Local Leguminoceae Profile Of Pea Pigeon (*Cajanus Cajan*) In Indonesia And Its Potential On The Bone Tissue Structure Of Female Rats

ABSTRACT

Aims: The aim of the research was to analyze isoflavone levels and the estrogenic potential of pigeon pie (*Cajanus cajan*) on bone tissue structure preclinically.
Study Design: Analysis of genistein and daidzein pigeon pie powder using the HPLC method. Preclinical tests were carried out using rat. 24 female white Sprague Dawley rats aged 8-9 months body weight at the start of treatment 150-180 grams. Rats were divided into 3 treatment groups. Control (P_0), treatment given a solution of pigeon pie seed powder 24 grams : 24 ml of distilled water (P_1), and treatment given a solution of pigeon pie seed powder 8 grams : 24 ml of distilled water. The solution administration treatment was for 36 days. On the 37th day, surgery was performed and blood and bones were taken. Blood 17 β estradiol analysis using HPLC method. HE staining bone micro technique.
Result: Genistein and daidzein pigeon pie were 247.89828 µg/g and 188.61309 µg/g respectively. 17β-estradiol P_0 level 237.231 pg/ml; P_1 603.926 pg/ml P_2 486.153 pg/ml. Increased proliferation of osteoblasts and fibroblasts in bone tissue structures.
Conclusion: Genistein and daidzein are isoflavone compounds found in pigeon pie seeds. Genistein and daidzein can increase 17β-estradiol levels. Pigeon pie seeds have the potential to act as natural estrogen.

Keywords: Bone tissue, pea pigeon, rats

1. INTRODUCTION

Osteoporosis in women is a factor caused by decreased estrogen levels, this results in a decrease in bone mineral density (BMD) **(Levin et al., 2018; Gosset, et al., 2021).** Estrogen deficiency causes rapid bone loss within 2-3 years post menopause **(Gosset et al., 2021).** The use of hormone replacement therapy (HRT) is a choice for many menopausal women to overcome this **(Gambacciani & Levancini, 2014; Eastell et al., 2019; Rozenberg et al., 2020; Gosset et al., 2021).**

Continuous use of hormone replacement therapy (HRT) for a long time can pose health risks, making the condition of osteoporosis increasingly complex. Therefore, there is a need for preventive measures and good practices in dealing with osteoporosis. Efforts to prevent osteoporosis can be made by administering natural ingredients containing estrogen **(QIN, et al., 2005; Martiniakova et al., 2020; Karimi et al., 2024).** The use of natural ingredients is a safer and more effective preventative measure than treatment using synthetic ingredients **(Pohl & Kong Thoo Lin, 2018; Scott et al., 2020; Karimi et al., 2024).**

The diversity of local Leguminoceae plants in Indonesia is a wealth of biological natural resources that have not been utilized optimally for health. Leguminoceae are plants that are grouped as phytoestrogens, because they contain high levels of isoflavone components **(Yoo et al., 2005; Nikolić et al., 2017; Das et al., 2020; Whitten et al., 2020).** Isoflavones as a compound with a chemical structure similar to 17 β estradiol can bind to estrogen receptors **(Vitale et al., 2013; Křížová et al., 2019; Fuentes & Silveyra, 2019).** The largest components of isoflavones are daidzein, genistein, glycitein, and coumestrol which are thought to be found in Leguminoceae which are distributed in plants and their products **(Nikolić et al., 2917; Whitten et al., 2020; Sun et al., 2022).**

The chemical structure of isoflavones is similar to 17 β estradiol, so its physiological properties resemble the hormone estrogen. Based on its physiological properties, isoflavones have been widely developed in the food and health sectors. Isoflavone can be used for prevention and treatment, as Hormone Replacement Therapy (HRT) **(Larkin et al., 2008; Pabich, & Materska, 2019).** Isoflavones can be used for the treatment of bone fragility, prevention of osteoporosis in postmenopausal women, therapy and prevention of reduction in bone density in postmenopausal women **(Perna et al., 2016; Lambert et al., 2017; Akhlaghi et al., 2019; Sansai et al., 2020).**

Pigeon pie (*Cajanus cajan*), one of Indonesia's local Leguminoceae, grows abundantly in the lowlands, at an altitude of around 1,800 – 2,000 m. Pigeon pie nutrition 22.6 g/100 g, with glutamic acid, aspartic acid and lysine **(Putra et al., 2021).** The abundance of pigeon peas has not been explored much, the health benefits of pigeon peas have not been explored.

