

Effects of *Lepidium meyenii* Walp and *Jatropha macrantha* on Blood Levels of Estradiol-17 β , Progesterone, Testosterone and the Rate of Embryo Implantation in Mice

Masami OSHIMA^{1,2}), Yeunhwa GU¹) and Sekihito TSUKADA¹)

¹)Graduate School of Medical Imaging, Suzuka University of Medical Science, 1001-1 Kishioka-cho, Suzuka-shi, Mie 510-0293 and

²)Kameyama Veterinary Clinic, 131-1 Tamura-cho, Kameyama-shi, Mie 519-0213, Japan

(Received 19 August 2002/Accepted 4 July 2003)

ABSTRACT. The effects of two Peruvian folk medicines, *Lepidium meyenii* Walp and *Jatropha macrantha*, on mouse sex steroid hormones and embryo implantation were investigated. Progesterone levels increased significantly in mice that received *L. meyenii* Walp, while testosterone levels increased significantly in mice that received *L. meyenii* Walp as well as in those that received both *L. meyenii* Walp and *J. macrantha*. However, there were no marked changes in blood levels of estradiol-17 β or the rate of embryo implantation.

KEY WORDS: *Jatropha macrantha*, *Lepidium meyenii* Walp, mouse.

J. Vet. Med. Sci. 65(10): 1145-1146, 2003

L. meyenii Walp is a root vegetable that only grows in the Peruvian Andes at elevations higher than 4,000 m [2, 4-7, 9-13, 16, 17, 19], while *J. macrantha* is sap collected from trees that grow naturally in the Peruvian Andes [3, 8, 18]. Because they are crude drugs, there are slight differences in components and compositions depending on where they are grown and when they are harvested. Both crude drugs have been widely used as folk medicines in Peru and have been administered to treat impotence, climacteric disorders and infertility in Europe and America [2-13, 16-19]. However, no reports have investigated administration of these natural substances to animals or determined changes in sex steroid hormone levels and rates of embryo implantation. Therefore, we conducted the present study to investigate whether these crude drugs are also effective in animals.

The level of estradiol-17 β and progesterone measured in female mice, and the level of testosterone was measured in male mice. Three week-old ICR mice were divided into the following four groups: Control group, L group (only *L. meyenii* Walp was administered), J group (only *J. macrantha* was administered) and LJ group (both *L. meyenii* Walp and *J. macrantha* were administered). Each group consisted of ten mice, and prior to the study, mice were acclimatized for seven days in the same cage (room temperature: 22 \pm 3°C, humidity: 60%, light: 14 hr, darkness: 10 hr). The crude drugs were administered for 30 days.

Three-week-old mice were used in the present study because although mice are not sexually mature at the age of 3 weeks, by the end of the study, mice were able to breed. The design of the present study also allowed the female mice to have similar estrous cycles, thus minimizing individual differences. Furthermore, a vaginal smear test was conducted on every female mouse, and a blood sample was collected in estrus.

Crude drugs were administered as follows: In the control group, 100 ml of plain water was poured into a 100 ml water bottle; in the L group, 5.0 g of *L. meyenii* Walp powder was

dissolved in 100 ml of water; in the J group, 5.0 g of *J. macrantha* powder was dissolved in 100 ml of water; and in the LJ group, 2.5 g of *L. meyenii* Walp powder and 2.5 g of *J. macrantha* powder were dissolved in 100 ml of water. In all groups, mice had free access to drinking water.

At the end of the administration period, each mouse was anesthetized using diethyl ether, and a blood sample was collected from the heart using a 1 ml heparinized syringe. Blood was then immediately centrifuged, and plasma was stored frozen at -20°C. An RIA measurement kit (Immunotech Inc., France) and a gamma counter (Minaxi γ Auto-Gamma 5000 series, Packard Inc. U.S.A.) were used to determine the levels of estradiol-17 β , progesterone and testosterone. The RIA kit that we used was designed specifically for mice, and its measurement accuracy and sensitivity have been proven. With regard to specificity, cross-reactions with other steroids did not occur in this study. The reason for utilizing the kit was to minimize fluctuations in test results attributable to the measurement technique. ANOVA and Student's *t*-test were used to analyze differences in levels of estradiol-17 β , progesterone and testosterone among the four groups.

