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**Estrogen replacement effect of Brazilian propolis in ovariectomized rats**

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**Purpose:** Many post-menopausal women experience some symptoms such as hot-flush, insomnia and osteoporosis, because of the decreased production of estrogens. To relief such symptoms, natural and synthetic estrogens have been subscribed for patients as the hormone replacement therapy. However, many epidemiological studies have revealed long-term treatment of estrogens increased the risk of several cancers. Therefore, alternative estrogens, which are estrogenic but no carcinogenic, are needed. One candidate of them would be phytoestrogens, some of which are reported to reducing breast cancer risk. Propolis, a natural product derived by honey bees from plants, is a mixture of several hundred-kinds of chemicals including phytoestrogens. In this study, we determined estrogenic activity of propolis using several in vitro and in vivo assays.

**Methods:** Ethanolic extract of Brazilian propolis (EEP) was used for assays directly or after fractionation using HPLC. Estrogenic activity was determined using following assays, 1) estrogen receptor (ER) competitive binding assay, 2) estrogen-dependent luciferase reporter gene assay, and 3) epithelial cell proliferation of mammary gland and uterus in ovariectomized (OVX) rats.

**Results and discussion:** EEP induced epithelial cell proliferation in mammary gland and uterus in OVX rats. This effect was diminished by pretreatment of ICI182,780 (ICI). EEP induced ER binding and reporter gene expression in in vitro assays, and five fractions of EEP isolated by preparative HPLC showed unequivocal ER binding. One fraction contained kempferol and an unknown estrogenic compound. These results suggest the daily intake of EEP would relief post-menopausal symptoms.

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**Comparison of content of toxic components in Keishikabushito decocted by microwave oven and conventional method**

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**Objective:** We aim at developing a new method to decoct traditional medicine with a microwave oven for patients’ convenience. We previously investigated the effective components in Kakkonto to confirm the effectiveness of this new decoction method. In this study, we investigated toxic components in Keishikabushito to confirm the safety of this method.

**Method:** According to preliminary experiments, we decocted a daily dose of Keishikabushito containing 1 g of processed Aconite root (Bushi) by a microwave oven in 600mL water at 500 W for 30 min and conventional decoction device in 500mL water at 600 W for 40 min, respectively. Then, the contents of toxic components from Bushi (aconitine, mesaconitine, hypaconitine), and three other effective as well as toxic components (benzoylaconine, benzoylmesaconine, benzoylhypaconine), the hydrolysates of the three toxic components in heating process, were analyzed with HPLC method.

**Result:** In both decoctions obtained by the new and conventional methods, aconitine, mesaconitine and hypaconitine with strong toxicity couldn’t be observed because of hydrolysis in heating process. Benzoylaconine, also not observed, may be further hydrolyzed. The contents of benzoylmesaconine and benzoylhypaconine were 0.68±0.06 mg, 4.75±0.15 mg in decoction decocted by a microwave oven, and 0.70±0.01 mg, 4.89±0.12 mg in decoction decocted by the conventional method (n=5).

**Conclusion:** There was no significant difference of the contents of toxic components between the new and conventional methods. Decocting Keishikabushito with a microwave oven is as safe as the conventional method. In addition, the decoction time can be saved. This new decoction method may be applied widely.