Research Article

Effect of Bengkoang *(Pachyrhizus erosus)* Extract on Estrogen Receptor-β, Progesterone Receptor Expression, and Follicle-Stimulating Hormone Levels

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Abstract

Objective: Progesterone functions by inhibiting the release of Gonadotropin-Releasing Hormone (GnRH), which decreases Follicle-Stimulating Hormone (FSH) levels and converts them into hypoestrogens. This condition affects the expression of steroid, estrogen, and progesterone receptors, contributing to endometrial proliferation and secretion during the menstrual cycle.

Methods: This study aims to demonstrate that Bengkoang extract administration increases the expression of Estrogen Receptor (ER), Progesterone Receptor (PR), and Follicle-Stimulating Hormone (FSH) in Wistar model rats. This study divided 25 female Wistar rats into five groups: one control group without progesterone and Bengkoang extract and four treatment groups injected with progesterone. After exposure, Bengkoang extract was administered to three treatment groups at doses of 70 mg/200 g BW/day (treatment 1), 140 mg/200 g BW/day (treatment 2), and 280 mg/200 g BW/day (treatment 3).

Results: The results showed an increase in the expression of Estrogen Receptor- β (Erβ), Progesterone Receptor (PR), and Follicle-Stimulating Hormone (FSH) levels in treatment 1, treatment 2, and treatment 3 groups compared to the KP with $p < 0.05$

Conclusion: The study investigating the effect of Bengkoang (Pachyrhizus erosus) extract on estrogen receptor-β (ERβ), progesterone receptor (PR) expression, and follicle-stimulating hormone (FSH) levels has demonstrated significant findings.

Keywords: Estrogen, Estrogen receptor-β, Follicle-stimulating hormone, Pachyrhizus erosus, Progesterone, Progesterone receptor.

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INTRODUCTION

Hormonal balance plays a critical role in maintaining women's reproductive health and overall well-being[1]. Estrogen, progesterone, and follicle-stimulating hormone (FSH) are key regulators of the female reproductive system, influencing processes such as the menstrual cycle, fertility, and menopause[2,3]. Disruptions in the levels or activity of these hormones can lead to various reproductive disorders, including polycystic ovary syndrome (PCOS), infertility, and menopausal symptoms[3,4]. Estrogen receptor-β (ERβ) and progesterone receptor (PR) are essential components in the signaling pathways of these hormones, and their regulation is crucial for maintaining hormonal homeostasis[5,6].

Conventional hormone replacement therapies (HRT) are commonly used to manage symptoms of hormonal imbalances, particularly in menopausal women7. However, long-term use of synthetic hormones is often associated with an increased risk of side effects, such as cardiovascular diseases and certain types of cancers[5–7]. As a result, there has been growing interest in exploring safer, plant-based alternatives that could regulate hormone levels without adverse effects. Phytoestrogens, naturally occurring compounds in plants that mimic estrogen activity, have emerged as promising candidates for addressing hormonal imbalances. Among these, Bengkoang (Pachyrhizus erosus), also known as jicama, has gained attention due to its phytoestrogenic properties and traditional use in herbal medicine[3,8,9].

Bengkoang contains isoflavones, a class of phytoestrogens, which may exert estrogen-like effects by binding to estrogen receptors, particularly ERβ. This suggests that Bengkoang extract could potentially modulate estrogen and progesterone receptor activity, thereby influencing hormonal regulation. However, the effects of Bengkoang extract on specific hormone receptors and FSH levels have not been extensively studied. Understanding the impact of Bengkoang on ERβ, PR expression, and FSH levels could provide insights into its potential as a natural alternative for managing hormonal imbalances and improving women's reproductive health[5,6,10–12].

While Bengkoang is traditionally used for various health benefits, including skin care and anti-inflammatory effects, its potential role in modulating hormone receptors and balancing reproductive hormones remains poorly understood[12]. In particular, the specific effects of Bengkoang extract on estrogen receptor-β, progesterone receptor expression, and follicle-stimulating hormone (FSH) levels need to be explored[5,6]. This study aims to address this gap by investigating whether Bengkoang extract can influence these hormonal pathways, which are critical for maintaining reproductive health and managing conditions related to hormone imbalances. This study seeks to investigate the effect of Bengkoang (Pachyrhizus erosus) extract on the expression of estrogen receptor-β (ERβ), progesterone receptor (PR), and follicle-stimulating hormone (FSH) levels, aiming to evaluate its potential as a natural therapeutic agent for regulating hormonal balance in women. By elucidating the hormonal effects of Bengkoang extract, this research will contribute to the growing body of knowledge on plant-based alternatives for hormone regulation, with potential applications in treating conditions such as menopause, infertility, and hormonal disorders[2,3,8,10].

