Effect of Daily Treatment with Thai Herb, *Kaempferia parviflora*, in Hershberger Assay Using Castrated Immature Rats

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Abstract. The aim of the present study was to investigate the testosterone-like effect of *Kaempferia parviflora* (KP). Castrated immature rats were randomized and divided into two groups (control and KP-treatment groups). The rats (n=7–8) were treated daily for 5 days by oral route with water in the control group and 1,000 mg/kg of KP in the treatment group. All rats were decapitated 24 h after their last dose and then blood samples were collected for assay of serum FSH, LH, testosterone, progesterone and corticosterone levels. The seminal vesicles plus coagulating glands, ventral prostate, levator ani muscle plus bulbocavernosus muscle, glans penis, kidneys and the adrenal glands were collected and weighed for organ wet weight. Body weight and weight of food intake were recorded throughout the study period. The results show that relative body weight gain in the KP-treatment group was significantly increased 24 and 48 h after the first dose (P<0.05) and then was indistinguishable from the control group. There were no significant differences in the relative reproductive and non-reproductive organ weights between the groups, although all organ weights, except for the glans penis, tended to increase in the KP-treatment group. The serum testosterone levels were significantly increased in the KP-treatment group. There were no significant differences in the serum FSH, LH, progesterone, or corticosterone levels between the groups, even though the serum progesterone level tended to increase and serum LH level tended to decrease in the KP-treatment group. The present study indicates that KP has no testosterone-like effect on reproduction in male rats.

Key words: Body weight, Castrated immature rat, Gonadotropins, *Kaempferia parviflora*, Testosterone

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flavone, 5-hydroxy-7,4'-dimethoxyflavone, 5-hydroxy-3,7,3'4'-tetramethoxyflavone, 3,5,7-trimethoxyflavone, 5,7,4'-trimethoxyflavone and 5,7,3'4'-tetramethoxyflavone [1]. In vitro study has shown that the 5,7,4-trimethoxyflavone, 5,7,3,4-tetramethoxyflavone and 3,5,7,4-tetramethoxyflavone isolated from KP are anti-bacterial agents [1]. KP extract exhibited anti-gastric ulcer activity by increasing the amount of gastric wall mucus content in adult male rats [2]. KP treatment at doses of 20, 200, 1,000 or 2,000 mg/kg/day for 6 months had no toxic effects in adult rats. Rats that received 2,000 mg/kg had a significant increase in liver weight and a significant decrease in eosinophil numbers, although these parameters were within the normal ranges (unpublished data). Until now, however, there are no scientific reports showing a testosterone-like effect for KP in humans or animals. Hence, we investigated the effect of KP on reproductive organs and related hormones using castrated immature rats as an animal model.

Materials and Methods

Animals
Immature male Wistar Imamichi rats were purchased from Imamichi Institute for Animal Reproduction (Ibaraki, Japan). They were housed in metal cages, maintained in a room with controlled illumination (14L:10D, lights-on at 0500 h) and temperature (22 to 24 C) and had free access to commercial pellets and tap water. All procedures were carried out in accordance with the guidelines established by the Tokyo University of Agriculture and Technology, for use of laboratory animals.

Experimental protocol
Immature male rats, 23–24 days old, were orchidectomized 7-days prior to the start of this study and randomly assigned to two groups (control and treatment). To reduce the effect of stress from the experimental procedure, all animals were trained by handling them for 5 days prior to the start of the experiment. In the experiment, the 30–31-day-old rats were treated daily for 5 consecutive days by oral route with water in the control group (n=7) and 1,000 mg of KP/kg body weight (BW) in the treatment group (n=8). A high dose of KP (1,000 mg/kg) was used in this study to examine the potential effect of KP on male reproduction and related hormones. The animals in both groups were decapitated 24 h after their last dose, and blood samples were collected and separated for serum by refrigerated centrifugation at 1,500 × g for 30 min. Serum samples were stored at −20 C until assay for the follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone, progesterone and corticosterone levels. At the same time, the seminal vesicles plus coagulating glands, ventral prostate, levator ani muscle plus bulbocavernosus muscle, glans penis, kidneys and adrenal glands were collected and weighed for organ wet weight. In addition, body weight and weight of food intake were recorded daily.