The effects of the two crude drugs on the rate of embryo implantation were assessed by dividing the mice in the same manner as described above and sub-dividing them as follows: male administration group (M group); female administration group (F group); and male and female administration group (MF group).

On the last day of administration, one male mouse and one or two female mice were placed in the same cage for copulation, and pregnancy was confirmed by the formation of a vaginal plug. The results of vaginal smear tests showed that all female mice used for copulation were in either proestrus or estrus. At 15 days after copulation, implantation was confirmed by counting the number of corpora lutea and live fetuses.

The results showed that there was no significant differ-

Table 1. Blood levels of progesterone in female mice (ng/ml)

Control group	3.11 ± 0.21
<i>Lepidium meyenii</i> Walp group	*3.93 ± 0.23
<i>Jatropha macrantha</i> group	3.21 ± 0.32
<i>Lepidium meyenii</i> Walp + <i>Jatropha macrantha</i> group	3.45 ± 0.24

Each group included 10 mice. Data represent means ± S.E.M., and were analyzed by ANOVA, followed by Student's *t*-test. Significant differences were observed between the Control group and the *Lepidium meyenii* Walp group (* *p* < 0.05).

Table 2. Blood levels of testosterone in male mice (ng/ml)

Control group	3.39 ± 0.53
<i>Lepidium meyenii</i> Walp group	*4.37 ± 0.68
<i>Jatropha macrantha</i> group	3.35 ± 0.36
<i>Lepidium meyenii</i> Walp + <i>Jatropha macrantha</i> group	*4.92 ± 0.36

Each group includes 10 mice. Data represent means ± S.E.M., and were analyzed by ANOVA, followed by Student's *t*-test. Significant differences were observed between the Control group, the *Lepidium meyenii* Walp group and the *Lepidium meyenii* Walp + *Jatropha macrantha* group (* *p* < 0.05).

ence in blood levels of estradiol-17 β between the control group and the three administration groups. However, there was a significant difference in blood levels of progesterone between the C and L groups (*p* < 0.05) (Table 1). There was also a significant difference in testosterone levels between the C group and the L and LJ groups (*p* < 0.05) (Table 2). There was no significant difference in the rate of implantation between the control group and the three administration groups.

The results demonstrated that the levels of blood progesterone and testosterone were significantly elevated. *L. meyenii* Walp and *J. macrantha* both contain saponins [3, 4, 7, 8, 16–18], which play a very important role in sex hormones. Saponins have been shown to normalize hormone secretion and have been used to treat sexual dysfunction [1]. Due to these actions, saponins are called adaptogens. Adaptogens maintain the body's physiological functions [14, 15]. We believe that the levels of progesterone and testosterone increased in this study because of indirect action of the adaptogens. However, the mechanism of action for adaptogens has not been clarified [14]. A comparison between the LJ and L groups revealed that progesterone levels in the female mice decreased when *J. macrantha* was coadministered. The reason for this could be that *J. macrantha* also contains steroids [3, 8, 18], and these steroids would have lowered adrenal functions [3, 8], consequently suppressing progesterone secretion. When compared to the C group, blood levels of testosterone were significantly higher for the L and LJ groups. In addition to the above-mentioned indirect actions of adaptogens, increased blood testosterone could have been attributable to synergistic effects involving other *L. meyenii* Walp components, such as arginine, lead and vitamin E [2, 4, 7, 9]. Testosterone can also be biosynthesized from progesterone, and the increased progesterone levels are thought to have been the result of the elevated tes-

tosterone levels.