METHODS

The research was carried out at the Brawijaya University Institute of Biosciences laboratory, the pathology and anatomy laboratory, and the central biomedical laboratory at the medical faculty of Brawijaya University. This was a true experimental study with a post-test-only control group design in vivo. This research was conducted for 3 months and data analysis was conducted using the SPSS for windows 24 application. All procedures performed in studies followed the ethical standards of the Health Research Ethics Commission, Faculty of Medicine, Brawijaya University, with number 52/EC/KEPK-S2/02/2019, registered 12 February 2019-retrospectively registered, https://bit.ly/ethicalclearancesuryanti.

Material Animal attempt

The experimental animal was a white mouse strain of Wistar. As many as 25 animals, aged 8 to 10 weeks and weighing 160 to 225 grams, were sampled. The negative control (without progesterone and bengkoang extract exposure), the positive control (progesterone exposure without bengkoang extract), treatment 1 (progesterone exposure and bengkoang extract 70 mg/200grBW /day), treatment

2 (progesterone exposure and bengkoang extract 140 mg/200grBW/day), and treatment 3 (Bengkoang extract 280 mg/ 200grBW/day).

Bengkoang was obtained from the Kediri Regency in East Java. Bengkoang was dried and extracted at UPT Materia medica Batu integrated service unit , East Java, using a maceration method with 96% ethanol as the solvent.

Procedure

Each treatment group received four injections of progesterone hormone at a dose of 2.75 mg. Various doses of ethanol extract were administered orally to groups 1, 2, and 3 for 14 days using the sonde technique. After administering the extract, the rat's proestrus phase is surgically removed to extract the endometrium and blood from the heart. Immunohistochemistry was used to examine ERβ and PR expression in the endometrium, while ELISA measured FSH levels in the blood. ERβ and PR expression were observed in epithelial cells and endometrial stroma with ERβ and PR antibodies from Santacruz that were appropriate for Rattus norvegicus. The results were then observed using an Olympus dot slide with 400x magnification in 5 fields of view. ELISA kits for follicle-stimulating hormone (FSH) species of Rattus norvegicus were used to determine FSH levels.

Data evaluation

Data normality test using Shapiro-Wilk. Hypothesis testing using One-way ANOVA. Suppose Duncan's test follows significant differences (significant).

RESULT

The effect of bengkoang extract administration on the expression of Erβ and PR

The results of endometrial staining with hematoxylin-eosin were seen using an Olympus microscope with a magnification of 400 times. The calculation is done using five visual fields in each sample, then counting the number of glandular epithelium and the surface, and the results are averaged. The sample description can be seen as follows in Figure 1.

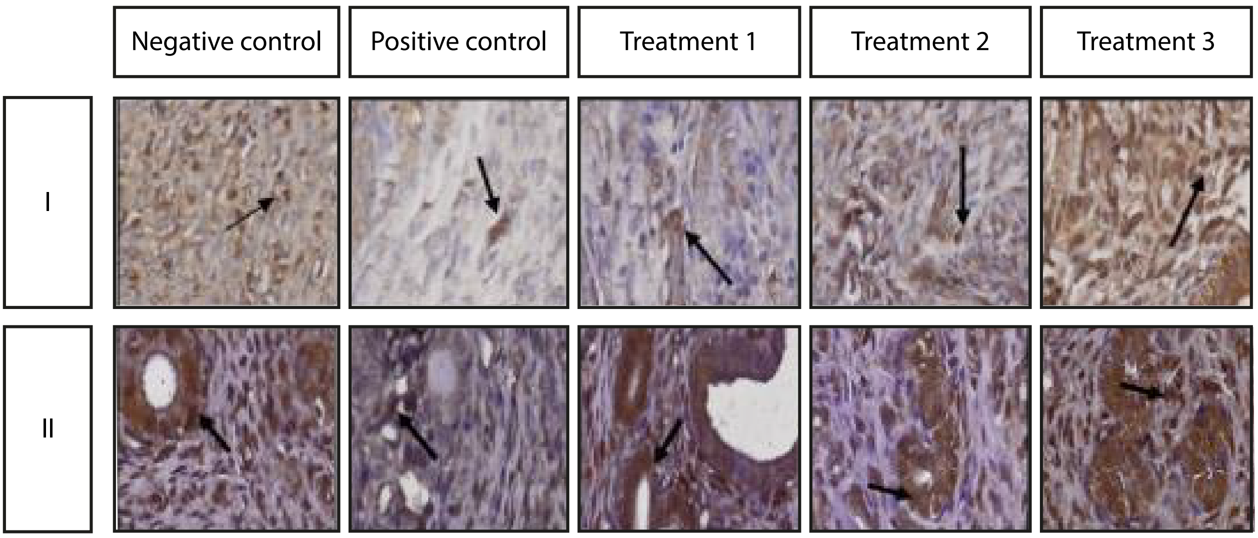

Figure 1. Overview of Erβ(I) and PR(II) expression in rat endometrium (400 times magnification)

