We investigated the effect of a hexane extract of *Curcuma comosa* Roxb., previously reported to mediate estrogenic activity, on fertility in adult male rats. Intragastric administration of the *C. comosa* extract at a dose of 500 mg/kg BW for 7 consecutive days significantly decreased weights of testes, ventral prostate and seminal vesicles. The decreased testicular weight corresponded with a marked regression of spermatogonia and spermatids in the seminiferous tubules. Biochemical analysis revealed significantly decreased activity of acid phosphatase in the ventral prostate. The activity of α-glucosidase in the cauda epididymides, and the fructose content in coagulating glands, were not significantly affected. Sperm concentration and motility in the cauda epididymides were also significantly suppressed. However, this 7-day treatment did not significantly affect fertility of the animals. Alterations by *C. comosa* extract were similar to effects mediated by estradiol. This suggests that the suppressing effect of *C. comosa* on male reproductive organs is mediated through the estrogenic-like action of the plant extract.

**INTRODUCTION**

Estrogen is known to affect the growth and development of male reproductive organs. Exogenous administration of estrogen causes atrophy of testes and male accessory sex organs, and decreases serum levels of testosterone (Moger, 1980). Its effect has been attributed to suppression of secretion of gonadotrophins as well as direct inhibition of the testes (Bendeck & Pomerantz, 1984). In addition, estrogen has also been reported to modulate the action of androgen in regulating epithelial growth of the accessory sex glands (Belis et al., 1977; Chinoy et al., 1984; Tenniswood et al., 1978), whose structure and functional integrity are essential for fertility (Mann, 1974; Queen et al., 1981). Estrogens have been reported to occur naturally in a number of plants and can depress fertility in animals on ingestion (Braden et al., 1967; Pelissero et al., 1991; Wong and Flux, 1962). In Thailand, *Curcuma comosa* Roxb (Zingiberaceae), which is widely used for relief of lower abdominal pain in males contains estrogen-like activity (Piyachaturawat et al., 1995a, b). We have recently noted that the hexane extract of *C. comosa* suppressed growth of accessory sex glands in immature rats in a dose-related manner (Piyachaturawat et al., 1997). In view of the use of this plant in adults and the potential of estrogen in suppressing testicular secretion and in modulating androgen action, it is not clear whether the function of the accessory sex glands and fertility in adult humans are disturbed after taking *C. comosa*. Therefore, the aim of the present study was to investigate the effects of the hexane extract of *C. comosa* on male fertility in intact adult animals. Accessory gland function, sperm motility and concentration, and fertility were also evaluated.

**MATERIALS AND METHODS**

**Plant Extract**

Extract of *Curcuma comosa* was prepared as described previously (Piyachaturawat et al., 1995a). Rhizomes
were dried, cut into small pieces, and ground into powder. The powder was extracted with n-hexane in a Soxhlet extractor to give a pale brownish viscous oil. For administration to animals, the extract was initially dissolved in DMSO and further suspended in corn oil. The final volume of administration was 0.5 ml, containing 10% DMSO.

Animals and Experiment
Mature male Wistar rats weighing 350–380 g and mature females weighing 150–180 g were supplied by the National Laboratory Animal Centre, Mahidol University, Salaya, Nakhorn Pathom, Thailand. They were randomly divided into groups and given free access to food and water. A controlled 12-h light/dark cycle was maintained. The C. comosa extract was intragastrically administered at a dose of 500 mg/kg BW for 7 consecutive days. This dose was selected on the basis of our previous studies (Piyachaturawat et al., 1997). Various doses of estradiol (E2) ranging from 1–10 µg/kg BW were subcutaneously injected for 7 days as a reference standard. On day 8, the rats were sacrificed. Testes and all accessory sex organs, including epididymides, ventral prostate and seminal vesicles were dissected free of fat and weighed. All organs were rapidly emptied and the contents were centrifuged. The fluid was kept for further evaluation of organ function. Activity of α-glucosidase in the epididymal fluid was determined by the method of Chapdelaine et al. (1978) and Grandmont et al. (1983). The acid phosphatase activity in the ventral prostate gland was determined from the release of p-nitrophenol from p-nitrophenylphosphate (PNPP) as described by Tenniswood et al. (1978). Prostatic phosphatase activity was calculated from total activity and non-prostatic acid phosphatase activity which was measured by inhibiting prostatic acid phosphatase activity with 20 mM D(-)-tartrate. Fructose content in the coagulating gland joined to seminal vesicles was also determined as previously described (Dische & Borenfreund, 1951).