Therefore, pigeion pie is suspected to contain isoflavone compounds, it is necessary to carry out an in-depth analysis, so that pigeon peas, a local bean in Indonesia, can be developed as a natural estrogenic ingredient, especially for preventing osteoporosis. The aim of this study was to analyze isoflavone levels and the estrogenic potential of pigeon pie (*Cajanus cajan*) in bone tissue structure preclinically. It is hoped that the research results will show that pigeon pie can be developed to prevent osteoporosis.

2. MATERIALS AND METHODS

Dried black pigeon pie seeds were obtained from pigeon pie plantations in Ponorogo Regency, East Java, Indonesia. Identification based on Number 0269/Taxo-Plant/Biology/XI/2023

2.1 Making pigeon pie seed powder

500 grams of pigeon pie seeds were dried in an incubator at 60^{o}C for 3 days, then ground using a blender, then ground using a 40 mesh sieve. The fine dry powder of pigeon pie was weighed by total weight.

2.2 Making pigeon pie seed extract

100 grams of pigeon pie powder was dissolved in 96% ethanol, the solution was homogenized with a vortex for 10 minutes. The solution was left for 30 minutes. Then filtered using Whatman filter paper no. 42, the filtrate was taken. The filtrate that was obtained was then centrifuged at a speed of 8000 rpm for 20 minutes, then 1 ml of the supernatant was taken and then dissolved in 10 ml of distilled water.

2.3 Analysis of genistein and daidzein from pigeon pie seed extract using HPLC method

Pigeon pie seed powder extract was purified through solid phase extraction using HPLC Shimadzu C18 Sep-Pak, system controller: SCL 10 AVP; solvent delivering unit LC 20 AT; column oven CTO 10 ASVP; SPD 20A UV-Vis detector; column temperature: 25oC; mobile phase: acetonitrile 20% in acetic acid 3%; mobile phase method: isocratic; flow rate: 0.8 ml/min; injection volume: 20 µl detector wavelength: 261 nm; run time: 60 minutes.

2.4 Serum 17β-estradiol analysis HPLC method

On the 37th day, blood was drawn. The 17β-estradiol analysis procedure was according to **Gatti et al., (1998)** and **Yamada et al., (2002).**

Grouping of experimental animals

Rats were divided into three treatment groups. The first group was a control (P_0), the second group (P_1) was given a solution of pigeon pie seed powder in the ratio of seed powder: distilled water (24 grams of powder:24 ml of distilled water) and the third group (P_2) was given a solution of pigeon pie seed powder in the ratio of seed powder:distilled water (8 grams of powder:24 ml of distilled water).

Treatment of experimental animals

24 female white Sprague Dawley rats aged 8-9 months were obtained from the Animal Breeding Unit Blitar, East Java, body weight at the start of treatment 150-180 grams, kept in group cages in the Biosciences experimental animal laboratory at Brawijaya University. Rats were kept in group cages at room temperature (± 27^o C), humidity 50-60%, 12 hour light cycle, daily feeding before treatment at 07.00 am, milk pellet composition A with a composition consisting of 12% water, 16% crude protein , crude fat 3-7%, crude fiber 8%, ash 10%, calcium 0.9%-1.2%, phosphorus 0.6%-1% with raw materials yellow corn, wheat bran, SBM, molasses, palm olein, essential amino acids, essential minerals, premixes, and vitamins. Providing water ad libitum. Husk replacement and cage maintenance are carried out every day. Rats were treated by direct induction into the stomach using a gavage tube with pigeon pea solution once a day for 36 days. On the 37th day of the rat, blood was drawn, surgery and bones were taken.