Figure 1 depicts Immunohistochemical examination of the rat endometrium to observe the expression of Erβ and PR was indicated by cells appearing brown in color in the cell nuclei and membranes of the rat endometrium. Black arrows indicated the cells expressing ERβ and PR in the negative control group (without DMPA and Bengkoang extract), positive control group (DMPA without Bengkoang extract), treatment 1 group (DMPA with Bengkoang extract at 70mg/200gBW), treatment 2 group (DMPA with Bengkoang extract at 140mg/200gBW), and treatment 3 group (DMPA with Bengkoang extract at 280mg/200gBW). There was a noticeable difference in ERβ and PR expression between the negative control, positive control, treatment 1,2, and 3 groups. The expression of ERβ in each group appeared to vary, with the brown staining being more prominent in the treatment 3 group compared to the others. The cells expressing ERβ in each image showed differences in the intensity of the brown color, with the treatment 3 group exhibiting the most brown staining, while the positive control group had the least. The brown staining was observed in both the epithelial and stromal compartments of the endometrium with more dominant staining in the stromal endometrium in the treatment 3 group. PR expression (II) results of immunohistochemical observations show that in the positive control group the expression of progesterone receptors is less than in the negative control group which is shown by brown discoloration with DAB staining. Whereas in groups treatment 1, treatment 2, and treatment 3 showed the results of changes in the number of expression of progesterone receptors in the endometrium after administration of ethanol extracts of Bengkoang with 3 different doses.

Table 1. Erβ, PR Expression and FSH Levels

	Erβ Expression (n=25)	**PR Expression (n=25)**	**FSH (n=25)**
Negative Control	19.02+3.36^{c}	68.176+6.5^{c}	182.75+19.49^{a}
Positive Control	5.62+1.23^{a}	43.988+8.1^{a}	47.59+7.89^{b}
Treatment 1	8.78+1.12^{ac}	50.936+5.1^{ab}	66.26+7.82^{c}
Treatment 2	15.2+1.87^{bc}	56.488+6.1^{b}	80.47+8.16^{d}
Treatment 3	39.9+11.48^{d}	66.98+3.8^{c}	102.96+12.05^{e}

Note: On the mean ± SD if loading different letters mean that there are significant differences (p-value <0.05) and if loading the same letters means there are no significant differences (p-value> 0.05).

Table 1 depicts the mean ER-β and PR expression in three groups of Rattus norvegicus female rats given DMPA and extracts. Bengkoang at 70 mg/200 grBW 140 mg/200 grBW and 280 mg/200 grBW. The image demonstrates that the highest average expression level of ER- β was seen in the treatment 3 group, meanwhile the lowest was seen in the positive control group This indicates that DMPA treatment promotes ER- β expression in female rats. The mean expression of ER- β appeared to be higher in the treatment 1, treatment 2, and treatment 3 groups than the positive control group; the expression of ER-β rose as the dose of Bengkoang extract was increased. Therefore, treatment of three doses of Bengkoang extract increases the expression of ER- in female Rattus norvegicus rats treated with DMPA. In contrast, the Bengkoang extract dose believed to boost ER- β expression the quickest is 280 mg/200 grBB, as the ER-β group in treatment 3 had the highest mean expression (39.9+11.48) compared to treatments 1 and 2. Compared to the positive control group with the lowest mean value, the PR expression increased in the treatment 1, treatment 2, and treatment 3 groups. The highest mean PR expression was found in KN, a condition typical of rats not administered DMPA injections and Bengkoang extract therapy. The expression of PR increases as the dose of Bengkoang extract is increased. Nonetheless, treatment 3 at a dose of 280 mg/200 gBW/day with an average value of 66.98 and the same homogeneity as evidenced by letter C on the average value is deemed to increase PR the most swiftly near standard conditions (negative control).

