Original Research Article

Anti-osteoporosis effect of *Notopterygium incisum* Ting ex H. T. Chang extract in rats

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Abstract

Purpose: *To investigate the effect of Notopterygium incisum Ting ex H.T. Chang extract (NICE) on ovariectomy-induced osteoporosis in rats.*
Methods: *Female Sprague-Dawley rats were randomly assigned to a normal group (control) and five ovariectomy (OVX) subgroups: OVX with vehicle (OVX), OVX with positive control drug alendronate sodium tablets (1.8 mg/kg/week), and OVX with varying NICE doses (45, 90, or 180 mg/kg/day). After rats were subjected to ovariectomy for 4 weeks, alendronate or NICE were administered orally daily for 16 weeks. The bone mineral density (BMD) of the L4 vertebrae and right femurs of all rats was estimated. The serum hormones estradiol (E2), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels and serum alkaline phosphatase (ALP), osteocalcin (OC) and telopeptides of collagen type I (CTx) levels of rats were detrmined.*
Results: *The results show that NICE dose-dependently inhibited bone mineral density (BMD) reduction of L4 vertebrae and femurs (p < 0.05). Compared with OVX group, serum E2, FSH and LH levels was significantly increased in osteoporosis rats (p < 0.01), but serum ALP, CTx, and OC concentrations were significantly lower (p < 0.05). On the other hand, bone trabeculae in the three NICE groups and nilestriol group were all wider, while the space and connections increased.*
Conclusion: *These findings indicate that NICE mitigates OVX-induced osteoporosis in rats, and hence can potentially be developed for the management of osteoporosis.*

Keywords: *Notopterygium incisum, Osteoporosis, Ovariectomy, Bone tissue morphology*

INTRODUCTION

Postmenopausal osteoporosis is a type of systemic bone disease characterized by a reduction in bone density, degradation of bone microstructure and an alteration in serum markers of bone metabolism such as alkaline phosphatase (ALP), estradiol (E2) and interleukin-6 (IL-6) in postmenopausal females. These results in bone fragility and an increased risk of fracture. Approximately one third of postmenopausal females suffer from osteoporosis usually due to marked reduction in estrogen levels [1].

According to data released by World Health Organization (WHO), osteoporosis affects approximately million people throughout Europe,

USA, and Japan [2]. Incidence of osteoporosis increases dramatically with life expectancy. Accordingly, the risk of osteoporotic fractures and their associated costs rising rapidly due to aging populations [3]. In the elderly, hip fractures are closely associated with mortality [4]. Hormone deficiency is known to impair cancellous metaphyseal and reduce BMD in humans and animals. Therefore, estrogen deficiency in post-menopausal women has been regarded as a critical cause of this population susceptibility to osteoporosis [5]. Osteoporosis is twice as common in women as in men [6], and approximately one in three women over 50 years old experiences an osteoporotic fracture in her life time [7].

Clinically, hormone replacement therapy (HRT) has been a popular therapeutic strategy designed for postmenopausal osteoporosis [8,9]. However, long-term application of HRT has potential malignant effects on reproductive tissues [10-12]. Other medicines that stimulate bone formation (e.g., growth hormone, sodium uoride, and parathyroid hormone) or inhibit bone resorption (e.g., bisphosphonates and calcitonin) may prevent bone loss in established osteoporosis. However, these drugs are not effective for a large proportion of the population, especially in developing countries, and some drugs have side effects, such as gastrointestinal reactions, cancers, osteonecrosis of the jaw, and reduced skeletal strength [13,14]. Consequently, to substitute or reduce the medicines used currently, there are efforts to develop new drugs with improved therapeutic efficacy, fewer undesirable side effects, and lower cost.

Notopterygium incisum Ting ex H.T. *Chang* is a traditional Chinese medicate herb, which has been commonly used as an anti-aging agent, anti-osteoporosis and aphrodisiac in China [15]. This study investigated the therapeutic effects of NICE on ovariectomy-induced osteoporotic rats.

EXPERIMENTAL

Preparation of NICE

Herbal samples of *Notopterygium incisum* Ting ex H. T. Chang were collected from Nanning City, Guangxi Province in China in May 2017. Taxonomic identification of the plant was performed by Professor Ling Wang of College of Pharmacy, Bengbu medical college, in Bengbu of China. A voucher specimen (no. NICE 201705014) was deposited in the herbarium of College of Pharmacy, Bengbu Medical College, Bengbu, China for future reference.

