Oral Administration of *Kaempferia parviflora* did not Disturb Male Reproduction in Rats

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**Abstract.** To investigate the androgenic effect of *Kaempferia parviflora* (KP), a Thai herbal plant, adult male rats were randomized into control and KP-treatment groups. Rats were treated orally with water in the control group and with 1,000 mg/kg/day of KP in the treatment group for 45 days. Blood samples were collected on days 10, 20, 30 and 45 for measurement of the serum follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone, progesterone and corticosterone levels. The reproductive and non-reproductive organs were dissected on day 45 and weighed. Mating behavior was also observed on days 20 and 30. Body weight was measured throughout the study period. The results showed that KP induced an increase in body weight compared with the controls. There were no significant differences in the weights of either reproductive (testis, seminal vesicle plus coagulating gland, levator ani muscle plus bulbocavernosus muscle and glans penis, except the prostate gland) or non-reproductive organs (kidney, adrenal gland and gastracnemius muscle). There were no significant differences in serum levels of either FSH or LH between the two groups. The serum testosterone and progesterone levels were insignificantly lower in the KP group during the first 30 days. The serum corticosterone levels in the KP group were lower than those in the controls throughout the study period and were significantly low on days 20 and 30. There were no significant changes in mating behavior in the rats treated with KP. Although KP affected the body weight and serum corticosterone level, it did not affect mating behavior, reproductive and non-reproductive organ weights or hormones related to the reproductive system in the adult male rats. Therefore, we conclude that the testosterone-like effect of KP did not disturb the hypothalamic-pituitary-testicular axis or male reproduction.

**Key words:** Body weight, *Kaempferia parviflora*, Luteinizing hormone (LH), Mating behavior, Testosterone (J. Reprod. Dev. 54: 375–380, 2008)

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*Kaempferia parviflora* Wall. Ex. Baker (KP), commonly referred to in Thai as Krachai Dum, is an indigenous herb that belongs to the Family Zingiberaceae. Nowadays, this plant is considered a Thai national herb. Based on studies using gas chromatographic techniques, many kinds of flavonoid substances, including 5-hydroxy-3,7-dimethoxyflavone, 5-hydroxy-7-methoxyflavone, 5-hydroxy-3,7,4’-trimethoxyflavone, 5-hydroxy-7,4’-dimethoxyflavone, 5-hydroxy-3,7,3’4’-tetramethoxyflavone, 3,5,7-trimethoxyflavone, 3,5,7,4’-tetramethoxyflavone, 5,7,4’-trimethoxyflavone and 5,7,3’4’-tetramethoxyflavone, have been found in KP roots [1, 2].

KP root has been reported to have anti-gastric ulcer activity by preservation of gastric mucous secretion without inhibition of gastric acid secretion in male rats gavaged with an ether extract of KP [3]. In addition, daily administrations of KP at 20, 200, 1,000 or 2,000 mg/kg is not toxic to adult rats; however, 2,000 mg/kg seems to produce an increase in liver weight and decrease in eosinophil numbers (Chewapat et al. unpublished data).

Native Thai people widely use this plant with alcohol or boiling water in traditional medicine to increase male libido and alleviative impotency because they believe this plant has androgenic activity although there are no reports that support this. Despite this, no scientific data has been collected concerning the effects of KP on male libido and reproduction. Therefore, to clarify whether KP has testosterone-like properties, the present study examined the effects of KP on mating behavior, the reproductive system and related hormones in adult male rats. Since 2,000 mg/kg might induce harmful effects in the animals, 1,000 mg/kg of KP was used in this study to avoid the potential of toxicological problems in the animals. Moreover, our previous study on castrated immature rats found that KP treatment for 7 days seemed to increase the weights of reproductive organs, including the seminal vesicle, prostate gland and levator ani muscle, although the changes were not significant [4]. At the same time, the serum LH level tended to decrease compared with the controls. Accordingly, this study examined effect of long-term treatment of KP over the course of 45 days.
Materials and Methods

Animals

Adult male Wistar-Imamichi rats, weighing 448.97 ± 8.96 g (mean ± SEM), were purchased from the Imamichi Institute for Animal Reproduction, Ibaraki, Japan. They were housed in metal cages and maintained in a room with controlled illumination (14L:10D), lights on 0500–1900 h, and temperature (22–24 °C) and free access to commercial pellets and tap water. The procedures were approved in accordance with the Guide for the Care and Use of Laboratory Animals of the Tokyo University of Agriculture and Technology.

