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

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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 / body weight per day for one week. Thereafter, changes 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,

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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* KOIDZOMI, *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.

Table I. Composition of Gamisoyosan

Medicinal Plants	Weight(g)
Atractylodes japonica KOIDZOMI	6
Paeonia moutan SIMS	6
Angelica gigas NAKAI	4
Fritillaria ussuriensis MAXIM	4
Paeonia albiflora BUNGE	4
Prunus persica BATSCH	4
Gardenia jasminoides ELLIS	3
Platycodon grandiflorum CANDOLLE	3
Scutellaria baicalensis GEORGI	3
Aurantil imnaturi PERICARPIUM	2
Glycyrrhiza uralenisis FISCHER	1
Total amount	40

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% CO_2 in air at 37°C overnight.

Table 1. Composition of TYH and MWM media

Ingredient	TYH (g/mL)	MWM (g/mL)
NaCl	0.6976	0.6400
KCl	0.0356	0.0356
$CaCl_2$	0.0190	-
KH_2PO_4	0.0162	0.0162
$MgSO_4 \cdot 7H_2O$	0.0293	0.0294
$NaHCO_3$	0.2106	0.1900
Calcium lactate, 5-hydrate	-	0.0460
Glucose	0.1000	0.1000
Na-pyruvate	0.0110	0.0025
Penicillin G	0.0075	0.0075
Streptomycin	0.0050	0.0050
0.1% Phenol red	200 μL	100 μL
β-Mercaptoethanol (20 mM)	-	50 μL
EDTA·2Na (100 mM)	-	50 μL
BSA	0.3200	0.2400

Preparation of sperm suspension

According to standard procedures (Oh *et al.*, 1998; Toyoda and Chang, 1974), a dense mass of spermatozoa was isolated from the epididymis of 9-week-old B6C3F1 mice, and placed in 200 μL of TYH, which had been covered with paraffin oil (Fisher, USA) and then incubated at 37°C in a 5% CO_2 in air atmosphere for 1 h. Sperm concentrations were determined with a haemocytometer.

Oocyte collection

Female mice were superovulated by intraperitoneal injection of 5 IU PMSG, followed by injection of 5 IU of hCG 48 h later. Oocytes were collected from oviducts between 13.5- and 14-h after post-hCG administration. The oviducts were isolated and placed in a dish containing paraffin oil. The cumulus-oocyte complexes were dissected from swollen ampulla and transferred to the TYH medium under paraffin oil, followed by preincubation at 37°C in a 5% CO_2 in air atmosphere.

In vitro Fertilization

In vitro fertilization was carried out in drops of MWM under paraffin oil. A preincubated, capacitated sperm suspension was gently added to the freshly ovulated oocytes to give a final motile sperm concentration of 1×10^6/mL. The combined sperm-oocyte suspension was incubated for 5 h. The oocytes were then washed with the medium several time and finally incubated in 40 μL drops of medium under paraffin oil. Fertilization was confirmed by recording the number of Two-cell embryos 24 h after completion of *in vitro* fertilization (Summers *et al.*, 2000; Kim *et al.*, 2004).

Body and adrenal weights

Changes in body and adrenal weights were measured at 7 days after experiments started. After all animals used were sacrificed by cervical dislocation, weights of bilateral adrenal glands were measured.

Hormone measurements

Serum 17β-estradiol was measured by a commercially available radioimmunoassay (Diagnostic Products, Los Angeles, CA). Estradiol was first extracted from serum under diethyl ether. Samples were run using a Beckman