Preparation of the KP suspension
KP roots, harvested from Loei Province, Thailand, were sliced, desiccated, and then ground into 100-mesh powder. The dry powder stock used in the study was kept in a desiccator and protected from exposure to light. A fresh suspension of KP was prepared daily by suspending dry KP powder in water.

Hormonal assays
The serum FSH and LH levels were measured using NIDDK radioimmunoassay (RIA) Kits (Torrance, CA, USA) for rat FSH and LH. The iodinated preparations were rat FSH-I-5 and LH-I-5. The antisera used were anti-rat FSH-S-11 and anti-rat LH-S-11. The results were expressed as rat FSH RP-2 and rat LH RP-3. The intra- and interassay coefficients of variation were 4.8 and 11.4% for FSH and 5.4 and 6.9% for LH, respectively.

The serum progesterone, testosterone and corticosterone levels were measured using a double-antibody RIA system with 125I-labeled radioligands as described previously [3, 4]. The antisera against progesterone (GDN 377), testosterone (GDN 250) and corticosterone (UCB) were provided by Dr. G. D. Niswender (Colorado State University, Fort Collins, CO, USA). The intra- and interassay coefficients of variation were 5.4 and 6.9% for progesterone, 5.9 and 5.8% for testosterone and 9.8 and 17.5% for corticosterone, respectively.
Statistical analysis

The data were expressed as the mean ± SEM. Statistic differences between the two groups were analyzed using the independent t-test. Results were considered statistically significant when the P value was equal to 0.05.

Results

Changes in body weight and food intake

The body weights on the first day of the experiment were 82.14 ± 1.77 and 79.54 ± 0.73 g in the control and KP-treatment groups, respectively. Relative body weight gains were adjusted from the percent body weight gain of the first dosing (day 1) as shown in Fig. 1. At 24 and 48 h after the first dose, body weight gain in the KP-treatment group was significantly higher than in the control group (106.58 ± 0.29 vs. 103.39 ± 0.81% after 24 h and 114.42 ± 0.82 vs. 111.27 ± 1.19% after 48 h). Subsequently, the body weight gains of both groups were indistinguishable. The weight of food intake in the KP-treatment group was lower than in the control group but there was no significant difference throughout the period of study (data not shown).

Changes in organ wet weights

The organ wet weights of all the non-reproductive and reproductive organs were adjusted to the ratio of the individual’s organ weight per body weight. As shown in Table 1, there were no significant differences in the weights of the seminal vesicles plus coagulating glands, ventral prostate gland, levator ani muscle plus bulbocavernosus muscle or glans penis between the KP-treatment and control groups. In addition, the weights of the kidneys and adrenal glands were

<table>
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<tr>
<th>Table 1. Organ wet weights of the castrated immature rats in the KP-treatment group compared with the control group</th>
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<td>Control (n=7)</td>
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<tr>
<td>Seminal vesicles plus coagulating glands (µg/kg)</td>
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<td>Ventral prostate (µg/kg)</td>
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<td>Levator ani muscle and bulbocavernosus muscle (µg/kg)</td>
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<td>Glans penis (µg/kg)</td>
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<td>Kidneys (µg/kg)</td>
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<td>Adrenal glands (µg/kg)</td>
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The body weights of the control and KP-treatment groups on the day of autopsy were 112.67 ± 3.05 and 108.91 ± 1.97 µg/kg, respectively.

<table>
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<th>Table 2. Serum FSH, LH, testosterone, progesterone, and corticosterone levels of castrated immature rats treated with KP compared with the controls</th>
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<td>Control</td>
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</tr>
<tr>
<td>FSH (ng/ml)</td>
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<td>LH (ng/ml)</td>
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<td>Testosterone (pg/ml)</td>
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<td>Progesterone (ng/ml)</td>
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<td>Corticosterone (ng/ml)</td>
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The results are expressed as the mean ± SEM. The asterisk indicates a significant difference compared with the controls (P<0.05).
also indistinguishable between two groups. Nevertheless, all organs, except the glans penis, tended to have higher weights in the KP-treatment group compared with the control group.

Changes in the hormonal levels of serum

As shown in Table 2, there were no significant differences in the serum FSH and LH levels between the KP-treatment group after treatment with 1,000 mg of KP for 5 days and the control group. The serum testosterone level in the KP-treatment group was significantly high compared with the control group. At the same time, the serum progesterone and corticosterone levels were not significantly different between the two groups.