Animals and treatments

Healthy six-month-old female Sprague-Dawley rats (weighing 220 ± 20 g) were provided by Anhui Province Experimental Animal center (certificate no. SYXK2006-0006). The animals had free access to feed and water, and were allowed to acclimatize for at least one week before use. The rat experiment was approved by Animal Care and Use Committee of the First Affiliated Hospital of Bengbu Medical College (approval ref no. 200805832) and carried out in compliance with the Directive 2010/63/EU on the handling of animals used for scientific purposes [16].

Sixty rats were randomly divided into six groups of ten rats each: normal control, OVX with vehicle (OVX), OVX with positive control drug Alendronate Sodium Tablets (ASD, 1.8 mg/kg/week), and OVX with different NICE doses 82, 164 or 328 mg/kg/day.

BMD measurement

The BMD of the L1-5 vertebrae and right femurs was estimated using dual-energy X-ray absorptiometry scanning (DEXA, GE Healthcare, USA). The measurements were expressed as mineral grams per cm^2 of surface area. Scans were performed by the same blinded technician.

Serum E2, FSH and LH levels in rats treated with NICE

After the rats were sacrificed by cervical dislocation, rat serum was obtained by excising the eyeball. Serum E2, FSH and LH levels of rats were determined by ELISA method (Nanjing, Biological Engineering Research Institute Co. Ltd, Nanjing, China).

Evaluation of serum bone markers in rats treated with NICE

After the rats were sacrificed by cervical dislocation, rat serum alkaline phosphatase (ALP), osteocalcin (OC) and telopeptides of collagen type I (CTx) levels were determined by ELISA method (Nanjing Jiancheng Biological Engineering Research Institute Co. Ltd Nanjing, China).

Statistical analysis

The data are expressed as mean ± SD. Statistical analysis was performed using one-way ANOVA combined with Bonferroni's multiple comparison test using SPSS 16.0. Differences

were considered statistically significant at $p < 0.05$.

RESULTS

BMD of L1-5 vertebrae and femurs

The BMD of the L1-5 vertebrae and femurs is presented in Table 1. These results demonstrate that OVX significantly decreased BMD in the L4 vertebrae and femurs compared to the control group ($p < 0.05$). Compared to the OVX group, NICE treatment significantly increased the BMD in OVX L4 vertebrae and femurs ($p < 0.05$) in a dose-dependent manner. The positive control drug Fosamax also significantly increased the BMD of the L4 vertebrae and femurs (both $p < 0.05$), which was similar to that observed in the H-NICE group ($p > 0.05$).

Effect of NICE on serum E2, FSH and LH levels

Compared to control group, serum E2, FSH and LH levels decreased significantly in OVX group

rats ($p < 0.01$). Compared to the OVX group, NICE treatment significantly increased serum E2, FSH and LH levels ($p < 0.05$) in a dose-dependent manner in rats (Table 2).

Effect of NICE on serum ALP, CTx and OC concentrations

Serum ALP, CTx, and OC concentrations in the OVX group were significantly higher when compared to the control group. After four weeks of treatment, the NICE 300 mg/kg-treated group showed significantly lower serum ALP, CTx and OC concentrations when compared to the OVX group ($p < 0.01$) (Table 3).

DISCUSSION

Postmenopausal osteoporosis is a systematic imbalance in which the speed of bone resorption is greater than the bone formation. This disease is caused by estrogen deficiency and results in microarchitectural changes, particularly bone remodeling.

