Journal of Ethnopharmacology xx (xxxx) xxxx–xxxx

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Protective effects and mechanisms investigation of Kuntai capsule on the ovarian function of a novel model with accelerated aging ovaries

Jinjin Zhang[a,1], Li Fang[a,2], Liangyan Shi[b,3], Zhiwen Lai[c,4], Zhiyong Lu[a,5], Jiaqiang Xiong[a,6], Meng Wu[a,7], Aiyue Luo[a,8], Shixuan Wang[a,*,9]

[a] Department of Obstetrics and Gynecology, Tongji Hospital, Huazhong University of Science and Technology, Wuhan, Hubei, People's Republic of China
[b] Department of Obstetrics and Gynecology, Hubei Province, Maternity and Child Health Care Hospital, Wuhan, Hubei 430030, PR China
[c] Maternal and Child Health Hospital of Zigong, Sichuan 643000, China

ARTICLE INFO

Chemical compounds studied in this article:
Estradiol (PubChem CID:5757)
Progesterone (PubChem CID:5994)

Keywords:
Kuntai capsule
Accelerated aging ovary
Model
Ovarian function

ABSTRACT

Ethnopharmacological relevance: Kuntai capsule, a traditional Chinese medicine, has been widely used for the clinical treatment of menopausal syndrome. However, its mechanisms remain poorly understood. Considering that aging ovaries are the primary cause of menopause, this study was designed to investigate the effects and mechanisms of Kuntai capsule on ovarian function in a novel mice model with accelerated aging ovaries.

Materials and methods: Seventy-five female C57BL/6 mice were chosen for this study. Fifteen of the mice were separated into the normal control group (NC). The remaining sixty were used to establish the novel accelerated aging ovary model by superovulation and oxidative stress and then by randomly dividing the mice into four equal groups. One group was considered the model group (Mod). The other three groups were treated with low (0.4 g/kg), middle (0.8 g/kg) and high (1.6 g/kg) doses of Kuntai capsule intragastrically every day for 4 weeks. During the treatment, the body weight and fur condition of all mice were recorded. All the mice were forced to swim to record their exhaustive swimming time (EST), which measures their strength. Mice were then sacrificed for sampling. Ovarian reserve was evaluated using follicle counts and AMH expression. Ovarian function was evaluated using estrous cycle, sex hormone level and litter experiments. Ovarian follicles were categorized and counted to estimate ovarian reserve, and ovarian histologic sections were stained for terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) to detect apoptotic cells. The ultrastructure of ovarian cells was observed using transmission electron microscopy. Western blotting was used to measure expression of Bax, Bcl2, AMH and SOD2 protein.

Results: Compared with the NC GROUP, the Mod group clearly displayed worse fur condition and ovarian function. These situations showed some improvement after Kuntai capsule treatment. Specifically, the fur condition and the EST of the Kuntai capsule groups were superior to the fur condition and EST of the Mod group. In cases of damaged ovarian function, Kuntai capsule can regulate the estrous cycles, increase hormone secretion and fertility and significantly decrease atretic follicles. The transmission electron microscopy results revealed that Kuntai capsule rescued the ovarian ultrastructure of mice. TUNEL staining confirmed that the apoptotic cells were reduced after Kuntai capsule treatment. Western blotting revealed that Kuntai capsule can increase AMH, SOD2, and Bcl2 protein expression and decrease Bax expression.

Abbreviations: NC, normal control group; Mod, the model group; Low, the low-dose group; Mid, the middle-dose group; High, the high-dose group; EST, exhaustive swimming time; AMH, anti-mullerian hormone; HRT, hormone replacement therapy; E_2, 17β-estradiol; P, progesterone; ELISA, enzyme-linked immune sorbent assay; PMSG, pregnant mare serum gonadotropin; hCG, human chorionic gonadotropin; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling; POF, premature ovarian failure; Fig., Figure; DAPI, 4',6-diamidino-2-phenylindole; TdT, terminal deoxynucleotidyl transferase

* Corresponding author.
E-mail addresses: 824754662@qq.com (J. Zhang), 1228043956@qq.com (L. Fang), 416234083@qq.com (L. Shi), 124075212@aa.com (Z. Lai), 553654372@qq.com (Z. Lu), 292539238@qq.com (J. Xiong), 651377251@qq.com (M. Wu), aiyueluo@163.com (A. Luo), sxwang@tjh.tjmu.edu.cn (S. Wang).

[1] Involved in the acquisition, analysis and interpretation of data, wrote the first draft of the manuscript.
[2] Involved in the acquisition of data.
[3] Involved in the acquisition of data.
[4] Involved in the acquisition of data.
[5] Involved in the acquisition of data.
[6] Involved in the analysis and interpretation of data.
[7] Involved in the analysis and interpretation of data.
[8] Involved in the analysis and interpretation of data.
[9] Designed the study, revised the drafts and reviewed the final manuscript. Jinjin Zhang wrote the first draft of the manuscript.

http://dx.doi.org/10.1016/j.jep.2016.11.014
Received 8 May 2016; Received in revised form 18 September 2016; Accepted 5 November 2016
Available online xxxx
0378-8741/

Conclusions: Kuntai capsule may improve damaged ovarian function, which may be related to its antioxidant and anti-apoptosis effects.

1. Introduction

With the increase in women's life-expectancy, a growing number of women will spend nearly one-third of their lives in a post-menopausal state (Daayana and Holland, 2009). During menopause, the drastic decline of estrogen production can lead to a decrease in life quality, including various perimenopausal and post-menopausal symptoms and pathologies (Cicero et al., 2013). Thus, many women use hormone replacement therapy (HRT) to relieve menopausal symptoms and to improve their quality of life. However, increasingly more studies have challenged the safety of HRT, citing the risk of venous thromboembolism or coronary event, breast cancer, stroke and gallbladder disease (Marjoribanks et al., 2012).