Discussion

The present study used the Hershberger assay to determine the androgenic activities of KP based on the response of androgen-dependent tissues and hormones in castrated immature rats. Testosterone has been shown to have an androgenic effect by increasing the accessory reproductive organ weights in studies using both castrated immature and adult male rats [5] and castrated adult male rats [6–8]. Testosterone propionate (TP) increases the reproductive organ weights, including those of the ventral prostate glands, seminal vesicles plus coagulation glands, glans penis, and bulbourethral glands, in F344, Sprague Dawley, and Wistar castrated immature rats [7, 9]. Dihydrotestosterone (DHT), a potent androgen that is converted from testosterone increase in the size of the increase in the weight of the seminal vesicles and prostate glands [6, 10]. In the present study, although the organ weights, including those of the seminal vesicles plus coagulation glands, ventral prostate, levator ani muscle and bulbocavernosus muscle, kidneys, and adrenal glands but not the glans penis, tended to increase in the KP-treatment group, there were no significant differences in the organ weights between the two groups. The inconclusiveness of this evidence may have been caused by the duration of KP treatment or inconsistencies in the experimental model.

After castration of immature rats, there is a significant reduction in serum testosterone level [7]. KP treatment significantly increased the serum testosterone levels compared those of the control group. Although there were no significant differences in the serum progesterone or corticosterone levels between the KP-treatment and control groups, both the progesterone and corticosterone levels tended to be higher in the KP-treatment group. We already know that progesterone converts to 17 αOH progesterone, androstenedione, and testosterone, respectively [11]. Moreover, progesterone is also a precursor for corticosterone synthesis [12]. The changes in serum testosterone, progesterone, and corticosterone seemed to be parallel, but there were no significant changes in the serum progesterone and corticosterone levels. Therefore, KP may have a direct effect on either testosterone synthesis in the adrenal gland or testosterone metabolism. In addition, there was no difference in the serum FSH and LH levels between the two groups. In previous reports, testosterone implantation induced an increase in the serum testosterone levels and suppressed serum LH levels by negative feedback action in both intact mature male and testosterone-treated, castrated, mature rats [5, 13]. On the other hand, flutamide, an androgen receptor antagonist, increased the serum LH levels in testosterone-treated castrated rats [5]. In the present study, although the serum LH levels of the KP-treatment group tended to be lower than those of the control group, no significant differences were observed between the two groups. It is possible that although KP provoked serum testosterone secretion, it did not disrupt the pituitary-gonadal axis by suppression of the FSH or LH secretions in the castrated immature rats.

The body weights of rats treated with KP increased significantly compared with those in the control group during the first two days of the study. The amount of food intake in the KP-treatment group tended to be lower than in the control group. Therefore, the results imply that the body weight gain of the rats treated with KP is not the result of food intake. According to the previous reports, TP treatment produces significant increases in rat body weight [14, 15]. Testosterone has been to have an anabolic effect during development stages such as puberty [16] and to produce greater increases in muscle size and strength in humans [17]. Furthermore, adult male and female rats injected daily with growth hormone (GH) have significant gains in body weight [18, 19], suggesting that GH stimulates the
rate of body weight gain. In hypophysectomized male rats, administration of GH combined with TP results in greater body weight gain than GH administration alone [20]. This study indicated that testosterone synergized with GH to promote body weight gain [20]. According to a prior report concerning the effect of KP, KP has an anti-gastric ulcer effect in rats by increasing the gastric wall mucus content and reducing the size of gastric ulcers induced by indomethacin, HCl/EtOH, and water immersion restraint [2]. GH stimulates hypertrophy and proliferation of epithelial cells in the stomach and heals induced gastric ulcer in rats [19]. Based on this evidence, we assumed that KP-induced body weight gain may result from testosterone and/or GH mechanisms. However, the assumption that KP acts to increase body weight in rats was not proved in this study. Thus, further research is required.

Although the results of the study did determine a KP effect in terms of increasing testosterone levels and body weight gain, we were unable to determine a testosterone-like effect on gonadotropins and reproductive organ weight. Thus we concluded that KP has no testosterone-like effect on reproduction in male rats.

Acknowledgements

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