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Submission date: 01-Nov-2019 08:51AM (UTC+0700)
Submission ID: 1204665387
File name: 9._BERKALA_HAYATI_CICILIA.pdf (558.79K)
Word count: 3331
Character count: 17751

THE DYNAMICS OF DAIDZEIN CHEMICAL COMPOUND FROM YAM TUBER (*Pachyrhizus erosus*) TUBER AND ITS POTENTIAL IN MYOMETRIUM

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ABSTRACT

Daidzein compounds dynamic and the effect of yam tuber (Pachyrhizus erosus (L)) Urb on myometrium rats model of estrogenic were investigated. Twentyfour of 5 months-old female Sprague Dawley rats were randomly assigned to three groups as followed, control, Daidzein administraton and yam tuber juice administration for 24 days. The administration of yam tuber juice were given by orally using a stomach with gavage tube in the morning. Every 8 h, the rats blood and urine were collected from tail until 24 h. The treatment were continued until 24 days. The result showed that the level of daidzein in blood and urine of rats that administratered by Yam tuber juice is lower than pure daidzein treatment rats every 8 until 24 hours of collection. The results demonstrated that the administration of yam tuber juice significantly myometrium proliferated in rats. It is assumed that genistein and quercetin is estrogenic as found in daidzein so that the miometrium is potentially more effecctive. This study suggest that phytoestrogen compound from Pachyrhizus erosus may offer a potential as natural estrogenic in women.

Keywords: *compounds dynamic, daidzein, myometrium, Pachyrhizus erosus*

INTRODUCTION

The diversity of plants has the potential to be developed as medicinal plants, one of which is the yam tuber (*Pachyrhizus erosus*). The usefulness of yam tuber as a medicinal plant has not been widely used, the people often use it as a salad and pickles, as well as the flour is used as a facial mask and skin whitening. The effication of natural ingredients as drugs are often analyzed by isolation of active compounds and its mechanisms in the body. The discussion on the concept of a single compound that considered active, as well as its potential in organs is an effective conclusion.

Based on HPLC analysis of yam tuber contain daidzein compound 110,454 mg/100 g (Tabel 1), as one of Phytoestrogen compound because the structure similar to 17 β-estradiol and the activity similar to estrogen (Murphy, 1982; Price and Fenwick, 1985; Wang and Murphy, 1994; Setchell, 1998; Bayer et al., 2001; Kim et al., 2008; Orhan et al., 2011; Gaete et al., 2012). The Daidzein has ability to bind with esterogen receptor (Setchell and Cassidy, 1999; Bayer et al., 2001; Burow et al., 2001; Hwang et al., 2006; Nynca et al., 2009; Gaete et al., 2012) in some of part body such as uterus, ovary, mammary gland, bone, hipotalamus, pituitary gland, leydig cell, prostate, and epididymis (Kim and Park, 2012).

The utilization of yam tuber as phytoesterogens agent has not been done, some of yam tuber studies conducted as preclinical trials. The adinistration of yam tuber with dose 400 mg/kg and 800 mg/kg for 4 weeks at ovariectomy rats model can prevent the fragility of bones Nurrochmad et al., (2010). The phytoesterogen has ability to increase the uterus mass (Ford et al., 2006). The administration of yam tuber with dose 0,3 g/kg, 0,6 g/kg, and 0,9 g/kg female premanopause mice model can promote the proliferation of the uterine endometrium, uterine gland, and maturation of follicle in the ovaries (Primiani, 2013[b]).

The studies about the metabolism of yam tuber have not been done, the possibility of daidzein were absorbed and further metabolized by intestinal microflora, excreted through urine and feces (Pottenger et al., 2000 dan Kim et al., 2008). The daidzein is converted into the structure *cis* dihydrodaidzein and *trans* tetrahydrodaidzein, O-desmethylangolensin (ODMA), dehydroequol, and equol by the intestinal microflora after consumption (Kelly et al., 1995; Wang et al., 2005; Kim et

al., 2008; Shimada et al., 2010). The present syudy was undertaken to investigate the effects and action of *Pachyrhizus erosus* (L) Urb tuber on serum, urin, and uterine tissue in this estrogenic rats model.

MATERALS AND METHODS

Animals

Female Sprague-Dawley rats, 5 months age, 150-200 g weight, were purchased from Animal Laboratory Center Unit Gadjah Mada University, Yogyakarta, Indonesia, were housed individually in polyacrylic cages and maintained under standard laboratory conditions at 25° C with 12-h light; 12-h dark cycles and allowed free acces to commercial pelled diet (PT Charon Phokpan, Indonesia) and water ad libitum. A ventilation system was provided to ensure the airflow in the room.

Plant and Chemical Meterials

The yam tuber (*Pachyrhizus erosus* (L) Urb) that used in this study study were collected from Takeran village Madiun, Indonesia and authenticated at the Laboratory of Chemistry Faculty of Science, Muhammadiyah University, Malang, Indonesia. Daidzein standard for HPLC analysis were purchased from Sigma (St. Louis, MO, USA).