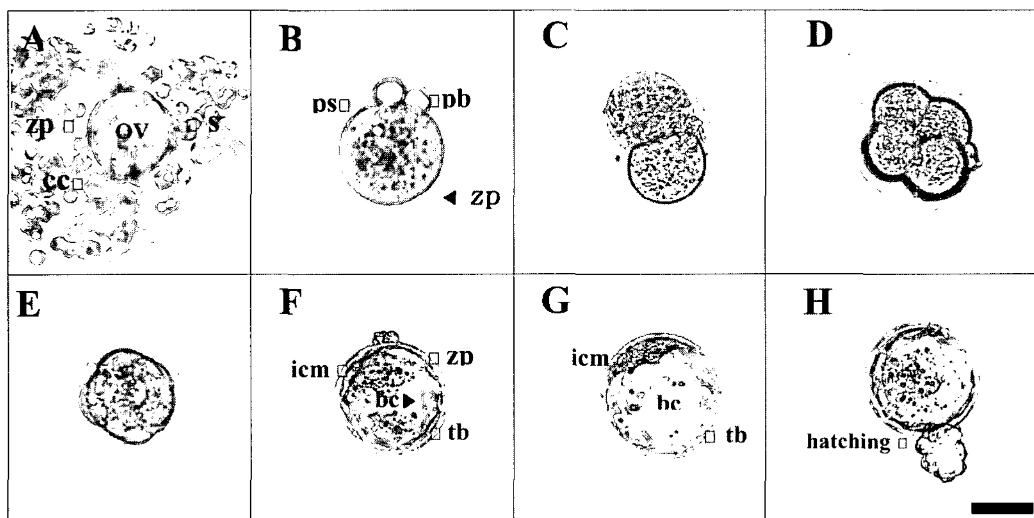

Fig. 1. Early embryonic development of fertilized oocyte in GSS-treated mice (A) fertilizing oocyte, (B) one-cell embryo, (C) two-cell embryo, (D) four-cell embryo, (E) morular embryo, (F) early blastocyst embryo, (G) late blastocyst embryo, and (H) hatching embryo. ov = ovum, zp = zona pellucida, cc = cumulus cells, s = sperm, ps = perivitelline space, pb = polar body, bc = blastocoel, tb = trophoblast, icm = inner cell mass (scale bar: 50).

Gamma 5500 counter (Beckman Coulter, Hialeah, FL) and results were analysed with Immunofit EIA/RIA analysis software (Version 2.00) using a logit-log curve fit. The interassay coefficient of variation (CV) and intraassay CV were 4.2-8.1% and 4.0-7.0%, respectively.

Statistical analysis

The values obtained in this study are expressed as mean ± SE. All data were initially analyzed using one-way ANOVA and the unpaired *t*-test and considered to be significant at $p<0.05$.

RESULTS

Changes in body weight

After treating the mice with or without GSS for a week, temporal changes in body weight were observed (Table III). Body weights of stressed mice were significantly reduced compared with normal control mice ($p<0.0001$). GSS did not significantly change the body weights of normal mice, but the body weights of GSS-treated stress mice were significantly increased compared with the control mice. There were no behavioral changes in stressed mice treated with GSS throughout the experimental period.

Changes in adrenal weight

Table IV shows the effects of GSS on adrenal weight in normal and stress-exposed mice. Electric shock caused a significant increase in the weight of adrenal glands (12.12 ± 0.02 mg/g, $p<0.05$). In contrast to these, GSS attenuated the adrenal weight increased by (from 12.12 ± 0.02 mg/g to 11.95 ± 0.06 mg/g, $p<0.05$ and from 0.591 ± 0.01 mg/g to 0.543 ± 0.02 mg/g, $p<0.001$).

Effects of gamisoyosan on ovulation

As shown in Table V, the number of ovulated oocytes in normal mice was not significantly increased by treatment with GSS. In contrast, stress caused a significant decrease in the number of ovulated oocytes, and this decrease was completely recovered by treatment of GSS (38.08 ± 0.48, $p<0.001$). GSS did not dramatically change the number of normal oocytes among ovulated oocytes in normal mice. However, in mice exposed to stress the number of normal oocytes among ovulated oocytes in GSS-treated mice (33.83 ± 0.51, $p<0.001$) was significantly higher than that in control mice.

Table III. Effects of GSS on the changes of the body weight of stressed mice by electric shock.

Group		GSS Treatment		BW Change (g)
		Before (g)	After (g)[a]	
Normal	Control[b]	18.16 ± 0.13	20.56 ± 0.30	+2.40
	GSS[c]	18.33 ± 0.16	19.13 ± 0.28	+0.80
SM	Control	19.60 ± 0.20	19.34 ± 0.20	-0.26*
	GSS	19.85 ± 0.30	19.99 ± 0.29	+0.14**

[a]At a week after the beginning of the experiment, body weight was measured. Results are expressed as mean ± SE.
[b]Animals were orally treated with saline.
[c]Animals were orally treated with GSS at 500 mg/kg body weight per day for a week.
*$p<0.0001$, significantly different from the normal control group.
**$p<0.0001$, significantly different from the SM control group.