Effect of Bengkoang extract administration on FSH levels

Table 1 shows the average of FSH levels in rats that were not exposed to DMPA (negative control), rats that were exposed to DMPA (positive control), and three groups of hypoestrogenic rats administered Bengkoang ethanol extract at doses of 70 mg/200 g BW, 140 mg/200 g BW, and 280 mg/200 g BW. The fastest-acting dose of Bengkoang extract is 280 mg/200 g BW since the average FSH levels in the treatment 3 group (102.96 12.05 ng/mL) are closest to the intermediate FSH level in the negative control group (182.75 + 19.4 ng/mL).

DISCUSSION

Effect of Bengkoang extract administration on ER-β expression

ER-β is closely related to ER-α, and the positions of these estrogen receptors can complement one another [13]. ER-α is believed to prevent estrogen-mediated actions mediated by ER-α [14]. ER-β has the opposite movement on the same gene promoter as ER-α in response to estrogen's effects [15]. Ovariectomized rats exhibit a distorted hyperproliferation response without estrogen and progesterone, but their adrenals produce relatively high levels of androgens. Androgens regulate ER-β via hydrogen receptors (AR) and inhibit breast cancer cell proliferation [16]. If a similar mechanism exists in the endometrium, androgens with high ER-β expression may inhibit excessive cell proliferation. Increased expression of ER-β in stromal cells is observed during the proliferative phase, with the color index halving during the secretory phase and remaining low during pregnancy [6,16].

Bengkoang is a plant that contains daidzein, phytoestrogens, and genistein, all of which can reduce cardiovascular risk and menopause-related symptoms [17,18]. Hypoestrogenic conditions in progesterone hormone users can reduce estrogen receptor expression and cause menopausal-like conditions[19]. As phytoestrogens in Bengkoang can bind to ER and stimulate cell proliferation, administering Bengkoang extract at the appropriate dose can enhance the effects of DMPA. Bengkoang phytoestrogens have an identical chemical structure to estrogen. Phytoestrogens can bind to estrogen receptors, particularly ER-β. His in vitro study on MCF-7 cells found that phytoestrogen content has differential and potent transactivation of ER-α and ER-β induced transcription, with ER-β activation 100 times stronger [17,20].

According to additional studies, genistein has no effect on the expression of ER-α mRNA in cultured pig granulosa cells. Aside from that, ER-β concentrations are higher in genistein-cultured cells than in control cells, and even higher genistein doses tend to increase ER-β in examined cells. Using IHC, ER-β is detected in the nucleus of granulosa cells, whereas ER-α is not detected [13]. In general, derivatives induce transcription-dependent receptors and are significantly more influenced by ER-β than ER-α. The concentration of genistein required to induce transcription is 104 times that of 17-beta estradiol. Considerably higher concentrations are necessary to stimulate cell growth.

All types of endometrial cells express ER-β, including canary epithelium and stromal cells. ER-β may be essential for the maintenance of the endometrial lining in rats. In contrast, ER-β mRNA expression throughout the menstrual phase is significantly lower than ER-α, despite ER protein expression being elevated throughout the menstrual cycle [14]. ER-β expression in the epithelium is linked to the menstrual cycle, which can increase estrogen levels and ER expression. This is due to the action of estrogen via ER-α and progesterone via PR, which has been demonstrated to increase ER-β transcription [15,16]. Therefore, the ER-β mechanism is the primary safety mechanism by which estrogen's intense mitogenic action can be limited to normal endometrium.

The influence of Bengkoang extract on the expression of PR

DMPA's interaction with progesterone receptors is also mediated by prostaglandin. Progesterone receptors A (PRA) also inhibits estrogen receptor activity (ER). Progesterone receptor A (PRA) and Progesterone Receptore B (PRB) are crucial for reproductive and non-reproductive activities in women. During the estrous cycle in rats or the menstrual cycle in women, estrogen and progesterone control the expression of progesterone receptors on epithelial cells, stroma, and uterine myometrial compartments[21]. High levels of synthetic progesterone that persist after DMPA administration reduce the amount of endogenous estrogen in the body by regulating the synthesis of progesterone receptors; PRA and PRB also decrease.

In this study, an immunohistochemical examination revealed that the expression of progesterone receptors was more significant in rats treated with varying doses of Bengkoang ethanol extract than in the positive control groups without medication. The results of the One-Way ANOVA test also demonstrate the effect of Bengkoang extract ethanol extract on groups receiving treatments 1, 2, and 3. In addition, Duncan's test revealed a statistically significant difference between the positive control and treatment groups. However, 280 mg / 200 gBW / day is considered the optimal dose based on the trend of the average amount to increase the expression of progesterone receptors as in normal conditions in the treatment group[22]. Due to changes in estrogen and progesterone receptors, endometrial bleeding and amenorrhea are influenced by a state of low estrogen and high progesterone during DMPA use [16].

