The Phytoestrogenic Potential of Yam Bean (Pachyrhizus erosus) on Ovarian and Uterine Tissue Structure of Premenopausal Mice

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Abstract

The use of estrogen hormone by public has significantly been improved either as prevention or treatment of disease. Menopausal issues in women are often treated using hormone replacement therapy. In regard to this, yam bean is found to contain genistein and daidzein compounds with a chemical structure that resembles estrogen hormone, therefore yam bean is categorized in the phytoestrogen group. The purpose of this study was to identify the potential of yam bean on ovarian and uterine histology of mice. This research employed a Completely Randomized Design of experimental research approach of one factor namely yam bean in three different dosage treatment: 0.3 g/kg, 0.6 g/kg, and 0.9 g/kg of yam bean for 24 days. The surgery and organ harvesting of ovary and uterus were conducted on Day 25 along with the making of histological preparat using paraffin method and Hematoxylin-eosin (HE) staining. The data was then analyzed descriptively. This research found that there were both secondary and tertiary follicle proliferation as the antrum contains some estrogen level. Meanwhile, the endometrial tissue of the uterus experienced uterine glandular proliferation. To conclude, yam bean was found to be a natural estrogen source.

Keywords: yam bean, isoflavone, phytoestrogen, ovarium, uterus, menopause

Introduction

Hormone replacement therapy (HRT) is an action which is conducted through giving estrogen hormone especially to women who experience some reduction in the estrogen hormone. HRT is usually undergone by women experiencing menopause. In particular, Estrogen Replacement Therapy (ERT) can also be used for conducting cardiovascular disease therapy (Stampfer et al., 1991 and Sourander et al., 1998), reducing the risk of osteoporosis (Grady et al., 1992 and Nurochmad et al., 2010), and reducing the symptoms of menopause (Barrett, 1998). The use of synthetic estrogen compound in a long term often gives negative impacts so that a natural alternative is needed to replace estrogen hormone.

Yam bean plant (Pachyrhizus erosus) as a tuber-legume crop, is categorised as a legume with the dissemination area of Sumatera, Java, Bali, Sulawesi and East Nusa Tenggara (Karuniawan & Wicaksana, 2006). People usually prefer to consume fresh yam bean either in the form of salad or rujak (Indonesian traditional fruit an vegetable salad dish). Yam bean extract is used in the cosmetics industry in whitening, compact powder, and moisturizer products. Yam bean contains isoflavone compounds with estrogen-like chemical structure (Wanibuchi, 2003; Abid, 2005; and Lukitaningsih, 2009). The chemical isoflavones structure resembles 17β-estradiol and has the efficacy like estrogen hormone (Delmonte and Rader, 2006; Barlow et al., 2007) so that yam bean is also included in phytoestrogen group (Urasopon et al., 2008). The largest components of isoflavone are genistein and daidzein which are often found in Fabaceae family, including Pachyrhizus erosus (Kang et al., 2006). Pachyrhizus erosus at least contain isoflavones such as, daidzein and genistein (Primiani, 2013), the analysis of HPLC daidzein and genistein of yam bean is 110,454 mg/100 g and 165,530 mg/100 g respectively.

The chemical structure of genistein and daidzein can bind to estrogen receptor and compete with endogenous estrogen such that they may provide both estrogenic and anti-estrogenic effects (Adlercreutz, 1990; Griffiths et al., 1996; Adlercreutz and Mazur, 1997). Utilization of yam bean as phytoestrogen is not conducted much, a study by Nurochmad et al., (2010) showed that providing yam bean extract of 400 mg/kg and 800 mg/kg dosage for 4 weeks to mice through ovariectomy could prevent bone fragility; phytoestrogen was then proven to be able to improve uterine mass (Ford et al., 2006). Meanwhile, Genistein affects on the increasing of weight of uterus by improving uterine mass (Ford et al., 2006). Meanwhile, Genistein affects on the increasing of weight of uterus by stimulating uterine endometrial thickening (Santell, 1997). Genistein dose 26,6 mg/day equivalent with human dose 0,625 mg/day within 6 months on monkey cause vaginal maturation (Marquez et al., 2012).

Research Methods

Research Design

The research was conducted using experimental research approach with a completely randomized design, the treatment was completed through providing grated yam bean in three different dosage: 0.3 g/kg, 0.6 g/kg, 0.9 g/kg. The observation is focused on the changes of ovarian and uterine tissue structure.
Tools and Materials

The research tools used in this study were: a gavage tube, plastic mice cages (50 x 30 x 20cm size), drinking bottles for mice, a digital scale (HM-200 brand), 1ml syringes with 3ml disposable needles (G23), an incubator, optical microscope, digital optilab camera microscope, a triple beam balance ohaus 700 series scale, analytical balance type HM-200 with the capacity of 210 grams and accuracy level of 0.1 mg, PR-50 microtome, surgical equipment, a surgical board, object glasses and lenses, glass beakers, spiritus lamp, a cube made of calendar paper, blender, flour sifter, a nife, a pipette, and a couple.