Experimental protocol

Adult male rats were randomized and divided into control (n=5–6) and KP-treatment groups (n=6–7). Rats were orally administered 0.5 ml/day of water in the control group and 1,000 mg/kg/day of KP in the treatment group between 0930–1000 h for 45 consecutive days. To avoid stress from the experimental conditions, all rats were acclimated to the experiment by regular handling for 5 days prior to the onset of study. Both groups were first tested for mating behavior on days 20 and 30. Blood samples (0.5 ml) were collected from rats by cardiac puncture between 1300–1315 h under ether anesthesia on days 10, 20, 30 and 45. Serum samples were separated by refrigerated centrifugation at 1,500 × g for 30 min and stored at −20 °C until assayed for the follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone, progesterone, and corticosterone levels. On day 45, all rats were sacrificed and the testes, seminal vesicles plus coagulating gland, ventral prostate, levator ani muscle plus bulbocavernosus muscle, glans penis, kidneys, adrenal glands and gastracnemius muscle were removed from each to examine organ weights. In addition, body weight and food weight intake were measured between 0900–0930 h throughout the study period.

Preparation of KP suspension

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

Behavioral testing

In preparation for the mating behavior test, two 1-cm capsules of estradiol powder were implanted into ovariectomized rats 48 h before the start of testing. Mating behavior was examined between 2000–2200 h. At the onset of the test and under a dim red light, each male rat was left in a semicircular aquarium with sawdust bedding; after 5 min, a female was placed in the aquarium with the male. Mating behavior scores were evaluated using mounting, intromission and ejaculation as criteria. The following parameters were used: the first mount, intromission and ejaculation latency times (F_ML, F_IL and F_EL) from introduction of the female; first post-ejaculatory interval (F_PE), time from the first ejaculation until the next mounting; frequencies of first mounting and intromission (F_MF and F_IF); and the frequencies of mounting, intromission and ejaculation within 30 min for each test (MF, IF and EF).

Hormonal assays

Serum FSH and LH levels were measured using NIDDK RIA kits (NIDDK, Torrance, CA, USA) for rat FSH and LH. Iodinated preparations included 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 sensitivities of the assays were 0.24 ng/tube (1.2 ng/ml) and 0.006 ng/tube (0.06 ng/ml) for FSH and LH, respectively. The intra- and interassay coefficients of variation were 4.8 and 11.4% for FSH and 5.4 and 6.9% for LH, respectively.

Serum were extracted by diethyl ether for measurement of progesterone, testosterone and corticosterone levels using a double-antibody RIA system with ¹²⁵I-labeled radioligands as described previously [5, 6]. The antisera against progesterone (GDN 377), testosterone (GDN 250) and corticosterone (UCB) were provided by Dr. Niswender GD (Colorado State University, Fort Collins, CO, USA). The sensitivities of the assays were 2.5 pg/tube (25 pg/ml), 0.2 pg/tube (2 pg/ml), and 1.25 pg/tube (12.5 pg/ml) for progesterone, testosterone and corticosterone, respectively. The intra- and interassay coefficients of variations were 5.0 and 6.0% for progesterone, 5.9 and 5.8% for testosterone and 9.8 and 17.5% for corticosterone.

Statistical analysis

All data are expressed as the mean ± SEM. Analyses of relative body weight and hormonal levels and mating behavior were performed using independent sample Student’s t-tests. P<0.05 was considered to be statistically significant.

Results

Body weight and food intake

At the beginning of the study (day 1), the body weights of the rat control and KP groups were 459.11 ± 17.55 and 440.10 ± 6.80 g, respectively, with no significant difference between the groups.

Fig. 1. Relative body weights of adult male rats in the 1,000 mg/kg/day KP group (n=5) compared with the control group (n=7). Body weights were converted to percent body weight at the first dose. Results are expressed as means ± SEM. Stars indicate significant differences compared with the control (P<0.05).
However, the body weights were adjusted to the percent change in body weight from day 1, as shown in Fig. 1. The body weights of the rats in the KP group were higher than those of the rats in the control group throughout the study period and increased significantly in the early phase of the study (P<0.05). At the same time, there was no significant difference in food intake between the groups (data not shown).

Reproductive and non-reproductive organ weights

Table 1 shows the organ weights which were measured on day 45, as the ratio of organ weight per body weight. Our study did not detect significant differences in the weights of reproductive organs, including the testis, seminal vesicles plus coagulating gland, levator ani muscle plus bulbocarvernosus muscle and glans penis, except in the ventral prostate gland (P=0.0003). There was also no significant difference in the weights of organs, including the kidneys, adrenal glands and gastracnemius muscle, between the KP treatment and control groups.

Serum FSH, LH, testosterone, progesterone and corticosterone levels

The changes in the serum hormonal levels on days 10, 20, 30 and 45 were compared between the control and KP groups (Fig. 2). We did not detect any significant differences in the serum FSH or LH levels between the groups throughout the study period. The serum testosterone levels tended to be lower in the KP group but were not significantly different throughout the study period. The serum progesterone levels also showed insignificant lower levels in the KP group on days 10, 20 and 30 and a slight increase on day 45. The serum corticosterone levels in the KP group were, however, lower than those in the control group throughout the study period.