Preparation of *Pachyrhizus erosus* and Daidzein Dosage

1,5 ml of yam tuber juice were analyzed with HPLC method to determine the dose of daidzein that will given to rats.

Administration Procedure

Rats were acclimatized to laboratory condition for 1 week before treatment, rats were housed individually in metabolic cages. Every 8 h, the blood and urine of rats were collected from tail, then moved in polyacrylic cages for 23 days. The experimental protocol were approved by Animal Ethical Committee, all procedures described conducted in accordance with Guideline for Care and Use of Animals Laboratory Biosains Brawijaya University. A total of 24 rats were assigned randomly into 3 groups of 8 animals each. The induction treatment consist of three categories, i,e., the initial control (K), Daidzein (D), and Yam tuber juice 1,5 ml (B). The induction treatment were administered direct into the stomach with gavage tube in the morning, when the stomach is still empty. At the end of 24 days of experimental period, all rats were sacrificed and uterus were taken for histopathology analysis.

Preparation Serum and Urin Analysis by HPLC

Blood samples were centrifugated at 3000 rpm for 10 min to obtain serum after standing for 30 min at room temperature. The blood sample were collected as much as 50 µl, the urine sample were collected as much as 0,5 ml, then were placed into erlenmeyer. Each sample were added 10 ml acetonitrile, 2 ml HCl 0,1 M and 5 ml aquades, then were homogenated by using stirer for 2 hour at room temperature. The solution were filtered by using filter paper and were collected the filtrat. The filtrat were evaporated by using rotary evaporator at 30° C. The residu were dissolved with 10 ml metanol grade HPLC 80% in the water and then were filtered with polytetrafluoroethylene 0,45 µm filter for HPLC analysis colum C18, mobile phase solution with 0.1% glacial acetic acid in water and 0.1% glacial acetic acid in acetonitrile, 20 ml sample were injected. The flow rate solution 1ml minutes, photodiode τ 255-300 nm detector.

Preparation of Histological Specimen

Paraffin sections of uterus were prepared following the standard procedure (Cui et al., 2009). Uterus were then fixed in 10% neutral buffered formalin. After 24 h uterus were removed from formalin and stored in 70% ethanol, followed by embedding in parafin, sectioning at 5

microns, and staining with hematoxylin and eosin (HE). Histopathological changes were observed under conventional optical microscope (OLYMPUS BX 41, Japan) and dyno eye camera. For histopathologic assessment, transverse sections were taken through myometrium.

Statistical Analysis

The differences of myometrium tissue structure were analyzed by One-way analysis of variance (ANOVA), followed by LSD post-hoc tes analysis was used to pairwise compare differences between different groups using SPSS 16 for windows. P-values < 0.05 were considered significant.

RESULTS

The Concentration of Daidzein in Blood and Urine

The daidzein concentration in blood and urine at yam tuber juice treatment (B) is lower than daidzein treatment (D) at each 8 h collected for 24 h (Figure 1).

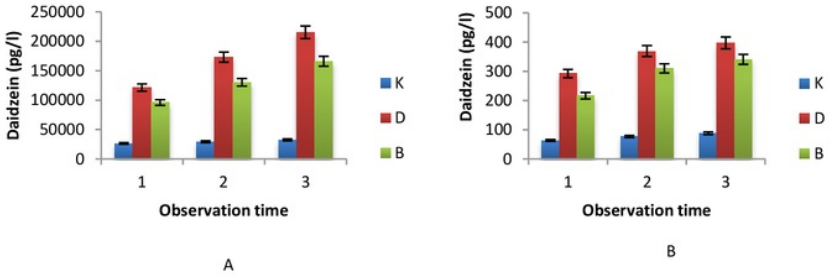

Figure 1. HPLC showing daidzein concentration of (A) serum and (B) urin
K=Control; D=Daidzein treatment; B=Bengkuang tuber treatment

Phytoestrogen of Yam Tuber

The analysis of HPLC daidzein and genistein of yam tuber is 110,454 mg/100 g and 165,530 mg/100 g (Table 1) respectively.

Tabel 1. Analisis HPLC Yam Tuber

Compound	Sample Weight(g)	RT (min)	Sample curve area	Standard curve area	Standard concentration	Result (mg/100)
Daidzein	37,553	18,29	27.082	163,228	50,000	110,454
Genistein	37,553	22,61	40,586	163,228	50,000	165,530

Histopathological Observations of Myometrium Uterus

Rats from control had normal tissue morphologies. We used histological paraffin from control to explain results. The myometrium tissue undergoig proliferation and increase in width of myometrium in treatment yam tuber (Figure 2).

A B C

Figure 2. Myometrium change; H&E-stained sections; 100x. (A) Normal structure myometrium (K); (B) Proliferation myometrium (D); (C) Proliferation myometrium and glandula uterina (B).

The yam tuber juice treatment (B) showed that there are significant different at myometrium tissue structure compared with control (K) and pure daidzein treatment (D) (Tabel 2).

