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Antioxidant effects of cultured wild ginseng root extracts on the male reproductive function of boars and guinea pigs

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ABSTRACT

The main objective of this study was to investigate the effects of cultured wild ginseng root extracts (cWGRE) on the sperm of boars and the reproductive system of guinea pigs. Firstly, semen collected from boars ($n = 10$) were incubated in 38 °C for 1 h with xanthine and xanthine oxidase to generate ROS. The cWGRE was added to the sperm culture system to test its antioxidant effect on the boar sperm. The amount of Reactive Oxygen Species (ROS) was measured by a chemiluminescence assay using luminol. The results indicated that the addition of cWGRE to boar sperm culture inhibited xanthine and xanthine oxidase-induced ROS concentrations. Treatment with cWGRE also had a positive effect on maintaining sperm motility. Effects of cWGRE administration on vitamin C-deficient guinea pigs were further investigated. Hartley guinea pigs ($n = 25$) at 8 weeks of age were randomly divided into five groups. With the exception of the positive control group, each group was fed vitamin C-deficient feed for 21 days (d). Respective groups were also orally administered cWGRE, ginseng extract, or mixed ginsenosides for 21 days. In comparison to the control group, oral administration of cWGRE reduced ($P < 0.05$) amount of lipid peroxidation and increased ($P < 0.05$) both glutathione peroxidase concentrations and the trolox equivalent antioxidant capacity. In addition, administration of cWGRE induced increases ($P < 0.05$) in body weight, testosterone concentrations, and spermatid populations. The results of the present study support our hypothesis that cWGRE has positive effects on male reproductive functions via suppression of ROS production.

1. Introduction

ROS such as superoxide radicals (O_2^-), hydroxyl radicals (•OH), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) are generated as normal metabolites in the biological system of males. However, excessive amounts of Reactive Oxygen Species (ROS) can have adverse effects on cells by reacting with phospholipids and oxidizing sulfhydryl groups in enzymes and proteins (Bathgate, 2011). In addition, DNA in cells can be damaged by ROS (Bathgate, 2011). In comparison to other types of cells, sperm cells are more sensitive to peroxidative damage because these cells are composed of greater concentration of polyunsat-

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urated fatty acids and have a lesser antioxidant capacity than other cells (Vernet et al., 2004). The ROS induce lipid peroxidation (LPO) of the plasma membrane of sperm, and this is followed by a reduction in fluidity of membranes, increasing non-specific permeability to ions, and inactivating membrane-bound receptors and enzymes (Tremellen, 2008). The LPO of the plasma membrane subsequently reduce sperm motility (Aitken and Clarkson, 1987; Iwasaki and Gagnon, 1992). Thus, depleted antioxidant capacity and excessive concentrations of ROS are typically evident in abnormal sperm (Aitken and Clarkson, 1987). The ROS also cause single- and double-stranded DNA breaks, which results in abnormal embryonic development (Aitken and De Iuliis, 2007; Badouard et al., 2008). The steroidogenic capacity of Leydig cells is also reduced by ROS (Garcia et al., 2012). Therefore, medicines or dietary supplements that provide antioxidants may be useful to the male reproductive system, by preventing loss of fertility due to ROS generation.

Suspension cultures of ginseng roots in bioreactors are the primary alternative method for large-scale production of wild ginseng. Various ginsenosides have been discovered as major compounds of both ginseng and cWGRE (Li et al., 2008). However, active compounds of wild ginseng differ from those of cultured ginseng because of differences in environmental factors (Li and Mazza, 2000). Therefore, the effects of cWGRE on the male reproductive system should be evaluated with respect to the antioxidant constituents of wild ginseng. In the present study, it was hypothesized that cWGRE has antioxidative effects on the male reproductive system. The results of the present study may be useful in the development of medicines or nutritional supplements for both enhancing fertility and preventing the loss of fertility in males. Results of the present study may also contribute to a greater understanding of ROS and the scavenger system for ROS of the male reproductive system.