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Fig. 2. Comparison of the serum corticosterone, progesterone, testosterone, FSH and LH levels in adult male rats in the KP and control groups. Hormonal levels are displayed as means ± SEM. Stars indicate the significant differences between the two groups (P<0.05).
and were significantly lower on days 20 and 30 (P=0.003 and 0.019, respectively).

**Mating behavior test**

We did not detect any effect of KP on mating behavior using F_ML, F_IL, F_EL, F_PE, F_MF, F_IF, MF, IF or EF as parameters. There were no differences between the control and KP treatment groups when male rats were tested on days 20 and 30 (Table 2). One male rat treated with KP was excluded on day 30 of the study period. We did not detect any effect of KP on mating behavior using F_ML, F_IL, F_EL, F_PE, F_MF, F_IF, MF, IF or EF as parameters. There were no differences between the control and KP treatment groups when male rats were tested on days 20 and 30 (Table 2). One male rat treated with KP was excluded on day 30 of the study period, since the rat did not show intromission within 15 min of the test.

### Discussion

The aim of the present study was to examine the testosterone-like effects of KP ingestion on reproduction using the hormone response, organ weights and mating behavior of adult male rats as indicators.

KP treatment caused an increase in the body weights of the adult male rats throughout the 45-day study period without an increase in food intake as shown in our previous report [4]. The anabolic steroid testosterone has been reported to increase body mass by increasing muscle protein synthesis [7, 8] and to decrease protein breakdown [9]. Intake of anabolic steroid increases the body weight and lean body mass of humans [10]. The present results seem to indicate that KP might have behaved like anabolic testosterone and increased the body weights of the rats.

In addition to testosterone, it has been reported that growth hormone (GH), which also has an anabolic effect, increases body mass [10]. Injection of GH significantly increases the body weight in both adult male and female rats [11–13]. There is positive correlation between testosterone and GH [14–16]. Testosterone appears to stimulate expression of growth hormone-releasing hormone (GHRH) mRNA in the arcuate nucleus of the hypothalamus [14] and GH secretion in both humans [15] and rats [14]. Neonatal gonadectomy of adult male rats results in suppression of the plasma GH level and a decrease in body weight, whereas testosterone replacement reverses these effects [17].

A previous report showed that GH stimulates hypertrophy and proliferation of the epithelial cells of the stomach and heals gastric ulcer in rats [12]. Consistent with the GH effects, KP has a gastro-protective effect and inhibits gastric ulcer formation by increasing gastric mucus and reducing gastric ulcer size in rats [3]. Hence, this reinforces the suggestion that KP increases rat body weight by directly or indirectly acting as testosterone.

In relation to hormone response, testosterone acts directly on the pituitary gland [18] and suppresses gonadotropin secretion by a negative feedback mechanism and reduction of pituitary gland response to gonadotropin releasing hormone (GnRH) stimulation [19, 20]. Administration of testosterone results approximate reductions in the levels of LH and FSH of 50 and 30%, respectively, in both humans [19, 20] and rats [21]. Flutamide, an androgen receptor antagonist, increases in serum LH levels in both intact mature male and testosterone-treated castrated mature rats [22, 23]. In pituitary cell culture, testosterone inhibits GnRH-stimulated FSH and LH release, and the inhibitory effect of testosterone is inhibited by treatment with flutamide [Trisomboon et al., unpublished data]. In addition, a significant decrease in LH levels causes a decrease in testosterone production and decreases in the testosterone levels in both serum and the testis [24]. In our study, although the serum testosterone levels tend to decrease in the treatment group, there were no the significant changes of serum gonadotropins or testosterone. We assumed that although KP had a testosterone-like effect in increasing rat body weight, it did not disturb the pituitary-gonadal axis in the rats.

Nevertheless, the serum corticosterone levels decreased significantly on days 20 and 30 and increased on day 45 of the treatment period. At the same time, the serum progesterone levels, also produced mainly in the adrenal gland, tended to be low beginning on day 10 of the study period. Many previous reports have shown the suppressive effect of testosterone on corticosterone production by the adrenal gland [25–27]. Orchietomy induces an increase in corticosterone concentration in rats, and testosterone replacement recovers the effect [26]. Long-term treatment of testosterone

### Table 2. Parameters of male mating behavior in the rats of the 1,000 mg/kg KP group compared with the control group on days 20 and 30 of the study period