Table 1: Effect of NICE on BMD of L4 vertebrae and femurs (n = 10)

Group	Dose (mg/kg)	BMD of vertebrum (g/cm^2)	BMD of femur (g/cm^2)
Control	-	0.89±0.04[*]	0.82±0.03[*]
OVX	-	0.18±0.03	0.15±0.04
ASD	1.8	0.38±0.04[*]	0.42±0.03[*]
L-NICE	45	0.30±0.02[*]	0.27±0.04[*]
M-NICE	90	0.41±0.03[*]	0.23±0.04[*]
H-NICE	180	0.76±0.03[*]	0.62±0.03[*]

[*]$P < 0.05$ and [**]$p < 0.01$ compared OVX group. L-NICE = low dose of NICE, M-NICE = middle dose of NICE, H-NICE = high dose of NICE, ASD: alendronate sodium tablets

Table 2: Effect of NICE on serum hormone levels (n = 10)

Group	Dose (mg/kg)	E2 (pmol/L)	FSH (IU/L)	LH (mIU/mL)
Control	-	9.32±0.11[**]	2.86±0.03[**]	3.81±0.09[*]
OVX	-	1.58±0.10	0.64±0.04	1.42±0.06
ASD	1.8	4.54±0.12[**]	1.92±0.05[*]	2.44±0.03[*]
L-NICE	45	2.63±0.18	0.86±0.04	1.96±0.11
M-NICE	90	3.91±0.19[*]	1.70±0.03[*]	2.52±0.08[*]
H-NICE	180	4.72±0.13[**]	1.91±0.04[**]	2.98±0.06[*]

[*]$P < 0.05$ and [**]$p < 0.01$ versus OVX group. L-NICE: low dose of NICE, M-NICE: middle dose of NICE, H-NICE: high dose of NICE, ASD: alendronate sodium tablets

Table 3: Effect of NICE on serum ALP, CTx, and OC levels (n = 10)

Group	Dose (mg/kg)	ALP (U/L)	CTX (ng/mL)	OC (ng/mL)
Control	-	135.3±3.0	21.8±1.5	34.5±1.6
OVX	-	272.5±5.1[**]	87.4±1.1[**]	86.1±2.2[**]
ASD	1.8	178.7±4.5[**]	52.4±2.4[**]	58.6±1.5[**]
L-NICE	45	223.2±5.1	76.9±2.0	79.1±1.6
M-NICE	90	168.7±4.0[**]	66.7±1.4[*]	74.8±1.5[*]
H-NICE	180	154.3±3.4[**]	55.2±1.6[**]	63.7±1.1[**]

[*]$P < 0.05$ and [**]$p < 0.01$ versus OVX group. L-NICE: low dose of NICE, M-NICE: middle dose of NICE, H-NICE: high dose of NICE, ASD: Alendronate Sodium Tablets

Certain molecules co-ordinate the actions of osteoblasts and osteoclasts during bone remodeling. Therefore, new anti-osteoporosis drugs is urgently needed. In recent years, Chinese medicinal herbal extracts have been extensively investigated for their pharmacological effects related to bone protection [17].

Decreased BMD is one of the major factors that jeopardize bone strength, resulting in increased susceptibility to fractures. Thus, BMD measurement can best predict fracture risk [18]. The results presented showed that OVX reduced BMD in the right femurs and L4 vertebrae which are rich in trabecular bone, while treatment with NICE dose-dependently prevented the decreases in BMD. Although BMD is among the strongest predictors of facture resistance, empirical observations and theoretical analyses show that the biomechanical properties of bone and trabecular microarchitecture influence trabecular bone strength as well [19].

Estradiol plays an important role in sclerotin of humans. When female serum estradiol level decreases significantly, osteoporosis will occur [20]. Compared to the OVX group, NICE treatment significantly increased serum E2, FSH and LH levels in a dose-dependent manner in rats. In the experiments, OVX resulted in significant decrease in femur BMD after four weeks. The BMD loss was accompanied by a significant increase in bone remodeling, as evidenced by the increased biochemical bone turnover markers such as serum ALP, CTx, and OC levels [21].

In the present study, oral administration of NICE at the dose 328 mg/kg significantly decreased BMD loss, which was accompanied by the decrease in serum ALP, CTx, and OC levels compared to a OVX control group. These results suggest that Bushen Jiangu Decoction could decreases bone loss by inhibiting bone turnover induced by OVX.

CONCLUSION

The findings of this study indicate that NICE prevents OVX-induced osteoporosis in rats, and therefore can potentially be used for the treatment of postmenopausal osteoporosis in elderly women.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Hai Ding designed all the experiment and revised the manuscript. Wenju Chang, Jian Yao and Fendou Liu performed the experiments, Qingliang Zeng and Zhenxing He wrote the manuscript.

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