Bengkoang extract contains phytoestrogens called genistein, which regulate the expression of estrogen and progesterone receptors. [23]The phytoestrogens found in pumpkin seeds can increase estradiol production by decreasing ER--β and PR[24]. The administration of phytoestrogen-containing Bengkoang ethanol extract can increase the expression of progesterone receptors by increasing estradiol and regulating estrogen and progesterone receptors, as demonstrated by this study[25].

Impact of Bengkoang Extract on FSH Concentrations

High progesterone levels will stimulate neurons in the central nervous system to release opioid, dopaminergic, and gabaergic neurotransmitters, thereby inhibiting the release of GnRH. Low GnRH secretion stimulates the anterior pituitary to release low levels of FSH and LH. Low levels of FSH inhibit follicular development and maturation, thereby preventing ovulation [4,26]. Users of DMPA have FSH levels comparable to the early follicular phase. This state is maintained as a contraceptive effect of DMPA to prevent the development of follicles and ovulation [27,28]. The level of FSH in a Depomedroxiprogesterone-Acetat (DMPA) is equivalent to that of the luteal phase. This indicates that FSH levels are low during the luteal phase[28].

The research found that Bengkoang contains isoflavones with estrogen-like chemical structures [8]. Isoflavones have a chemical structure similar to 17-estradiol and possess estrogen-like properties. Bengkoang isoflavones (daidzein and genistein) have a system and estrogen-like activity identical to 17-estradiol. Thus, Bengkoang can be concluded to be a natural estrogen source[11].

They have demonstrated that consuming isoflavone-containing foods (genistein and daidzein) can affect ovarian function [29]. This is because ovarian function is regulated by hormones circulating throughout the body. Estrogen is the primary hormone responsible for the female reproductive cycle. Estrogen is primarily produced in the ovaries, enters the bloodstream, and sends a response signal to the brain. The hypothalamus and anterior pituitary are the brain regions responsible for regulating reproductive hormones. Estrogen stimulates the hypothalamus to generate GnRH, which then signals the anterior pituitary to generate FSH and LH. The hormone enters the bloodstream and signals the ovaries to produce an egg. Compounds with estrogenic activity can influence this signaling and elicit a response. When consumed, food containing estrogenic compounds (phytoestrogens) can bind to estrogen receptors. The hypothalamus and pituitary gland produce the ovulation-regulating gonadotropins FSH and LH in response to estrogen. Estrogen signaling in the ovary regulates gene expression required for follicular development and presentation of FSH and LH receptors, which respond to gonadotropin signals from the hypothalamus and pituitary[2]. In addition, phytoestrogens reduce the formation and activity of free radicals in the hypothalamus, preventing a decrease in hormone secretion [30,31].

CONCLUSIONS

The study investigating the effect of Bengkoang (Pachyrhizus erosus) extract on estrogen receptor-β (ERβ), progesterone receptor (PR) expression, and follicle-stimulating hormone (FSH) levels has demonstrated significant findings. Bengkoang extract, particularly at higher doses, was found to modulate hormone receptor activity and hormone levels in a dose-dependent manner.

1. ERβ Expression: The immunohistochemical analysis revealed a marked increase in ERβ expression in the endometrium, particularly in the P3 group (DMPA with Bengkoang extract at 280 mg/200gBW), as evidenced by the greater intensity of brown staining compared to other groups. This suggests that Bengkoang extract may enhance estrogenic activity by increasing ERβ expression, indicating its potential as a phytoestrogenic agent.
2. Progesterone Receptor Expression: Bengkoang extract also showed an effect on progesterone receptor expression. Higher doses of the extract, particularly in the P3 group, may have contributed to a regulatory effect on PR, aligning with the extract's potential role in balancing estrogen and progesterone signaling pathways.
3. FSH Levels: The study indicated that Bengkoang extract influenced FSH levels, suggesting that the extract may contribute to the modulation of pituitary-gonadal axis activity. The changes in FSH levels observed in the treatment groups point toward the extract's ability to affect overall hormonal regulation.

In conclusion, Bengkoang extract exhibits potential as a natural therapeutic agent for modulating estrogen and progesterone receptor activity as well as FSH levels. These findings support its potential use in managing hormone-related disorders, such as menopause and other reproductive health issues, offering a plant-based alternative to conventional hormone therapies. Further research is needed to fully understand its mechanisms of action and clinical applications.

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