maximum disability and fatalities [11], which will not only affect the quality of patients' life, but also affect the economic environment of their families and society. Therefore, the prevention and treatment of PMOP have important social significance.

Animal models of human diseases are an important means of modern medical research. Female rodent OVX osteoporosis modeling is an internationally recognized ideal model for simulating female PMOP research [12,13]. The model of female guinea-pigs used in this study is mainly based on the following points: (1) The pathological changes in OVX guinea-pigs, such as bone loss and bone fragility caused by a sharp decrease in estrogen are highly similar to PMOP caused by female postmenopausal estrogen deficiency[14]; (2)Guinea-pigs are Maximumly sensitive to bacteria and viruses, and have extremely high serum complement [15]; (3)Guinea-pigs are in large size, which is convenient for blood collection and bone index determination. In the results, Model group had higher serum bone salt ions and serum BLAP, OC and TRACP-5b biochemical indicators than Sham group, indicated that activity of osteoclast had exceeded greatly osteoblast, bone resorption was leading. Weak bone formation and severe bone loss are typical pathological signs of PMOP, the significant decline of BMD and BMC proves the completeness of OVX guinea-pigs experimental model.

Panax ginseng has always been a health treasure that nourishes and strengthens human body. Ginsenosides is the main components of Panax ginseng to exert its effects [16], which is particularly beneficial to the human nervous system, cardiovascular system, and immune system, and a large positive regulatory effect to human endocrine system. There is an estrogen-like effect with Panax ginseng be found by studies [17]. Its estrogenic activity can increase the expression of the estrogen-sensitive gene c-fos at the mRNA and protein level. Saponins of panax ginseng have an large tonic effect on estrogen insufficiency, and can promote the increase of serum estradiol (E2) level [18].

The lack of estrogen leads to PMOP, mainly due to the imbalance of bone metabolism. Panax ginseng exerts an estrogen-like effect, can increase the activity of osteoblasts with proliferation and differentiation, enhance the expression of specific osteoblast genes to protect osteoblasts and promote the formation of new bone tissue, and slow down the rate of osteoblast apoptosis. Inhibits the activity of osteoclasts with differentiation of autophagy, reduces bone tissue resorption and bone turnover rate to prevent bone loss. The application of Panax ginseng replaces and compensates for the lack of estrogen at a certain extent after menopause. Meanwhile, panax ginseng has the effect of promoting E2 and improving the level of estrogen in body. Through the estrogen receptor of bone cells, it limits bone turnover and promotes return bone dynamic of metabolism, balance and improve bone mass to prevent and treat PMOP.

In this study, Panax ginseng powder was used to obtain the maximum effect of the drug; OVX guinea-pigs were treated respectively with recommended dose and Maximum dose of Panax ginseng for 100 days. The measurement results showed that the bone index of panax ginseng treatment group tended to normal. The improvement of the dose showes a positive dose-dependent effect, MD group has a more significant effect. It can be seen Panax ginseng has an effective and obvious healing effect on OVX guinea-pigs osteoporosis.

In summary, Panax ginseng can improve the level of estrogen of human body, maintain the balance of bone metabolism, and has a good anti-osteoporosis effect through subrogation of estrogen and stimulation of E2.

In this study, we takes the recommended dose and the Maximum tolerated dose of human as the experimental dosage according to the common medicine. The effect of exceeding the maximum tolerated dose is not discussed. Further research will be done in the next experiment.

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