Testes and accessory sex glands were randomly selected for fixation for histological study. Paraffin sections were stained with hematoxylin and eosin.

Sperm Concentration and Motility Counting
Spermatozoa collected from the cauda epididymides were diluted 40 times with modified Tyrode’s solution. After incubation at 37°C in a temperature bath for 10 min, the suspension was further diluted and then examined under a phase-contrast microscope for assessment of number and motility of sperm.

Fertility Test
Fertility of each male was assessed by allowing cohabitation with a young proestrous female before and after the treatment. The presence of spermatozoa in a vaginal smear on the following morning was used as evidence of mating and was considered as day zero of pregnancy. The pregnant rats were separated and were sacrificed on day 13 of pregnancy. The number of implantation sites and corpora lutea were counted and used as an index of fertility of the male. Percent fertility was the number of implantation sites over the number of corpora lutea.

Chemicals
17-β-Estradiol acetate, p-nitrophenyl α-D-glucopyranoside, glutathione (reduced form), p-nitrophenyl phosphate (disodium), p-nitrophenol (standard solution), carbazole, L-cysteine hydrochloride monohydrate and D(-) fructose were purchased from Sigma Chemical Co. (St. Louis, Missouri, U.S.A.). Testosterone (Testoviron Depot) was purchased from Schering Ltd. (Thailand). All other reagents and solutions were also commercially obtained and were of analytical grade.

Statistical Analysis
All data were expressed as mean ± SEM. Statistical analysis was performed by using one-way analysis of variance (one way ANOVA) and differences between pairs of means were made by using the Student’s Newman-Keuls test.

RESULTS
Effect of C. comosa Extract on Organ Weights
Table 1 shows the effects of treatment with hexane extract of C. comosa and estradiol on body weight and reproductive organ weights in intact adult male rats. Intragastric administration of the extract at a dose of 500 mg/kg for 7 consecutive days significantly reduced body weight and weights of testes, ventral prostate and seminal vesicles, but had no significant effect on epididymal weight (P > 0.05). Estradiol treatment (1–10 µg/kg), which was employed as a reference standard, caused a dose-dependent decrease in the weights of most organs in the reproductive system. The suppressing effect of the extract at a dose of 500 mg/kg on the organ weights was comparable to the effect of estradiol at a dose of 2 µg/kg BW.
Effect on Biochemical Markers of Accessory Sex Gland Functions

Administration of hexane extract had no significant effect on the activity of α-glucosidase in the cauda epididymides or on the content of fructose in the coagulating gland, but it significantly decreased prostatic acid phosphatase activity (Table 2). Likewise, administration of a low dose of estradiol (2–5 µg/kg BW) did not significantly alter α-glucosidase activity, whereas a higher dose of estradiol (10 µg/kg BW) significantly decreased both prostatic acid phosphatase activity and fructose content in the coagulating gland.

Sperm Concentration and Motility

The concentration of sperm in the cauda epididymides of control animals was $1.80 \pm 0.04 \times 10^9$/ml, and motility was $62 \pm 1.4\%$ (Table 3). Treatment with the hexane extract (500 mg/kg BW) for 7 days caused a significant decrease in sperm concentration to $1.4 \pm 0.8 \times 10^9$/ml and motility to $29 \pm 3.1\%$. Estradiol

Effect of *Curcuma comosa* extract (500 mg/kg) and estradiol (E2) on body weight (g) and weights of testes and accessory sex organs (mg/100 g BW) in adult male rats. Animals received treatment for 7 consecutive days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Testes (mg/100 g BW)</th>
<th>Epididymides (mg/100 g BW)</th>
<th>Ventral prostate (mg/100 g BW)</th>
<th>Seminal vesicles (mg/100 g BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>374.0 ± 2.2</td>
<td>1051.2 ± 16</td>
<td>250.7 ± 3.9</td>
<td>94.8 ± 4.2</td>
</tr>
<tr>
<td><em>Curcuma comosa</em></td>
<td>15</td>
<td>352.2 ± 5.3**</td>
<td>1035.8 ± 5.3*</td>
<td>254.7 ± 3.3</td>
<td>66.6 ± 5.1**</td>
</tr>
<tr>
<td>E2 1 µg/kg BW</td>
<td>10</td>
<td>368.4 ± 2.3</td>
<td>1051.4 ± 17.2</td>
<td>251.3 ± 5.7</td>
<td>98.4 ± 6.7</td>
</tr>
<tr>
<td>E2 2 µg/kg BW</td>
<td>11</td>
<td>370.5 ± 2.8</td>
<td>1036.1 ± 25.0</td>
<td>241.2 ± 5.3</td>
<td>62.8 ± 2.8**</td>
</tr>
<tr>
<td>E2 5 µg/kg BW</td>
<td>15</td>
<td>364.2 ± 2.1**</td>
<td>984.1 ± 17.0*</td>
<td>202.2 ± 5.5**</td>
<td>50.1 ± 3.4**</td>
</tr>
<tr>
<td>E2 10 µg/kg BW</td>
<td>15</td>
<td>355.8 ± 2.1**</td>
<td>976.1 ± 13.1**</td>
<td>175.4 ± 6.8**</td>
<td>40.5 ± 2.7**</td>
</tr>
</tbody>
</table>

