Effects of Gamisoyosan on In Vitro Fertilization and Ovulation of Stressed Mice by Electric Shock

Ji Yeun Kim, Dong Hoon Kwak, Eun Jin Ju, Sung Min Kim, Dae Hoon Lee, Kyung Su Keum¹, Seo Ul Lee², Kyu Yong Jung³, Byoung Bu Seo³, and Young Kug Choo

Department of Biological Science, College of Natural Sciences, Wonkwang University, Iksan, Jeonbuk 570-749, Korea, ¹Department of Oriental Medical Informatics, College of Oriental Medicine, Wonkwang University, Iksan, Jeonbuk 570-749, Korea, ²Department of Pharmacology, Wonkwang University School of Medicine, Iksan, Jeonbuk 570-749, Korea, and ³Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, California 92037, USA

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Exposure to stress is known to precipitate or exacerbate many reproductive dysfunctions such as dysmenorrhea and infertility. Abnormalities of the reproductive system, as shown by reduced ovulation, fertilization and early embryonic development, are frequently seen in dysmenorrhea and infertility. It has been generally accepted that Gamisoyosan (GSS) is a useful prescription for treating insomnia, dysmenorrhea and infertility induced by a stress. Also GSS has been used traditionally to improve systemic circulation and biological energy production. Based on these, this study investigates whether GSS improved ovarian dysfunction caused by stress in mice. Mice were subjected to stress by electric shock on the foot for 30 min daily for a week and treated with GSS at 500 g body weight per day for one week. Thereafter, changes in body weight, adrenal weight, ovulation rate, in vitro and in vivo fertilization, embryonic development and estradiol concentrations were measured. GSS markedly increased the body weight of mice with stress, but not normal mice. The administration of GSS caused a reduction in adrenal weight in stressed mice. GSS also had significant positive effects on ovulation rate, estradiol production, in vivo and in vitro fertilization rates and embryonic development. These results indicate that GSS can improve the reproductive dysfunctions caused by stress, and these may production biological energy.

Key words: Gamisoyosan, Stressed mice, In vivo and in vitro fertilization, Embryonic development

INTRODUCTION

Stress is a fundamental adaptive response that enables the organism to cope with daily threatening environmental stimuli. If prolonged and uncontrolled, the stress response might become inadequate and ultimately result in various health problems (Selye, 1979). In humans, stress is thought to have adverse physiological and behavioral effects on both males and females (Selye, 1979) and has been reported to cause amenorrhea or alterations of the menstrual cycle in women (Genazzani et al., 1991; Rabin et al., 1988). In animals, stressors have been reported to affect several aspects of reproductive function (Axelson, 1987; Briski and Sylvester, 1988; Gonzalez et al., 1994; Norman et al., 1994; Rodriguez Echandia et al., 1988). During the exposure to a stressor, the whole system of stress regulation, that is, the hypothalamus-pituitary-adrenal cortex system (HPA axis) and the sympathetic nervous system-adrenal medulla system, is activated (Arana et al., 1985; Burns et al., 2003; Carroll et al., 1981; Holsboer, 1983; Kalin et al., 1982; Shores et al., 2001). The degree of stress response depends on genetic factors, personality characteristics, previous experience, support from the social environment and the way of coping with stress. Stress has important consequences for the reproductive system, which is dependent on adequate nutrition and body weight (Butler, 2000; McGrady and Chakraborty, 1983; Warren and Shantha, 2000). Epidemiological evidence clearly shows that stress contributes to menstrual disorders,
reduced numbers of follicles, infertility, poor pregnancy outcome and impaired fetal well-being (Dobson and Smith, 2000; Mulder et al., 2002; Sanders and Bruce, 1999). As a result, stress is a major risk factor for a number of clinical disorders of the female reproductive system.

Traditional herbal medicines have been employed for thousands of years and have contributed greatly to the prevention and treatment of various diseases. They are still valuable for human health and have received much attention as potential sources of new therapeutic agents due to their varied biological activity and low toxicity. Gamisoyosan (GSS) consists of eleven herbs with the addition of *Paeonia moutan* SIMS and *Gardenia jasminoides* ELLIS in the Soyosan. Soyosan consists of nine herbs; *Atractylodes japonica* KOIZOMI, *Angelica gigas* NAKAI, *Fritillaria ussuriensis* MAXIM, *Paeonia albiflora* BUNGE, *Prunus persica* BATSCH, *Platycodon grandiflorum* CANDOLLE, *Scutellaria baicalensis* GEORGI, *Aurantil imnaturi* PERICARPIUM and *Glycyrrhiza uralenisis* FISCHER. GSS has been widely used in the treatment for dysmenorrhea, insomnia and anxiety in Hanbang, Korean traditional medicine. In addition, one study has shown that GSS is effective in reducing stress (Choi and Lee, 1996).

The present study was designed to clarify the biological efficacy of GSS on the abnormalities of the female reproductive system using mice with stress induced by electric shock. In order to this, the present study investigates whether GSS will affect the ovulation rate, both the *in vivo* and *in vitro* fertilization rates and embryonic development in mice subjected to stress.

**MATERIALS AND METHODS**

**Preparation of gamisoyosan**

Each component of GSS is listed in Table I. All materials were obtained from the College of Oriental Medicine, Wonkwang University. This prescription was prepared according to the method by Yu et al. (1994). The medicinal plants were added to 2000 mL of distilled water and boiled for 2 h and then concentrated to 1720 mL. This was centrifuged at 3500 rpm for 20 minutes, and the supernatant was filtrated (510 mL) through a 0.45 μm filter (Millipore, France). The extract was stored at 4°C until use. The yield was about 15% of the dry weight of the herbal constituents.

**Animals**

Female B6C3F1 mice aged 8 weeks were supplied by the Samtako Bio Korea and allowed free access to a commercial diet and tap water. Animals were housed under controlled conditions of 14 h light and 10 h darkness, at a temperature of 23 ± 2°C and relative humidity of 55-66%. The mice were fed with laboratory pellet chow (Samtako, Korea; protein 24%, lipid 3.5% and carbohydrate 60.5%) for 8 weeks. After the introduction of stress by electric shocks, the animals were orally treated with GSS at 500 mg/kg body weight per day. Control animals were received an equal volume of physiological saline instead of GSS. After treatment of GSS for one week, the mice were injected with pregnant mares serum gonadotropin (PMSG; Sigma, St Louis, USA) and human chorionic gonadotropin (hCG; Sigma) to induce multi-ovulation, and the ratio of ovulated oocytes to normal oocytes was counted. Both the *in vivo* and *in vitro* fertilization rates and embryonic development were examined.

**Induce of stress by electric shocks**

Stress was induced using a wooden box (30×30×40 cm high) with a steel-rod base floor (29 parallel rods, 0.3 cm in diameter set 1 cm apart) (Homayoun et al., 2002). Mice were exposed to intermittent electric shock (75 mV) on the foot for 30 minutes daily for a week (8-second pulses delivered every 10-seconds).

**Induction of superovulation**

Superovulation was induced using the methods described by Summer et al. (2000) with slight modification. Mice aged 9 weeks were intraperitoneally treated with 5 IU of PMSG. After 48 h, 5 IU of hCG were intraperitoneally administered. The mice were sacrificed by a cervical dislocation at 15 h after hCG administration.

**Media**

Table II shows the composition of the basic media, TYH and MWM, used for fertilization of oocytes. MWM was used as the culture medium for early development of mouse embryos. All fertilization and culture media (each 200 μL) were covered with paraffin oil (Fisher, USA) and equilibrated in an atmosphere of 5% CO₂ in air at 37°C overnight.