Making histological preparations for HE staining

Includes the stages of fixation, washing, dehydration, clearing, infiltration, blocking, trimming, sectioning, mounting and staining. Procedure for making histological preparations **(Feldman & Wolfe, 2014; Sampedro-Carrillo, 2022).** Changes in bone tissue structure were observed using an optilab microscope.

Data analysis

The data obtained, namely data on genistein and daidzein levels from the HPLC test, were analyzed based on the chromatogram results, sample curve area and retention time. Data on bone tissue structure preparations were analyzed for changes in bone tissue structure, osteoblasts and osteoclasts.

3. RESULTS AND DISCUSSION

The results of High Performance Liquid Chromatography (HPLC) analysis of isoflavones (genistein and daidzein) showed genistein and daidzein levels of 247.89828 µg/g and 188.61309 µg/g, respectively. The results of the HPLC analysis are in Figure 1.

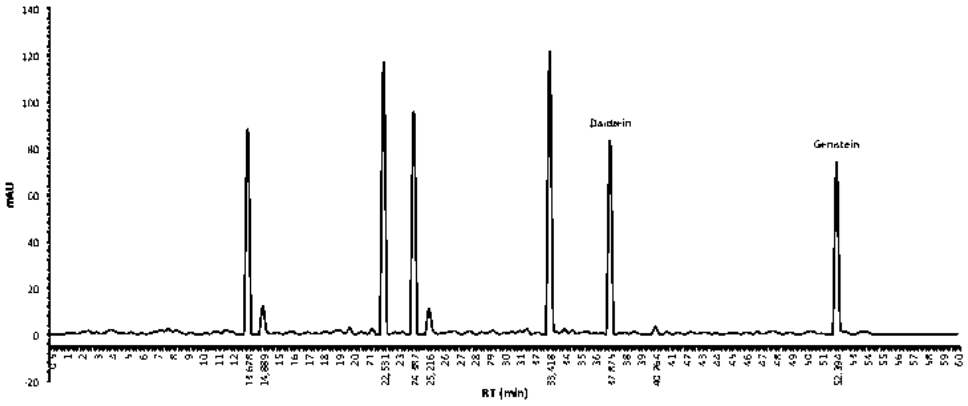


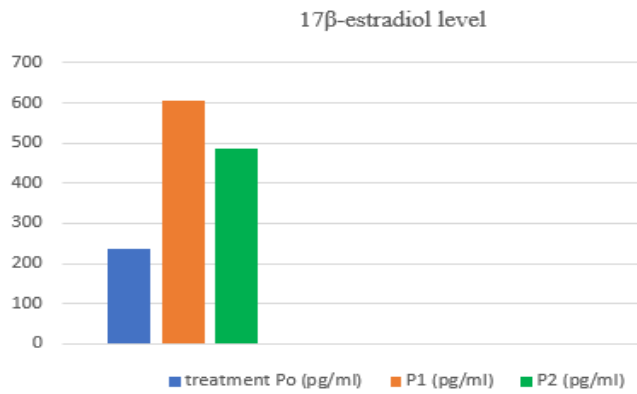
Figure 1. HPLC with specifications: Shimadzu, injection volume 20 µl, running time 60 minutes, wavelength detector 261 nm, column temperature 25°C, column C 185 µm Shimadzu 120x4.6 mm, mobile phase acetonitrile 20% in acetic acid 3% , Flow rate: 0.8 ml/min. HPLC pigeon pie contained genistein 247,898 µg/g and daidzein 188,613 µg/g.

HPLC analysis Figure 1 and Table 1, based on sample weight, retention time, and sample curve area.

