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—Research Note—

Pfaffia paniculata-Induced Changes in Plasma Estradiol-17β, Progesterone and Testosterone Levels in Mice

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Abstract. The present study undertook chemical analysis of components of *Pfaffia paniculata* roots. In addition, an animal experiment was conducted in which mice had *ad libitum* access to water enriched with powdered *P. paniculata* root for 30 days. Changes in plasma concentrations of estradiol-17β and progesterone in female mice and of testosterone in male mice were ascertained. The results revealed that *P. paniculata* roots contain two types of phytosteroids, β-sitosterol and stigmasterol, in addition to other compounds such as pfaffic acid, allantoin, saponins, β-sitosteryl-β-D-glucoside, and stigmasteryl-β-D-glucoside. Regarding changes in plasma concentrations of hormones, levels of the sex hormones estradiol-17β, progesterone and testosterone were clearly higher for mice that drank *P. paniculata* root-enriched water than for mice that drank plain water. Powdered *P. paniculata* root is easily dissolved in feed or water, and as no adverse reactions were seen in mice within 30 days of oral intake, consumption of *P. paniculata* for long periods of time appears safe.
Key words: *Pfaffia paniculata*, Brazilian ginseng, Estradiol-17β, Progesterone, Testosterone

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In Brazil, *Pfaffia paniculata* is known as Brazilian ginseng, and due to its tonic action, it has long been used to treat various human ailments [1–7], but, since few studies have examined *P. paniculata*, its components and actions have not been fully elucidated. In recent years, the use of folk remedies [4, 5], particularly Chinese herbal remedies, for the treatment of human ailments such as climacteric disorders, osteoporosis, prostate hypertrophy and sexual dysfunction has increased dramatically. The present study was conducted to ascertain whether a crude drug used to maintain homeostasis of sex hormones in humans could be used in animals. In the relatively new field of theriogenology, reduced fertility of cattle remains a serious problem, and little improvement has been achieved [8–12]. In milking cows, cross breeding has noticeably increased milk production in recent years, but fertility has declined [13, 14]. Reduced fertility is correlated with adrenal dysfunction caused by feeding and stress, and raising cattle under appropriate conditions is therefore important [15–17]. Nevertheless, a large amount of capital investment is necessary to set up and maintain optimal breeding conditions, making the operation of financially viable ranches difficult. In addition, several recent studies have shown that sperm cell counts have decreased due to such factors as hormone disruptors [18]. We therefore studied whether *P. paniculata* has effects on sex hormones. Chemical analysis of the components of powdered *P. paniculata* root was undertaken, and the concentrations of estradiol-17β and progesterone in female mice and testosterone in male mice were then measured by radioimmunoassay (RIA). This research is fundamental research on herbal remedies for animals.

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Materials and Methods

Test sample preparation (Pfaffia paniculata)

After washing and cleaning, natural *P. paniculata* roots were cut into about 3 mm chips and sterilized by exposure to ozone for 3 h. The sterilized chips were then desiccated in a drier for 18 h. Dried *P. paniculata* chips were pulverized into 120-mesh powder in a stainless steel mill.

Component analysis (Pfaffia paniculata)

First, methanol was added to powdered *P. paniculata* root to heat extract components, and the methanol was then evaporated. Butanol and water were then added to the extract to obtain aqueous and butanol fractions. The butanol was evaporated from the butanol fraction, and water was added. After separating the resulting aqueous solution from the residue, either hexane-ethyl acetate-methanol or chloroform-methanol was added to the residue, and subjected repeatedly to silica gel column chromatography. Furthermore, to the aqueous solution a water-methanol-ethyl acetate solution was added, and after charcoal column chromatography, a chloroform-methanol-water solution was added. The resulting solution was subjected to silica gel column chromatography, and then, each component was made clear with the spectroscope.

Animals

Three-week-old male and female ICR mice weighing 10 to 12 g were acclimatized for 1 week (room temperature, 22 ± 3 C, relative humidity 60%, daily lighting for 14 h, lights off for 10 h), then used in the study at 4 weeks old. Each cage housed 10 mice.

Grouping

Female mice were divided into three groups (control, estradiol-17β measurement and progesterone measurement), and male mice were divided into two groups (control and testosterone measurement). All groups were 10 mice to a cage. At the end of the acclimatization period, mice had *ad libitum* access to water for 30 days. Male and female mice in control groups were supplied water without *P. paniculata*, but mice in test groups were supplied water containing *P. paniculata* (5 g of powdered root per 100 ml of water) in water bottles.

