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Korean red ginseng protects against doxorubicin-induced testicular damage: An experimental study in rats

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ABSTRACT

The protective effects of Korean red ginseng (KRG; *Panax ginseng* Meyer) aqueous extract against doxorubicin (DOX)-induced testicular damage in rats was investigated. DOX (1 $mg \cdot mL^{-1} \cdot kg^{-1} \cdot week^{-1}$) was administrated intraperitoneally for 8 weeks and KRG extract was administrated orally for 9 weeks (100 or 200 mg/kg/day). The serum sex hormone levels, sperm kinematic parameters, histopathological parameters and protein expression levels were determined. DOX distinctly damaged the histology of the rat testes and decreased the sex hormonal levels, spermatogenesis, seminiferous tubular diameter, and testes weight. Further, the protein expression levels of peroxiredoxin, glutathione-S-transferase, nectin-2, inhibin-α, and cAMP response element binding protein were suppressed in DOX-treated rats. In contrast, KRG extract ameliorated the changes induced by DOX. Data indicated that KRG ameliorated DOX-induced testicular damage in rats by modulating the antioxidant system and hormonal imbalance. In conclusion, KRG can be used as a functional food and/or adjuvant for the prevention of reproductive damage caused by DOX.

1. Introduction

Ginseng, one of the most frequently used herbs in both Eastern and Western countries, has been used in traditional medicine for over 2000 years to restore and enhance normal well-being. In addition, it is often referred to as an adaptogenic agent that enhances physical performance including sexuality, promotes vitality, controls hypertension, and increases resistance to stress and ageing (Lee & Kim, 2014; Nocerino, Amato, & Izzo,

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2000). The most commonly used and researched ginseng species is the *Panax ginseng* called the Asian or Korean red ginseng (KRG). KRG dominates the functional food market in Korea and other Asian countries as the top-selling functional food ingredient for the past several years. Further, a number of randomized clinical trials have demonstrated its effect on physical and psychomotor performance, cognitive function, immunomodulation, diabetes mellitus, anxiety, and herpes simplex type-II infections (Mahady, Gyllenhall, Fong, & Farnsworth, 2000; Vogler, Pittler, & Ernst, 1999; Zhao et al., 2014).

Much attention has been focused on KRG for its potential effects on sexual function (Hong, Ji, Hong, Nam, & Ahn, 2002; Jang, Lee, Shin, Lee, & Ernst, 2008; Kim et al., 2006). Previous reports from our group and others indicate that KRG relieves senile testicular dysfunction (Hwang et al., 2010; Won et al., 2014), erectile dysfunction (Choi, Seong, & Rha, 1995; de Andrade et al., 2007), decreased sexual arousal, menopausal symptoms in women (Oh, Chae, Lee, Hong, & Park, 2010), and acts as a potent aphrodisiac (Shamloul, 2010). It was also reported that human consumption of KRG extract improved the sperm count, motility, and testosterone level in patients with fertility problems (Salvati et al., 1996). However, to the best of our knowledge, there is no molecular study reporting the restorative potential of KRG against testicular impairment induced by natural and artificial damage.

The decline in the birth rate is one of the gravest concerns in industrialized countries. Although several psychological, social, and economic issues exist, reproductive abnormalities in both partners are a major problem (Bak et al., 2012; Merritt et al., 2013; Wu, Elliott, Katz, & Smith, 2013). Particularly, male related factors are estimated to account for approximately 20% of the infertility of couples (Anderson, Farr, Jamieson, Warner, & Macaluso, 2009). The most significant of these factors are low sperm concentration, poor sperm motility, and abnormal sperm morphology. Male sexual dysfunctions including testicular failure, subfertility, erectile dysfunction, hypogonadism, and aspermia may also contribute to reproductive disorders, which affect men and are more common with increasing age (Parmet, Lynm, & Glass, 2004). Testicular impairment can be caused by several physical, chemical, and psychological problems, resulting in the production of abnormal sperm or sex hormones. Decreased sperm quality including the sperm count, motility and other parameters such as DNA fragmentation, abnormal sperm zona binding and acrosome integrity reaction are also some of the major causes of male subfertility and infertility (Aitken, Irvine, & Wu, 1991; Barroso, Morshedi, & Oehninger, 2000; Liu & Gordon, 1992).