Table 1. HPLC analysis of pigeon pie seed extract

Sample name	Sample weight (g)	RT (min)	Sample curve area	Result (µg/g)	Compound
Pigeon pie	5,0003	37,875	83,28266	188,61309	Daidzein
	5,0003	52,394	74,22387	247,89828	Genistein

Figure 2: HPLC analysis of 17β-estradiol for each treatment was P_0 237.231 pg/ml; P_1 603.926 pg/ml P_2 486.153 pg/ml



Isoflavones, lignans, stilbens, koumestans have an aromatic ring structure similar to 17 β-estradiol **(Dixon, 2004; Ahmad Hairi et al., 2018; Lee, & Tseng, 2020; Chavda et al., 2024),** this is often referred to as estrogen like molecules **(Vaya & Tamir, 2004; Kiyama, 2023).** Therefore, these compounds are often called phytoestrogens. Genistein and daidzein are the main isoflavones, which are aglycones that are widely distributed in plants of the Leguminoceae family **(Chavda et al., 2024).**

Genistein and daidzein are thought to be able to bind to estrogen receptors on ER α and ER β in the body system **(Dhananjaya et al., 2012; Chan et al., 2018).** The phytoestrogen compounds genistein and daidzein have a mechanism similar to estradiol so they can provide potency in organ systems. Figure 3 shows that giving pigeon pie powder solution to mice for 36 days was able to increase the proliferation of osteoblasts and fibroblasts in bone tissue structures.

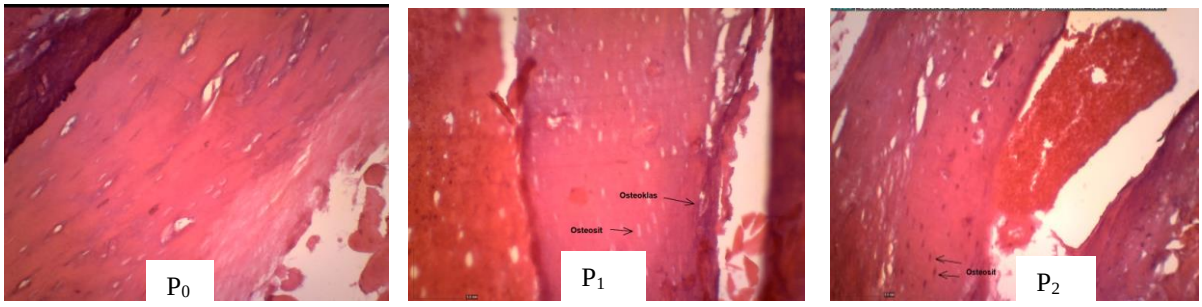


Figure 3. Bone tissue structure of white rat, HE staining, 100x

P_0 Normal bone tissue, no osteoblast proliferation; P_1 Osteoblasts experience proliferation and increase in fibroblast tissue; P_2 Osteoblasts experience proliferation although not all of them proliferate, there are some osteoclasts.

Osteoblasts are part of the mesenchymal cells that are responsible for bone formation and development. Osteoblasts produce osteoprotegerin (OPG) as a receptor to prevent bone loss, while 17β-estradiol can stimulate OPG production **(Rickard et al., 2003; An et al., 2016).** Genistein and daidzein pigeon pie solution can increase the binding of estrogen receptors so that they can increase bone mineral density (BMD) **(Abdi et al., 2016; Nayeem et al., 2019).** The hormone estrogen can inhibit bone resorption, suppress the production of IL-1, IL-6 and TNFα, and inhibit RANK-RANKL interactions by stimulating stromal cells to produce OPG **(Drugarin, et al., 2003; Hooshiar, et al., 2022).**

Genistein and daidzein in pigeon pie are phytoestrogens, and can act as substitutes for the hormone estrogen. Therefore, these two phytoestrogen compounds can act as estrogen hormones, especially as estrogen replacement therapy in menopausal conditions to prevent osteoporosis.

4. CONCLUSION

Genistein and daidzein pigeon pie were 247.89828 µg/g and 188.61309 µg/g respectively. 17β-estradiol P_0 level 237.231 pg/ml; P_1 603.926 pg/ml P_2 486.153 pg/ml. Giving pigeon pie can increase 17β-estradiol levels in mice, this can cause proliferation of osteoblasts and fibroblasts of bone tissue structures. Pigeon pie has the potential to act as a natural estrogen.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

This article based on our research and did not used any AI technology

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