Each value represents mean ± SEM.

*P < 0.05; **P < 0.01 significant difference from control.

n indicates number of animals used in each experiment.

Effect of *Curcuma comosa* extract (500 mg/kg) and estradiol (E2) on activities of α-glucosidase from cauda epididymis, acid phosphatase from ventral prostate and fructose concentration from coagulating gland in adult rats. Animals received treatment for 7 consecutive days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>α-Glucosidase (mU/mg prot/30 min)</th>
<th>Prostatic acid phosphatase (µmol/mg prot/30 min)</th>
<th>Fructose (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.1 ± 0.3</td>
<td>0.86 ± 0.03</td>
<td>5.1 ± 0.2</td>
</tr>
<tr>
<td><em>Curcuma comosa</em></td>
<td>7.0 ± 0.3</td>
<td>0.56 ± 0.07**</td>
<td>5.0 ± 0.2</td>
</tr>
<tr>
<td>E2 2 µg/kg BW</td>
<td>7.6 ± 0.8</td>
<td>0.88 ± 0.06</td>
<td>4.5 ± 0.4</td>
</tr>
<tr>
<td>E2 5 µg/kg BW</td>
<td>7.0 ± 0.3</td>
<td>0.75 ± 0.02</td>
<td>4.1 ± 0.5*</td>
</tr>
<tr>
<td>E2 10 µg/kg BW</td>
<td>6.9 ± 0.2</td>
<td>0.60 ± 0.06**</td>
<td>3.7 ± 0.4**</td>
</tr>
</tbody>
</table>

Values are means ± SEM obtained from 7–12 animals.

*P < 0.05; **P < 0.01, significant difference from control.

Effect of *Curcuma comosa* extract (500 mg/kg) and estradiol (E2) on sperm concentration and motility in adult rats. Animals received treatment for 7 consecutive days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(n)</th>
<th>Sperm concentration ($\times 10^9$/ml)</th>
<th>Sperm motility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20</td>
<td>1.80 ± 0.04</td>
<td>62 ± 1.4</td>
</tr>
<tr>
<td><em>Curcuma comosa</em></td>
<td>10</td>
<td>1.41 ± 0.08*</td>
<td>29 ± 3.1*</td>
</tr>
<tr>
<td>E2 1 µg/kg BW</td>
<td>10</td>
<td>1.74 ± 0.08</td>
<td>53 ± 2.8</td>
</tr>
<tr>
<td>E2 2 µg/kg BW</td>
<td>10</td>
<td>1.78 ± 0.06</td>
<td>32 ± 2.1*</td>
</tr>
<tr>
<td>E2 5 µg/kg BW</td>
<td>15</td>
<td>1.56 ± 0.07*</td>
<td>23 ± 2.0*</td>
</tr>
<tr>
<td>E2 10 µg/kg BW</td>
<td>20</td>
<td>1.36 ± 0.09*</td>
<td>29 ± 3.1*</td>
</tr>
</tbody>
</table>

Values are means ± SEM.

* P < 0.05 significant difference from control.

n indicates number of animals used in each experiment.
treatment caused a dose-related decrease in both sperm concentration and motility.

**Fertility**

Fertility of male animals was evaluated before and after treatment with the hexane extract of *C. comosa*. As shown in Figure 1, treatment with the hexane extract at a dose of 500 mg/kg, or estradiol at 10 µg/kg, for 7 days, did not significantly affect the fertility of rats.