Table IV. Effects of GSS on the adrenal weight of stressed mice

Group		Final body (g)[a]	Adrenal glands (mg)[b]	Ratio (adrenal glands/body)
Normal	Control[c]	22.11 ± 0.01	11.65 ± 0.05	0.526 ± 0.02
	GSS[d]	21.50 ± 0.01	11.26 ± 0.06	0.523 ± 0.03
SM	Control	20.50 ± 0.01	12.12 ± 0.02*	0.591 ± 0.01**
	GSS	21.97 ± 0.04	11.95 ± 0.06#	0.543 ± 0.02***

[a]At a week after the beginning of the experiment, body weight was measured.
[b]At a week after the beginning of experiment, adrenal glands weight was measured. Results are expressed as mean ± SE.
[c]Animals were orally treated with saline.
[d]Animals were orally treated with GSS at 500 mg/kg body weight per day for a week.
*$p<0.05$, **$p<0.001$; significantly different from the normal control group.
#$p<0.05$, ***$p<0.001$; significantly different from the SM control group.
SM = stress mice; GSS = gamisoyosan

Table V. Effects of GSS on ovulation of stressed mice.

Group		No. Examined (%)[a]	No. of Oocytes per Mouse Ovulated[b]	No. of Normal Oocytes per Mouse Ovulated[b]
Normal	Control[c]	15/17 (88.2)	35.21 ± 0.33	31.92 ± 0.37
	GSS[d]	16/17 (94.1)	36.28 ± 0.50	32.35 ± 0.49
SM	Control	16/17 (94.1)	32.50 ± 0.32*	23.50 ± 0.29*
	GSS	15/17 (88.2)	38.08 ± 0.48**	33.83 ± 0.51**

[a]At 8 weeks after the beginning of the experiment, ovulation was measured.
[b]Data are expressed as mean±SE.
[c]Animals were orally treated with saline.
[d]Animals were orally treated with GSS at 500 mg/kg body weight per day for a week.
*$p<0.001$; significantly different from the normal control group.
**$p<0.001$; significantly different from the SM control group.

In vivo fertilization and early embryonic development

After treating mice with or without GSS for a weeks, *in vivo* fertilization and the rate of embryonic development to the blastocyst stage were examined (Table VI). In mice given stress, *in vivo* fertilization rates in GSS-treated mice were significantly higher compared with those of control

mice, being 93.6%, $p<0.01$ for GSS-treated mice and 84.3%, $p<0.001$ for control mice. Also, embryonic development to the blastocyst stage was significantly higher in GSS-treated mice (81.0%, $p<0.01$) compared with control mice (68.7%, $p<0.01$).

In vitro fertilization and early embryonic development

After treating mice with or without GSS for a weeks, *in vitro* fertilization and the rate of embryonic development to the blastocyst stage were examined (Table VII). In mice exposed to stress, *in vitro* fertilization rates in GSS-treated mice were significantly higher compared with those of control mice, being 86.1%, $p<0.001$ for GSS-treated mice and 76.3%, $p<0.001$ for control mice. In mice exposed to stress, embryonic development to the blastocyst stage was significantly higher in GSS-treated mice (70.7%, $p<0.001$) compared with control mice (57.2%, $p<0.001$).

Effects of Gamisoyosan on estradiol production

As shown in Table VIII, the estradiol concentration in normal mice was significantly increased by treatment with GSS (Table VIII). In contrast, stress caused a significant decrease in the estradiol concentration, and this decrease was completely recovered by treatment of GSS (19.2 ± 1.5, $p<0.001$).

Table VIII. Effects of GSS on serum estradiol in stressed mice

Group		No. of Mice	Conc. of Estradiol (pg/mL)
Normal	Control[a]	10	19.4 ± 1.8
	GSS[b]	11	21.5 ± 1.5*
SM	Control	11	17.8 ± 1.6*
	GSS	10	19.2 ± 1.5**

Estradiol was measured in female mice at 16 hours after hCG injection.
Experiments were replicated 3 times.
[a] Animals were orally treated with saline.
[b] Animals were orally treated with GSS at 500mg/kg body weight per day for a week.
*$p<0.001$; significantly different from the normal control group.
**$p<0.001$; significantly different from the SM control group.