Blood sample collection

At the end of 30 days, each mouse was anesthetized with diethyl ether, and about 1 ml of blood was collected from the heart with a 1 ml heparinized syringe. Each blood sample was immediately centrifuged to separate plasma from blood cells, then stored frozen at –20 C until testing.

RIA measurement of plasma estradiol-17β, progesterone and testosterone

First, each plasma sample was treated with a mouse plasma RIA kit (Immunotech Co., Ltd.), and then two measurements were taken with a gamma counter (Minaxi γ Auto-Gamma 5000 Series, Packard Co., Ltd.). The 5 standard samples included in the kit to prepare standard curves were each measured twice to plot a standard curve. Concentrations of sex hormones were then calculated from this standard curve.

Statistical analysis

All numerical results were expressed as the mean ± SD, and analysis of variance (ANOVA) was performed to assess significant differences.

Results

Component analysis of Pfaffia paniculata

The results revealed pfaffic acid and two phytosteroids, β-sitosterol and stigmasterol, were isolated from the residue, whereas allantoin, saponins, β-sitosteryl-β-D-glucoside and stigmasteryl-β-D-glucoside were isolated from the aqueous solution (Fig. 1).

Plasma concentration of estradiol-17β

The plasma concentration of estradiol-17β for *P. paniculata*-fed mice (4.89 ± 1.77 pg/ml, n=10) was significantly higher than that for control mice (3.55 ± 2.12 pg/ml, n=10) ($p<0.05$) (Table 1, Fig. 2).

Plasma concentration of progesterone

The plasma concentration of progesterone for *P. paniculata*-fed mice (23.71 ± 10.73 pg/ml, n=10) was significantly higher than that for control mice (16.97 ± 9.25 pg/ml, n=10) ($p<0.05$) (Table 1, Fig. 3).

Plasma concentration of testosterone

The plasma concentration of testosterone for *P. paniculata*-fed mice (8.64 ± 4.57 pg/ml, n=10) was