2. Materials and methods

2.1. Preparation of cultured wild ginseng root extracts (cWGRE)

Primary roots of wild ginseng in a state of hibernation when collected, (plants approximately 70–120 years of age, and 50 cm long) and selected for culture. The root material was cleaned with 70% ethanol and incubated in a modified wood plant medium containing sucrose 30 g/L, agar 7 g/L, and 1–5 ppm 3-indole acetic acid (IAA) for 40–60 days in a dark room (pH 5.8; temperature, 25 °C). After initial incubation, the material was transferred to a 500 mL bioreactor (INNOBIO, DAST 500, South Korea) and grown for 20 days (d) for mass production. The end product (ginseng root material) produced from the bioreactor was freeze-dried using an evaporator (EYELA, N-1200A, Japan) and used in the preparation of cWGRE.

2.2. Experiment 1

2.2.1. Boar sperm culture

Semen was obtained from boars (between 1 and 2 years, Duroc, $n = 10$) during a routine semen collection procedure for artificial insemination at a pig-breeding farm in Korea. The boars used in this study were certified to use for breeding, and the sperm met the criteria for normospermia (data not shown). A single ejaculates from each boar was used for the experiment (in total of 10 semen samples). The semen was collected in a 50 mL conical tube and stored in a portable refrigerator (17 °C) and was subsequently delivered to the laboratory within approximately 1 h. The semen was centrifuged to remove seminal plasma and the pellet was extended in Beltsville Thawing Solution (BTS, Minitube^{TM}, Tiefenbach, Germany) at the concentration of 2.5×10^6 spermatozoa/ml. The samples were stored at 32 ± 1 °C, which maintained the pH and osmolality at 7.6–7.9 and 315–318 osmol/kg, respectively. Sperm were treated with both Xanthine (X, 1000 μM, X4002, Sigma, USA) and Xanthine oxidase (XO, 25 mM, T65404, Sigma), followed by incubation at 38 °C for 1 h to generate ROS. To determine the individual or synergistic effects of the antioxidants, sperms were treated with superoxide dismutase (SOD, S5398, Sigma), catalase (CAT, C1345, Sigma), cWGRE, and/or ginsenosides (Rb_1, Rc, Re, Rg_1 Rd, Ambo Institute, South Korea) as presented in Table 1.

2.2.2. Measurement of ROS in cultured sperm

The concentration of ROS was measured by a chemiluminescence assay using luminol (5-amino-2,3-diydro-1,4-phthalazinedione; A8511, Sigma) according to the methods outlined in a previous report (Duru et al., 2001) with a slight modification as follows: sperm suspensions (400 μL) were at a concentration of 2.5×10^6/mL for each experimental group was placed in the 15 mL conical tube (Falcon) and allowed to equilibrate in the luminometer (Atolumat LB953, Berthold Technology, Bad-Wildbad, Germany) for 5 min. The background chemiluminescence was monitored, then 4 mL of 25 mM dimethyl sulfoxide (276855, Sigma) was added and the chemiluminescence signal was detected. Results were expressed as the difference between the integrated counts per minute (cpm) before and 5 min after the addition of luminal dimethyl sulfoxide.

2.2.3. Semen evaluation

Sperm motility was evaluated with the use of the Hamilton-Thorne computer-aided semen analyzer (CEROS II), software version 12.3CEROS (HTR Ceros 12.3, Hamilton-Thorne Research, USA), with different sperm motility variables being assessed simultaneously.

2.3. Experiment 2

2.3.1. Animals and diets

Hartley guinea pigs ($n = 25$; 8 weeks of age) purchased from Japan SLC, Inc. (Seoul, South Korea) were selected for the present study. The guinea pigs were individually housed in stainless steel cages in a room maintained at a temperature of 24 ± 2 °C and relative humidity of 50 ± 5% under a 12 h alternating light and dark cycle. Animals were fed a commercial diet (Cargill Agri Purina, Inc., South Korea) and provided with sterile water *ad libitum*. After 7 days of acclimation, the animals were randomly divided into five groups ($n = 5$ in each group) and the individual groups were fed and treated for 21 days as follows: (1) vitamin C-