<table>
<thead>
<tr>
<th></th>
<th>Control (n=6)</th>
<th>KP group (n=6)</th>
<th>Control (n=5)</th>
<th>KP group (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F_ML</td>
<td>39.83 ± 35.44</td>
<td>6.67 ± 1.12</td>
<td>3.20 ± 0.80</td>
<td>4.80 ± 2.48</td>
</tr>
<tr>
<td>F_IL</td>
<td>395.83 ± 245.63</td>
<td>152.00 ± 93.81</td>
<td>287.80 ± 270.23</td>
<td>118.60 ± 80.43</td>
</tr>
<tr>
<td>F_EL</td>
<td>268.67 ± 176.05</td>
<td>591.00 ± 332.57</td>
<td>161.00 ± 55.38</td>
<td>264.00 ± 94.58</td>
</tr>
<tr>
<td>F_PE</td>
<td>365.33 ± 80.17</td>
<td>184.20 ± 77.04</td>
<td>220.40 ± 55.83</td>
<td>213.40 ± 56.12</td>
</tr>
<tr>
<td>F_MF</td>
<td>7.67 ± 4.07</td>
<td>18.50 ± 9.40</td>
<td>11.80 ± 3.81</td>
<td>29.00 ± 7.85</td>
</tr>
<tr>
<td>F_IF</td>
<td>6.33 ± 3.31</td>
<td>3.83 ± 1.74</td>
<td>5.60 ± 1.63</td>
<td>8.00 ± 2.53</td>
</tr>
<tr>
<td>MF</td>
<td>52.83 ± 9.04</td>
<td>54.00 ± 53.73</td>
<td>46.00 ± 5.81</td>
<td>67.60 ± 9.00</td>
</tr>
<tr>
<td>IF</td>
<td>15.67 ± 5.08</td>
<td>9.00 ± 3.17</td>
<td>15.80 ± 4.06</td>
<td>15.00 ± 3.39</td>
</tr>
<tr>
<td>EF</td>
<td>1.33 ± 0.61</td>
<td>0.67 ± 0.33</td>
<td>2.80 ± 0.73</td>
<td>3.00 ± 0.63</td>
</tr>
</tbody>
</table>

F_ML, F_IL and F_EL are the first mount, intromission and ejaculation latency times from introduction of female (sec.). F_PE is the first post-ejaculatory interval (sec.); that is, the time from first ejaculation until the next mounting. F_MF and F_IF are the frequencies of first mounting and intromission (times). MF, IF and EF are the frequencies of mounting, intromission and ejaculation within 30 min for each test (times).
Testosterone has been reported to play a role in increasing reproductive organ weights in a dose-treated rats and the controls. Testosterone has been reported to play a role in relation weight of adrenal glands in castrated immature rats. These prior reports clearly show that testosterone plays a role in relation to androgen receptor and induces regrowth of the prostate gland in hypophysectomized rats. The effects of daily testosterone treatment in terms of increasing prostate gland weight in castrated rats is inhibited by flutamide, suggesting that testosterone has a proliferative effect on the prostate.

In addition to the reproductive organs, the kidney and adrenal gland have been shown to be the targets of androgen. Androgen receptor and induces regrowth of the prostate gland in hypophysectomized rats. The effects of daily testosterone treatment in terms of increasing prostate gland weight in castrated rats is inhibited by flutamide, suggesting that testosterone has a proliferative effect on the prostate.

In regard to the mating behavior in the males, we did not observe any differences in mating behavior in the adult male rats treated with KP or the controls. Male mating behavior in mammals, including rats, is regulated by androgen at the levels of both the central nervous system and peripheral systems. Daily treatment with GnRH antagonist decreases the plasma testosterone levels and abolishes mating behavior in male rats within 2 weeks. The effects of the GnRH antagonist were abolished by treatment with testosterone propionate.

A reduction in mating behavior is observed in castrated hamsters and can be recovered by treatment with testosterone. The treatment of the medial amygdala with fadrozole, a nonsteroidal aromatase inhibitor, in intact male rat in order to inhibit the aromatization of testosterone to estradiol reduces mating behavior. In addition, mating behavior is not promoted in DHT-treated castrated rats when the rats receive estrogen plus bone serum albumin to inhibit the transfer of estrogen across the plasma membrane. These studies suggest that testosterone has a direct or indirect effect on mating behavior in rats. Therefore, our present study could not assume that KP had a testosterone-like effect on mating behavior. Moreover, adult male rats, which normally have high levels of serum testosterone, exhibit high sexual drives. This is therefore one possible reason why we did not observe an effect for KP on mating behavior in the rats in the present study.

In summary, although KP showed anabolic testosterone effects by increasing body weight and suppressing serum corticosterone levels, we found that there were no significant changes in mating behavior, the weights of reproductive and non-reproductive organs and the levels of serum hormones related to the reproductive system (FSH, LH and testosterone) in the adult male rats. Therefore, we concluded that KP did not disturb the hypothalamic-pituitary-testicular axis or male reproduction. Our findings strongly demonstrate that KP can be used in herbal medicine without disrupting male reproduction.

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