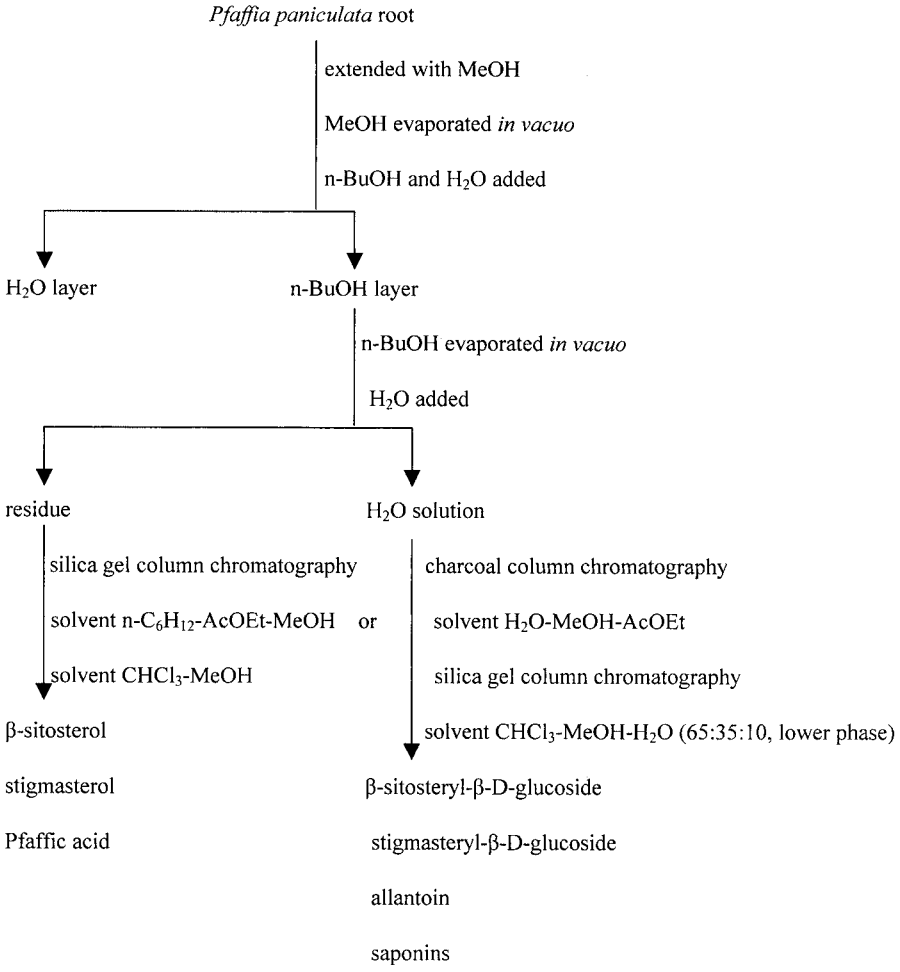

Fig. 1. Element analysis flow chart for *Pfaffia paniculata* root.

Table 1. Concentrations of sex hormones in plasma

Groups	estradiol-17β (pg/ml)	progesterone (ng/ml)	testosterone (ng/ml)
Control	3.55 ± 2.12	16.97 ± 9.25	4.37 ± 2.39
Pfaffia	4.89 ± 1.77[a)]	23.71 ± 10.73[a)]	8.64 ± 4.57[b)]

Each group is 10 mice. Data are the mean ± SD, and analysis by ANOVA.
Significant differences were detected between the control group and the *Pfaffia* group ([a)] $p < 0.05$, [b)] $p < 0.01$).

significantly higher than that for control mice (4.37 ± 2.39 pg/ml, n=10) ($p<0.01$) (Table 1, Fig. 4). This difference was the greatest among the three sex hormones.

Discussion

P. paniculata was used because the root of this vine is used to treat various human ailments in Brazil. After analyzing the components of *P. paniculata*, components were identified that could alter concentrations of sex hormones. In other words, we investigated whether *P. paniculata* could effect the concentrations of estradiol-17β and progesterone in female mice and testosterone in male mice. RIA was used to measure these concentrations. To the best of our knowledge, no

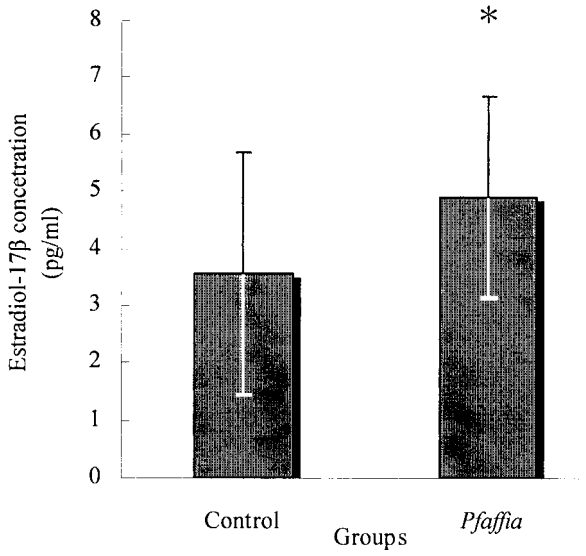

Fig. 2. Concentration of plasma estradiol-17β in female mice. Data are the mean ± SD, and analysis by ANOVA. Significant differences were detected between the control group and the *Pfaffia* group (* $p< 0.05$).

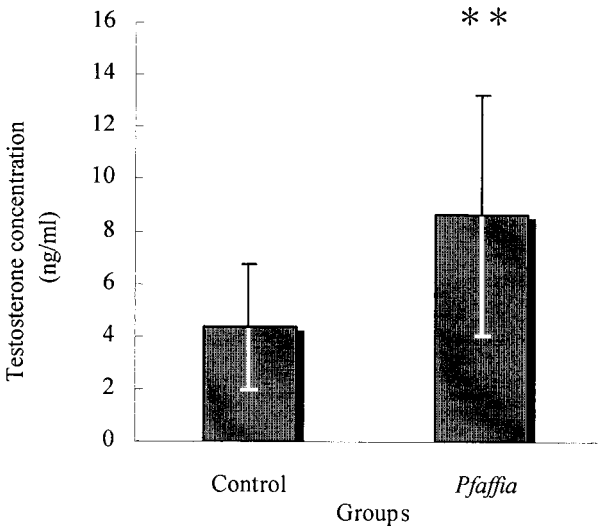

Fig. 4. Concentration of plasma testosterone in male mice. Data are the mean ± SD, and analysis by ANOVA. Significant differences were detected between the control group and the *Pfaffia* group (** $p< 0.01$).