Tabel 2. Effect of *Pachyrhizus erosus* on myometrium

	Sum of Squares	*df*	*Mean Square*	*F*	*Sig.*
Between Groups	4530.308	2	2265.154	6.384	.007
Within Groups	7451.402	21	354.829		
Total	11981.710	23			

sig (0.007) < 0.05

The myometrium at rat with yam tuber juice treatment is wider than control and pure daidzein treatment.

DISCUSSION

Based on the HPLC analysis show that the level of blood daidzein rats that administratered by yam tuber is lower than the blood rats that administratered by daidzein in the fraction of 8 h. Similary, the levels of daidzein rats that administratered by yam tuber is lower than the urine rats that administratered by daidzein in the fraction of 8 h (Figure 1). Though the potential of the yam tuber juice in the rats myometrium is higher than pure daidzein (Figure 2 and Table 2).

Yam tuber juice treatment is the treatment that is not only using daidzein but also other components, such as natural components. Based on the analysis of HPLC and GC-MS, yam bean contains genistein (Table 1) dan Quercetin (not reported in the present study). Their structures are similar to 17 β-estradiol (Figure 3) and their activities are like estrogen. Some compounds that are similar to daidzein becoming competitors, are considered as genistein and quercetin. Furthermore, daidzein has isomer structure (enantiomer) *cis*-tetrahydrodaidzein and *trans*-tetrahydrodaidzein which have chiral structure (Shimada *et al*., 2010). Since there was competitor compounds, the degree of daidzein in the rat's blood with the yam tuber juice treatment is lower than the degree of daidzein in the rat's blood with pure daidzein treatment. Thus, there is still needed more investigation to make sure an assumption about the degree of genistein, quercetin, dan enansiomer daidzein in the blood.

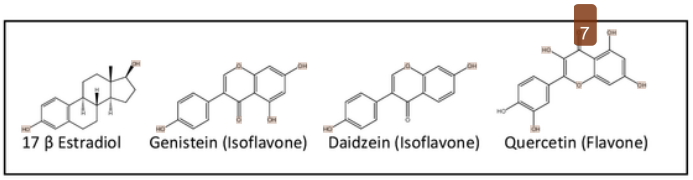

Figure 3. The Structure of 17β-Estradiol, Genistein, Daidzein, and Quercetin

Pachyrhizus erosus at least contain isoflavones such as, daidzein and genistein (Primiani, 2013[a]). Daidzein in *Pachyrhizus erosus* form in cojugate or glycoside which are degraded by gut microflora, and undergo biotransformation. Daidzein in the whole body turned into *cis* dihydrodaidzein (DHD) and *trans* tetrahydrodaidzein (THD), 2,3 dehydroequol and equol by intestinal microflora (Kelly et al., 1995 and Wang et al., 2005). Allegedly, the equol have estero[illegible]ic properties and become O-desmetylangolensin (ODMA) or dihydrodaidzein (Xu et al., 1994; Chang and Nair, 1995; Joannou et al., 1995; Rafii, 1999; Hur et al., 2000; Rafii et al., 2007). The equol as chiral structure cotained in two enantiomeric forms that is R-equol and S-equol that potential to provide biologicalactivity (Wang et al., 2005; Ishimi, 2010; Zheng et al., 2012).

Phytoesterogen as a component of the plant that have ability to bind to estrogen receptors, so that cause the biological activity in the target cell. The yam tuber juice treatment affected in the myometrium proliferation (Figure 2), although the blood levels lower than pure daidzein treatment. It is provide that pure daidzein had no effect as well as yam tuber treatment. The use of daidzein compound is estrogen delivery technique with a single compound concept, but the administration of the yam tuber juice is a technique of giving substance to multi component concept.

Yam tuber juice treatment in premenopause caused endometrium proliferation, myometrium and endometrium gland proliferation. (Primiani, 2013[b]). The administration of yam tuber with dose 0,3, 0,6, dan 0,9 g/kg cause ovary follicle proliferation and endometrium gland proliferation in premenopause mice (Primiani, 2013[a]). Other study reported that genistein with dose 26,6 mg/day eqiuvalen with human dose 0,625 mg/day within 6 months on monkey cause vaginal maturation (Marquez et al., 2012). Daidzein from yam tuber have similar activity with estrogen, that is were absorpted in the blood although that level are low and can be excreted through the urine.

In summary we have demonstrated that yam tuber (*Pachyrhizus erosus*) may offer a potential as natural estrogenic in women. The mechanism of phytoestrogen compounds of *Pachyrhizus erosus* on serum, urin, and uterus more than sinthetis compound. Further studies are needed to investigate the toxisity and efficacy of that phytoestrogen in human.

ACKNOWLEDGEMENTS

This study supported by DIKTI KEMENDIKNAS 2014. We thank you for all facilities of Biosains Laboratory Department of Biology, Brawijaya University. Thanks to Arisandy (Chemistry Department, Muhammadiyah Malang University) acknowledged for helpful analysis.

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