Many acquired factors such as effects of chemotherapeutic agents, genetic defects, unhealthy lifestyle, disease, and injury can play a malignant role in testicular function. Several classes of prescription drugs contribute to sexual dysfunction in men (Jang et al., 2008). Anticancer agents used in chemotherapy are well known to produce toxic side effects in multiple organ systems including the testes. Therapeutically effective doses of many anticancer drugs may produce irreversible changes in male reproductive tissues, causing testicular damage. It is well documented that doxorubicin (DOX), an anthracycline that is a cornerstone of many chemotherapeutic protocols, is used for treating a wide spectrum of malignancies. However, evidence suggests that one of the dose-limiting side effects of DOX is its toxic response in normal cells including testicular damage (Abdella & Ahmed, 2009; Nambu & Kumamoto, 1995).

The use of functional foods, nutraceuticals and herbal products including extracts, decoctions, fractions, and semi-purified compounds constitute a great promise to improve health, and these are used in the treatment of dysfunctional libido, sexual asthenia, erection, and sperm disorders (Nantia, Moundipa, Monsees, & Carreau, 2009; Nocerino et al., 2000). Therefore, the use of natural herbs or functional foods might be an appropriate alternative approach in reducing the adverse effects and aid in achieving natural fertilization. In the light of such reports, we employed a DOX-induced testicular impairment model in rats to investigate the beneficial role of KRG in testicular function related to spermatogenesis.

2. Materials and methods

2.1. *Preparation of KRG-aqueous extracts*

The Korea Ginseng Corporation, Daejeon, South Korea kindly supplied the six-year-old *P. ginseng* root extract. The extraction procedure was followed as described previously (Hwang, Kim, Wee, Choi, & Kim, 2004). Briefly, the roots were washed with tap water, steamed at 98 °C for 30 min and dried at 70 °C for 72 h to produce KRG. Then, the active constituent was obtained from the KRG by extraction with 10 volumes of water at 90 °C for 48 h followed by filtration. The filtrate was dried under reduced pressure to obtain the final water extract (WE) as a dark-brown syrup (KRG-WE).

2.2. *Experimental animals*

Twenty-eight 8-week-old male Sprague-Dawley rats (240 ± 20 g) were purchased from the Daehan Biolink Co. (Eumseong-gun, Korea) and allowed to acclimatize to the animal facility for 2 weeks before the experiment. The rats were divided into four groups and treated as indicated: vehicle (0.9% normal saline solution), control (CON), DOX-treated alone (DXR), KRG-WE 100 mg/kg plus DOX-treated (EX1), and KRG-WE 200 mg/kg plus DOX-treated (EX2) groups. Each group consisted of seven rats. The DOX was injected intraperitoneally (*i.p.*) once weekly for 8 weeks at a dose of 1 $mg{\cdot}mL^{-1}{\cdot}kg^{-1}$ (Prahalathan, Selvakumar, & Varalakshmi, 2005). The selected dose of KRG-WE (100 and 200 mg/kg b.w.) was based on our previous experiment (Hwang et al., 2004). The selected doses of KRG were mixed evenly with sterilized standard powder type diet and administered orally after pelletization. The dose of KRG was adjusted once every week by taking into account the body weight increment and the daily dietary intake. The KRG-WE was evenly mixed in sterilized standard powdered diet and then pelleted. The animals were provided with the standard diet pellets AIN-76A and water *ad libitum*, and kept at a constant temperature (23 ± 2 °C) and relative humidity (55 ± 10%) in a 12/12 h light/dark cycle. All the animal experiments were approved and conducted in accordance with the guidelines of the Institution of Experimental Animal Ethics, Konkuk University, South Korea.