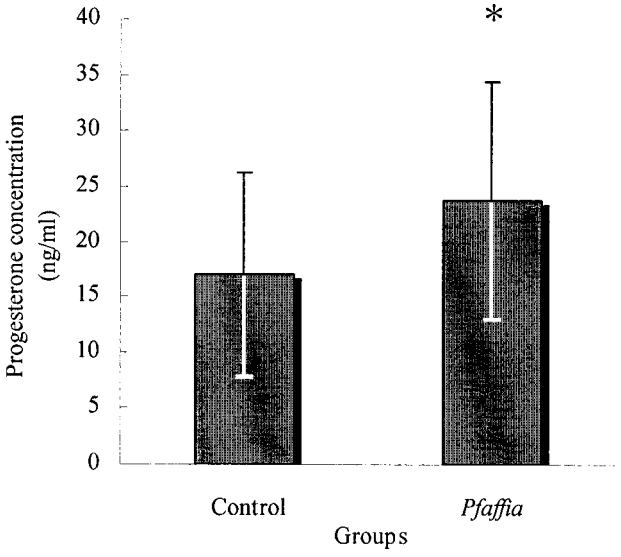

Fig. 3. Concentration of plasma progesterone in female mice. Data are the mean ± SD, and analysis by ANOVA. Significant differences were detected between the control group and the *Pfaffia* group (* $p< 0.05$).

previous studies have investigated the effects of *P. paniculata* on sex hormones. The *P. paniculata* used in the present study grows widely along the Amazon River in Brazil, and grows much faster than other cultivated plants. As the roots of *P. paniculata* are used as a crude drug, slight differences in components exist according to the growing region and time of harvesting.

Three-week-old mice were obtained because at this age they are not sexually mature, but will be fully mature and capable of breeding after one week of acclimatization and 30 days of administration. Particularly, in all female mice the sexual cycle would be largely the same, minimizing individual differences.

Component analysis revealed that powdered *P. paniculata* root contains two phytosteroids, β-sitosterol and stigmasterol, in addition to other compounds such as pfaffic acid, allantoin, saponins, β-sitosteryl-β-D-glucoside, and stigmasteryl-β-D-glucoside. Several studies have shown that β-sitosterol and stigmasterol inhibit absorption of cholesterol to suppress increases in plasma concentrations of cholesterol, stimulate production of estradiol-17β in the ovary [19, 20], and are effective in treating menstrual irregularities and climacteric disorders in humans [4]. We therefore believe that these phytosteroids increased plasma concentrations of estradiol-17β in female mice. In addition, after oral administration of moderate or high dose β-sitosterol in one study, the

weight of the uterus and plasma concentrations of progesterone increased [20]. The present study also confirmed significant increases in estradiol-17β and progesterone concentrations, and saponins in powdered *P. paniculata* root are known to play a very important role in what aspect of sex hormones. Saponins have been reported to normalize hormone secretions and to treat sexual dysfunctions [4]. These types of saponins are also referred to as adaptogens [21, 22]. Adaptogens normalize the physiological functions of the body, and we believe that they also indirectly increase plasma concentrations of progesterone. But how and where these adaptogens function have yet to be elucidated. In the present study, concentrations of estradiol-17β and progesterone for *P. paniculata*-fed mice were significantly higher than those for control mice. Testosterone can be synthesized from progesterone and used to synthesize estradiol-17β. Therefore, the fact that the plasma concentrations of estradiol-17β and progesterone increased in female mice suggests an increase in the plasma concentration of testosterone in male mice. In humans, saponins can relieve impotency [4], and this could be attributable to increased plasma testosterone levels. This is an indirect effect of saponins on the testis and an indirect effect of adaptogens on testosterone [21]. In the present study, plasma concentrations of testosterone for *P. paniculata*-fed mice were significantly higher than those for control mice. Oral intake of *P. paniculata* therefore significantly increases levels of male and female sex hormones (estradiol-17β, progesterone and testosterone). In addition, powdered *P. paniculata* root can easily be added to feed or water, and 30 days of oral intake induced no adverse reactions in mice, suggesting that *P. paniculata* can be consumed safely for long periods.

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