Table VI. Effects of GSS on *in vivo* fertilization and early embryonic development of stressed mice

Group		No. of Oocytes	Fertilized Embryos (%)[a]	%[b] of Embryos Developing to			
				Two-cell (24)[c]	Four-cell (48)[c]	Morula (72)[c]	Blastocyst (96)[c]
Normal	Control[d]	32	89.8 ± 1.5	84.5 ± 2.1	81.0 ± 1.5	78.3 ± 2.1	77.0 ± 1.9
	GSS[e]	33	89.7 ± 0.8	84.7 ± 1.2	82.1 ± 1.4	79.4 ± 2.3	77.9 ± 2.0
SM	Control	29	84.3 ± 1.5**	79.3 ± 1.7*	75.3 ± 2.6*	72.2 ± 2.1*	68.7 ± 2.1**
	GSS	30	93.6 ± 0.7***	89.0 ± 0.5***	86.0 ± 0.7***	83.4 ± 0.8***	81.0 ± 0.9***

[a] Oocytes were examined 5 hours after insemination and only fertilized embryos were cultured. Experiments were replicated 7 times.
[b] Data are expressed as mean ± SE.
[c] *Number in parentheses indicate the time of examination (hours after insemination).*
[d] Animals were orally treated with saline.
[e] Animals were orally treated with GSS at 500mg/kg body weight per day for a week.
*p 0.01, **$p<0.001$; significantly different from the normal control group.
***$p<0.01$; significantly different from the SM control group.

Table VII. Effects of GSS on *in vitro* fertilization and early embryonic development of stressed mice

Group		No. of Oocytes	Fertilized Embryos (%)[a]	%[b] of Embryos Developing to			
				Two-cell (24)[c]	Four-cell (48)[c]	Morula (72)[c]	Blastocyst (96)[c]
Normal	Control[d]	35	88.7 ± 0.7	82.2 ± 1.0	80.0 ± 1.3	77.1 ± 1.1	75.4 ± 1.3
	GSS[e]	36	88.3 ± 1.4	83.3 ± 1.5	80.1 ± 1.8	77.9 ± 1.8	76.6 ± 2.0
SM	Control	32	76.3 ± 1.2*	69.1 ± 1.3*	64.0 ± 1.2*	59.5 ± 1.4*	57.2 ± 1.5*
	GSS	32	86.1 ± 0.6**	78.9 ± 1.7**	75.3 ± 1.8**	73.5 ± 1.6**	70.7 ± 1.7**

[a]Oocytes were examined 5 hours after insemination and only zygotes were cultured. Experiments were replicated 7 times.
[b]Data are expressed as mean ± SE.
[c]Number in parentheses indicate the time of examination (hours after insemination).
[d]Animals were orally treated with saline.
[e]Animals were orally treated with GSS at 500mg/kg body weight per day for a week.
*$p<0.001$; significantly different from the normal control group.
**$p<0.001$; significantly different from the SM control group.

DISCUSSION

Stress is thought to be a major contributor to disease and dysfunction (Selye, 1979). Because stress can alter the release and/or function of neurotransmitters and hormones involved in the regulation of reproductive physiology and behavior, it is not surprising that stress has also been reported to affect reproductive function in females (Genazzani *et al.*, 1991). Stress may lead to a variety of disorders such as ovarian dysfunction. The present study investigated the effects of GSS, which is used for treatment of infertility caused by stress, on fertilization and early stage of embryonic development in stressed mice. GSS reduced the weight of adrenal glands and increased the body weight of mice subjected to stress as well as significantly increased the fertilization rates and the early stages of embryonic development. Therefore, although the active ingredients in GSS for the acute stress-induced abnormalities of the reproductive system have not yet been identified, these results suggest that ovarian dysfunction caused by stress can be rectified by oral administration of GSS.