The research materials were yam bean obtained from Takeran village Madiun Indonesia, ovarian and uterine tissue, milk A granule for the mice food produced by PT. Charoen Pokphand Indonesia, husk, cotton, tissue paper, aqua destillata, water tap, paraffin, 0.9% physiological saline, bouin fixative solution, 50%, 70%, 90% and absolute alcohol, pure xylol, xylol-alcohol mixture, with xylol and alcohol comparison consecutively 1:3, 2:2, and 3:1, Li2CO3 solution, 1% HCl, 3% formalin, and haupt adhesive.

Animal Experiments

The experimental animals used were mice (Mus musculus) strain Balb/c female, in a good health condition, aged 12 months, 24 mice in total. Every mouse had an initial body weight ranging from 20-25 grams before treatment, the mice were kept in a cage located in the Biology Education IKIP PGRI MADIUN, Indonesia

Maintenance of mice and manufacture of test materials

The mice were placed in the mice cages, given food and drink ad libitum and acclimatized for 14 days prior to induction treatment. The mice were maintained at the room temperature of (± 270C), relative humidity between 50-60% and 12 hour lighting cycle. Every day the mice were weighed as the basis for determining the provision of grated yam bean. The manufacture of grated yam bean as a research material was conducted through grating the yam bean using a grater. The amount of the yam bean was then weighed and complied with the amount given to the mice.

The provision of yam bean and manufacture of preparat of ovarian and uterine histology

The provision of yam bean was conducted through direct induction to the stomach using a gavage tube once a day for 24 days. The mice were then dislocated on the 25th day and dissected. The organ harvesting of their ovaries and uterus were then performed. The manufacture of the preparat of ovarian and uterine histology was completed using paraffin method in attempt to determine any ovarian and uterine tissue structure changes.

Data analysis

The ovarian and uterine tissue structure changes were analyzed descriptively based on the changes that happened in the ovarian follicle, myometrium layer, endometrium and uterine mucosa.

Results and Discussions

The results of observation on the ovarian tissue showed that there were changes in the ovarian follicles (Figure 1).
The results of observation on the uterine tissue showed that there were changes in the uterus endometrium tissue (Figure 2).
which may also occupy estrogen receptors and lead to estrogen-like effects such as endogenous itself (Harrison et al., 1999). Myometrium may develop due to the phytoestrogens yam bean effect.

Genistein and daidzein in yam bean have an effect on uterine epithelium resulting in proliferation and cornification of epithelial cells as well as in the optimization of the secretion of estrogen. Decreased estrogen level in premenopausal and postmenopausal period, resulting in an excess amount of estrogen receptors that are not bound. Genistein and daidzein ability to bind to estrogen receptors called sex hormone binding globulin (HGB) serves to increase the production of steroid hormones and is responsible for binding estrogen and circulating it through the blood vessels (Setchell et al., 1998). Genistein as one of the isoflavone compounds has the effect of increasing the weight of the uterus by stimulating uterine endometrial thickening (Santell et al., 1997).

Decreased levels of endogenous estrogen in premenopausal period can cause the estrus phase to not occur, thus giving yam bean to premenopausal mice can stimulate the estrus phase. Genistein and daidzein as a group of phytoestrogens bind to estrogen receptors in the ovaries and uterus. The resulting response when estrogen levels are low is that the receptor and phytoestrogens binding will help in balancing estrogen levels. The function of estrogen in relation to reproduction is causing proliferation to occur and tissue in the reproductive organs to grow.

The use of natural materials as hormone replacement therapy in postmenopausal women is one of the alternatives that can be done to overcome the side effects. Component compounds contained in natural materials are very complex and often interact each other to provide a physiological effect (Ioannides, 2002 and Zhou et al., 2003). Multi component contained in herbal medicine is a compounds complexity that provides optimal effect (Lan and Jia, 2010).

Conclusions

Yam bean can be used as natural estrogen hormone source which can increase the proliferation and maturation of ovarian follicles and proliferation of uterine endometrial glands of the uterus in premenopausal period. Following up the need for estrogen therapy for premenopausal and postmenopausal women, it is necessary to study the effectiveness and safety ranging from experimentin on animals to humans, so its use as hormone therapy can be trustworthy.

References

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