In humans, *L. meyenii* Walp and *J. macrantha* are administered to treat infertility and climacteric symptoms caused by reduced blood estradiol-17 β . Because they increase levels of blood estradiol-17 β , directly affects the ovary, nutrients and chemicals contained in these crude drugs [2, 4, 7, 9] appear to be responsible for improving infertility and climacteric symptoms.

The results of the present study show that although *L. meyenii* Walp and *J. macrantha* increased blood levels of progesterone in female mice and blood levels of testosterone in male mice, they did not directly affect levels of blood estradiol-17 β or the rate of embryo implantation in female mice.

ACKNOWLEDGEMENT. The authors wish to thank Hiroyuki Baba, President of Coperunix Japan Inc. for providing us with *L. meyenii* Walp and *J. macrantha*.

REFERENCES

- Arletti, R., Benelli, A., Cavazzuti, E., Scarpetta, G. and Bertolini, A. 1999. *Psychopharmacology* **143**: 15–19.
- Balick, M. J. and Lee, R. 2002. *Altern. Ther. Health. Med.* **8**: 96–98.
- Calixto, J. B. and Sant'ana, A. E. 1990. *Gen. Pharmacol.* **21**: 117–122.
- Canales, M., Aguilar, J., Prada, A., Marcelo, A., Huaman, C. and Carbajal, L. 2000. *Arch. Latinoam. Nutr.* **50**: 126–33 (in Spanish with English summary).
- Cicero, A. F. G., Bandieri, E. and Arletti, R. 2001. *J. Ethnopharmacol.* **75**: 225–229.
- Cicero, A. F. G., Piacente, S., Plaza, A., Sala, E., Arletti, R. and Pizza, C. 2002. *Andrologia* **34**: 177–179.
- Dini, A., Migliuolo, G., Rastrelli, L., Saturnino, P. and Schettino, O. 1994. *Food Chem.* **49**: 347–349.
- Duarte, D. F. P., Sant'ana, A. E. G. and Calixto, J. B. 1992. *Eur. J. Pharmacol.* **215**: 75–81.
- Ganzera, M., Zhao, J., Muhammad, I. and Khan, I. A. 2002. *Chem. Pharm. Bull.* **50**: 988–991.
- Gonzales, G. F., Cordova, A., Gonzales, C., Chung, A., Vega, K. and Villena, A. 2001. *Asian. J. Androl.* **3**: 301–303.
- Gonzales, G. F., Cordova, A., Vega, K., Chung, A., Villena, A. and Gomez, C. 2003. *J. Endocrinol.* **176**: 163–168.
- Gonzales, G. F., Cordova, A., Vega, K., Chung, A., Villena, A., Gomez, C. and Castillo, S. 2002. *Andrologia* **34**: 367–372.
- Gonzales, G. F., Ruiz, A., Gonzales, C., Villegas, L. and Cordova, A. 2001. *Asian. J. Androl.* **3**: 231–233.
- Kropotov, A. V., Lisakovskaia, O. V. and Khotimchenko, Iu. S. 2001. *Eksp. Klin. Farmakol.* **64**: 60–62 (in Russian with English summary).
- Kelly, G.S. 2001. *Altern. Med. Rev.* **6**: 293–302.
- Muhammad, I., Zhao, J., Dunbar, D.C. and Khan, I. A. 2002. *Phytochemistry* **59**: 105–110.
- Piacente, S., Carbone, V., Plaza, A., Zampelli, A. and Pizza, C. 2002. *J. Agric. Food Chem.* **50**: 5621–5625.
- Silva, A. M., Brum, R. L. and Calixto, J. B. 1995. *Life Sci.* **57**: 863–871.
- Zheng, B. L., He, K., Kim, C. H., Rogers, L., Shao, Y., Huang, Z. Y., Lu, Y., Yan, S. J., Qienb, L.C. and Zheng, Q. Y. 2000. *Urology* **55**: 598–602.