**DISCUSSION**

The present study demonstrated a marked suppression of weights and function of the adult male reproductive organ in rats by the *C. comosa* extract. These effects are consistent with the results of our earlier study in immature rats which the effects were suggested to be due to estrogen-like activity of the extract. The suppression by the extract, in addition to be the consequence of the impairment of testosterone secretion, was due to the direct action on the reproductive organs (Piyachaturawat et al., 1997). In the present study, changes in organ weight by the hexane extract and

**Histology**

Figure 2 shows testicular cross-sections obtained from adult rats. In control animals, seminiferous tubules showed clear organization of cells at various stages of spermatogenesis (Fig. 2a). After treatment with the hexane extract or estrogen, the seminiferous tubules showed a loose arrangement of degenerating spermatogonia and primary spermatocytes (Fig. 2b, c). These treatments also caused a marked reduction in the diameter of epididymal tubules and lower epithelial cell height. In addition, the epididymal lumens contained less sperm as compared with controls. Treatment with the hexane extract slightly affected the epididymides. The lumens were still filled with spermatozoa (data not shown).

Fig. 1. Implantation in female rats which cohabited with males before and after being treated with *Curcuma comosa* extract (500 mg/kg, i.g.) or estradiol (E2, 10 µg/kg, s.c.) for 7 days.

Fig. 2. Light micrograph of seminiferous tubules obtained from adult male rats treated with (a) corn oil, (b) *C comosa* extract (500 mg/kg, i.g.), (c) estradiol (E2, 10 µg/kg, s.c.) for 7 days. Arrows indicate pyknotic nuclei of spermatozoa (× 200).
estrogen corresponded with changes in histology of the tissues. In the testis, a large component of the weight of tissue is associated with spermatogenic function; hence suppression of testicular weight after the hexane extract and estradiol treatment might result from the large changes in the content of spermatids and spermatozoa. This was evident in the histological picture which showed a marked reduction of spermatogonia and spermatids in the seminiferous tubules (Fig. 2). Likewise, the reduction in epididymal weight caused by estradiol correlated with the marked reduction observed in the size of the tubules. The hexane extract only slightly affected the histological appearance of the epididymis and did not significantly affect the weight.

The reduction in the weights of accessory sex glands caused by the hexane extract and estradiol should lead to decreases in their glandular function. Indeed, both weight and acid phosphatase secretion from the prostate gland were decreased by the treatment. The observed effect of estradiol here was consistent with earlier studies which showed a dramatic reduction in the size and secretory activity of the prostate gland after estrogen treatment (Robinett et al., 1978; Tenniswood et al., 1978). As the secretory activities of all accessory glands are known to be specifically dependent on androgen stimuli (Tenniswood et al., 1978), those changes have been suggested to be probably due to inhibition of testicular biosynthesis.

Estradiol treatment for 7 days caused a dose-dependent decrease in both sperm concentration and motility in the cauda epididymides which serves as a sperm depot and site for sperm maturation. The hexane treatment also significantly decreased both parameters. Accordingly, histological study of the hexane extract and estradiol-treated animals also revealed fewer sperm in the seminiferous tubules and the epididymal lumen. Thus, severely affected spermatogonia and spermatids in the seminiferous tubules could also have been a cause of decreased sperm concentration in the epididymides. Sperm maturational activity and motility in the epididymides would have been impaired. All these abnormalities of sperm could eventually lead to impaired fertility. In this study, however, although both sperm count and motility were reduced by treatment with estradiol and hexane extract, fertility did not significantly change. Perhaps our relatively short period of treatment could account for this. In rats, the time required for a spermatogonium to yield spermatozoa in the ejaculate is approximately 60 days and thus our 7-day treatment may not have been long enough to affect fertility. In addition, we assessed sperm from the cauda epididymis which might not be the same as the ejaculated sperm in the fertility test. Alternately, it has been reported that mature spermatozoa in the cauda epididymides are able to retain their fertilizing capacity for several days in the absence of circulating testicular androgen after castration (Rissman & Crews, 1989). Therefore, although accessory organ function were impaired, fertility was not significantly affected within the short 7-day period of treatment. However, the apparent damage of spermatozoa in seminiferous tubules and decrease of sperm count and motility in cauda epididymides, would certainly lead to depleted sperm reserve and eventual infertility.

Therefore, we conclude that the hexane extract of C. comosa (500 mg/kg BW for 7 days) in all likelihood will affect fertility in male and mediated its effects through the estrogen-like action of the plant extract. A longer period of continuous use of C. comosa would probably lead to infertility.

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REFERENCES


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