Kuntai capsule (Heyan®), a traditional Chinese medicine produced by Guiyang Xintian Pharmaceutical Stock Limited Company (Guiyang, China), was derived from the Chinese masterwork *Shang Han Lun*, written by Zhang Zhongjing. Kuntai capsule comprises six different types of herbs. These herbs create the effects of Ziyin/Nourishing Yin, reducing pathogenic fire, stabilizing the mind, eliminating worries and regulating Yin and Yang, which could effectively relieve menopausal symptoms. In recent decades, Kuntai capsule has been used in clinics for alleviating menopausal symptoms and has had no apparent estrogenicity for decades (Chen et al., 2005). Recent clinical investigations have also suggested that Kuntai capsule can effectively ameliorate low estrogen levels caused by the add-back therapy of gonadotropin-releasing hormone agonist for endometriosis (Chen et al., 2015; Liu et al., 2014). Despite its wide use, the manner in which Kuntai capsule works on menopausal syndrome remains unclear.

Menopause results from ovarian aging, and the aging process of ovaries is characterized by a gradual decrease in both the quantity and quality of the oocytes. According to some studies, the many primordial follicles formed during the development of an embryo inevitably decline as women age and drop below 1000 approaching menopause (Alviggi et al., 2009; Broekmans et al., 2007; Djahanbakhch et al., 2007 ; Block, 1953; Faddy et al., 1992; Hansen et al., 2008; Markström et al., 2002; McGee and Hsueh, 2000; te Velde et al., 1998; te Velde and Pearson, 2002). In addition to the decrease in follicles, oocyte quality also diminishes with age. The results of daily metabolic processes, the metabolic products are supposed to be a primary cause of ovarian aging (Tarin, 1995, 1996; Sohal, 2002). Previous studies have indicated that oxidative stress has damaging effects on mitochondrial function and the development of oocytes, thus affecting the pregnancy rates of women who undergo IVF (Liu and Keefe, 2000; Liu et al., 2000; Tamura et al., 2008). As estrogen levels decrease with age, the absence of estrogen's beneficial effects against oxidative stress ultimately damages the ovary, which may explain the significantly increased rate of congenital birth defects in women older than 38 (Lass et al., 1997). The accumulation of oxidative damage and a synchronous decrease in antioxidant defense ultimately lead to ovarian aging.

Based on these findings, our laboratory established a new ovarian aging acceleration model to induce a decline in both the quantity and quality of the oocytes. This model used superovulation to reduce the primordial follicle pool and used ozone inhalation to increase oxidative damage throughout the entire body, including in the ovaries. Before that model, dozens of mouse models caused by single gene mutations or chemicals were available to investigate ovarian aging at different stages of development and regulation (Danilovich and Ram Sairam, 2006; Jiang et al., 2013; Terraciano et al., 2014; Tsuyoshi et al., 2015; Wang et al., 2013). No animal model can exactly duplicate all of the physiologic and pathologic aspects of ovarian aging, and the problem of time renders it difficult to explore the mechanism using a naturally aging model. This study was designed to evaluate the effect of Kuntai capsule on damaged ovarian function and identify its possible mechanisms on menopausal syndrome.

2. Materials and methods

2.1. Ethics statement

All of the experimental procedures used in this study were approved by the ethics committee of Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology in the People's Republic of China.

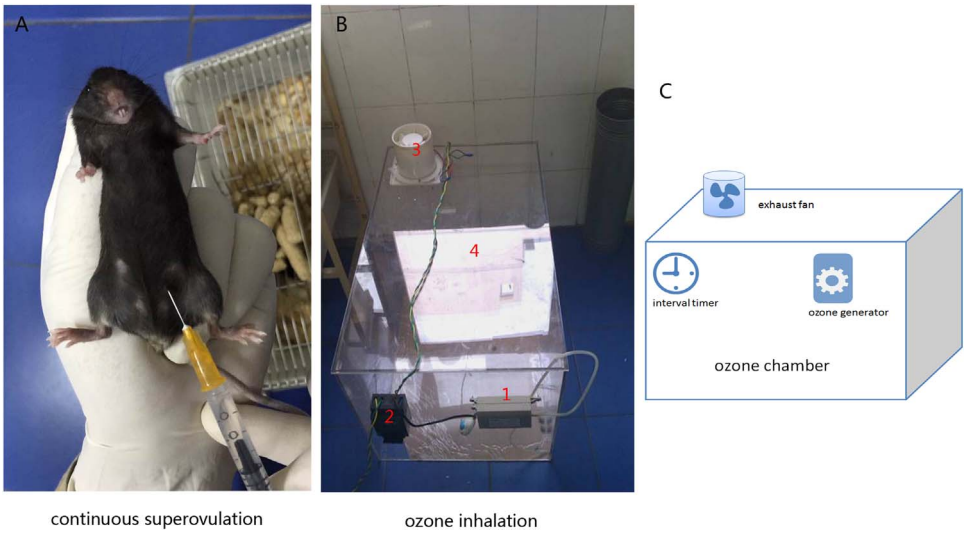

Fig. 1. The methods of establishing the model. A) Intraperitoneal injection. B) Ozone inhalation. C) The sketch map of the ozone chamber. Note: 1. ozone generator, 2. interval timer, 3. exhaust fan, 4. ozone chamber.