Reproductive function has been known to be impaired by various kinds of physical and emotional stress, but the mechanism by which stress impairs the reproductive axis has not been clearly understood (Kam *et al.*, 2000). Thus, dissolution of stress may reverse the abnormal alterations of the reproductive system. The effects of stress on body weight are determined both by the severity of the stress and by an individuals perception of the stress. In animals, mild stressors, such as tail pinch, may increase food intake (Levine and Morley, 1981), whereas more extreme stressors, such as immobilization (Kitayama *et al.*, 1989), and restraint (Harris *et al.*, 1998), can induce hypophagia and weight loss. In humans, sufficient weight loss will induce anovulation (Pirke *et al.*, 1989; Warren *et al.*, 1999). This experiment observed that the reduction of body weight caused by a stress was significantly improved by GSS (Table III). These results strongly suggest that GSS contain pharmacological property to dissolve the stress caused by electric shock in mice. Stress causes a shift in biological function, a shift that comes at a biological cost to the individual (Moberg, 1985). During stress, changes in metabolism help support the biological defenses an individual uses to maintain homeostasis (Chrousos and Gold, 1995; Elsasser *et al.*, 1997; Moberg, 1996; Stratakis and Chrousos, 1995). These metabolic alterations during stress often results in the mobilization of energy away from energy-sensitive functions, such as growth in adolescents and maintenance of body weight in adults (Chrousos and Gold, 1992; Elsasser *et al.*, 1997; Stratakis and Chrousos, 1995). As a result, stress can cause negative energy balance. For example, stress characteristically results in depressed body weight and food intake in rodents (Dallman *et al.*, 1993; Harris *et al.*, 1998; Krahn *et al.*, 1986). Previous reports have demonstrated that the stress-induced depression in body weight is maintained over time, even after exposure to stress has ceased (Harris *et al.*, 1998; Smagin *et al.*, 1999) and also both metabolizable energy intake and total heat energy production were reduced by repeated exposure to stress. Thus, this stress-induced increase in basal energy expenditure probably caused the overall expenditure to be higher than one would expect from an equivalent reduction of metabolizable energy intake in nonstressed mice. Although this study cannot explain the biochemical mechanism related to the gain in body weight and reduction of adrenal weight, we consider that it may be mediated, at least in part, by production of biological energy and dissolution of stress in mice which were suffered with stress. Further studies are needed to clarify this.

We also observed on increase in adrenal weight in stressed mice compared with normal mice, and this increase was reversed by treatment of the stressed mice with GSS (Table IV). It is well known that stress activates the hypothalamo-pituitary-adrenal (HPA) axis. Glucocorticoids are secreted from the adrenals following secretion of hypothalamic corticotrophin-releasing hormone (CRH) and pituitary adrenocorticotropic hormone (ACTH). The secreted glucocorticoids serve as a feedback inhibitor of CRH and ACTH to increase resistance to stress. Thus, the magnitude of the feedback action of glucocorticoids is thought to be an important factor to dissolve the influence of stress in organism (Mizoguchi *et al.*, 2002). Namely, attenuation of the feedback system increase weight of adrenal glands. Therefore, it is suggested that the acute stress caused decrease in the body weight, increase in the adrenal weight, and disruption of the feedback system. These results suggest that GSS has a therapeutic effect on the abnormalities of the neuroendocrine system caused by acute stress. The findings that GSS did not affect the adrenal weight or the glucocorticoid feedback in the normal mice indicate that this drug does not positively decrease the adrenal weight or accelerate the feedback, suggesting that this drug is effective against abnormalities of the neuroendocrine system that occur specifically under stressful conditions.

In the present study, we also observed a decrease of ovulation rate in stressed mice compared with normal mice, and this reduction was completely reversed by treatment with GSS (Table V). In the past few years, stress has been found to affect reproduction (Dobson and Smith, 2000) and to reduce pregnancy rate during infertility treatment (Boivin and Takefman, 1995; Facchinetti *et al.*, 1997; Klonoff-Cohen *et al.*, 2001). Stress had deleterious

effects on sex hormone concentrations. Women, may have ovarian dysfunction severe enough to cause infertility and still have regular menstrual cycles (Wu, 1990). Rensis and Scaramuzzi (2003) studied there is a reduction in LH secretion leading to reduced estrogen secretion, impaired detection of estrus, reduced oocyte quality, implantation failure and infertility. In terms of these suggestions, this study examined the effects of GSS on fertilization rate and embryonic development. GSS conspicuously improved the rates of *in vitro* and *in vivo* fertilization and embryonic development in stressed mice (Tables VI and VII). Based on these findings, we consider that improved rate of *in vivo* and *in vitro* fertilization may be directly and/or indirectly related to the pharmacological activities of GSS. We also observed a decrease in estradiol concentration in stressed mice compared with normal mice, and this decrease was reversed by treatment with GSS (Table VIII). These results suggest that the decrease in estradiol production caused by stress may be improved by oral administration of H-tang.

In conclusion, the present study discusses the effects of GSS on ovarian function in mice, which were exposed to stress caused by electric shock. GSS has ameliorating effects on weight gain, reduction of adrenal glands weights, ovulation rates, *in vitro* and *in vivo* fertilization rates and embryonic development in mice with stress caused by electric stressed, but does not significantly affect these function in normal mice. These findings suggest that GSS may improve the functions of the ovary and oviduct by adjusting internal secretions and metabolism in stressed mice.

Abbreviations with eight tables abbreviations

SM : stress mice
GSS : gamisoyosan
BW : body weight
hCG : human chorionic gonadotropin

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