Asian Journal of Health Research
Journal Homepage: https://a-jhr.com
Published by Ikatan Dokter Indonesia Wilayah Jawa Timur

OPEN ACCESS

Original Research

Genistein Daidzein in Kudzu Root (Pueraria lobata) Extract Decreased Malondialdehyde Plasma Levels in Hypoestrogenic Rats

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ARTICLE HISTORY
Received: 10 January 2022
Revised: 25 January 2022
Accepted: 22 February 2022

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KEYWORD
Daidzein, Genistein, Hypoestrogenic, Kudzu Root, Malondialdehyde, Ovariectomy

ABSTRACT

Introduction: Menopause can reduce women's quality of life and cause health issues. The decline in estrogen during menopause is thought to trigger oxidative stress. When the creation of antioxidants does not match the synthesis of reactive oxygen species (ROS) as an antidote, oxidative stress ensues, characterized by malondialdehyde. The aim of the research is to evaluate the effect of the dose of isoflavone genistein daidzein powder in Kudzu root (Pueraria lobata) extract on the decrease in plasma malondialdehyde (MDA) plasma levels in hypoestrogenic rats.

Methods: 30 rats Wistar rats were separated into five groups, namely: the control group, the ovariectomy (OVX) group, waited two weeks to produce hypoestrogenic rats. Of the four groups, the positive group was not given a Pueraria lobata powder containing the isoflavones genistein and daidzein (IGD), and the other group was given 15 mg, 30 mg, 60 mg per kg body weight per day, respectively. Twenty-one days later, the mice were killed, and plasma was taken to be checked for MDA levels using spectrophotometry.

Results: Genistein Daidzein in kudzu root extract was effective in reducing the oxidative stress. All IGD doses significantly reduced plasma MDA levels. At a dose of 60mg/kg BW, the plasma MDA levels were comparable to those in the control group, it could prevent a significant increase in plasma MDA levels in hypoestrogenic rats.

Conclusion: Isoflavone Genistein and Daidzein of kudzu root (Pueraria lobata) extract reduced MDA plasma levels in hypoestrogenic rats.

Cite this as: Noviana NN, Suprobo NR, Wiyasa IWA (2022) Genistein Daidzein in Kudzu Root (Pueraria lobata) Extract Rats. *Asian J Heal Res*. 1(1):7-11. doi:https://doi.org/10.55561/ajhr.v1i1.6

INTRODUCTION

Menopause is a physiological shift in women as they approach reproductive age caused by the end of ovarian endocrine activity with a significant drop in estrogen production and endometrial cycle decline [1]. When estrogen levels dropped significantly, it was shown to raise oxidative stress levels in the body [2]. Oxidative stress is characterized as an imbalance between antioxidant defense and free radical generation caused by a rise in reactive oxygen species (ROS) or reactive nitrogen species (RNS) concentrations, or a reduction in antioxidant defense mechanisms [3–5]. Antioxidants are substances that prevent oxidation by scavenging free radicals and subsequently aiding in their maintenance at an average level, hence minimizing potential harmful consequences and reducing cell death caused by oxidative stress [6,7].

Menopausal hypoestrogenic problems can be treated with hormone replacement treatment, which is known to be costly and, in the long run, can raise the risk of breast cancer and endometrial cancer [8]. Many therapies have been developed to address this biological drop in estrogen, and broadened to encompass lifestyle modifications including exercise and diet [2]. The usage of natural compounds containing hormones or phytohormones, such as phytoestrogens, has been widely established at this time. Phytoestrogens are plant compounds that have estrogen-like activity [8].

The kudzu root (*Pueraria lobata*) is one of Traditional Chinese Medicine's 50 essential herbs, and it has just been commercially accessible as a Western dietary supplement [9, 10]. Kudzu root has been used for fever reduction, headache treatment, and other therapeutic uses [11]. Chemical analysis of kudzu extract revealed three major isoflavones: puerarin, daidzein, and genistein [12]. Isoflavones are phytoestrogens having specific estrogenic action that has a similar backbone structure to β -estradiol [9]. As a result, the purpose of this study was to examine the influences of isoflavone genistein and daidzein kudzu root (*Pueraria lobata*) in Malonaldehyde plasma levels in hypoestrogenic rats.

MATERIAL AND METHODS

Study Design

This was an experimental study on an ovariectomy rat model that used a randomized post-test control group design, at Laboratory Pharmacology Brawijaya University in Malang, Indonesia. A total of 30 healthy female Wistar rats (Rattus Novergicus) aged 8-10 weeks and weighing 100-120 g were separated into five groups, namely: group I, control group (untreated and not underwent OVX); group II, OVX group (rats underwent OVX); groups III, rats underwent OVX and given IGD at dose of 15 mg/kg BW; group IV, OVX rats with administration of IGD at 30 mg/kg BW; and group V, OVX rats given IGD at dose of 60 mg/kg BW. Mice were acclimatized one week before being treated, given food and drink ad libitum, and kept in a standard cage. Then groups III, IV, and V administrated with IGD for 21 days per oral. The mice were sacrificed after day 21 later, and their plasma was taken to be checked for MDA levels using spectrophotometry.

Rat model of ovariectomy

Ovariectomy is the surgical excision of the ovaries from a person's or experimental animal's abdominal cavity as part of a surgical menopause model wherein estrogen levels decline quicker than in natural menopause [13]. Ketamine at a dosage of 40 mg/kg BW was used to anesthetize the rats through intramuscular injection, then the rats' abdominal hairs were shaved. A transabdominal incision was made approximately 1.5–2 cm above the uterus. The oviduct and ovary are freed from the adipose tissue and surrounding connective tissue. The distal oviduct and ovary are then ligated and removed. The incision wound is sutured layer by layer. Gentamicin injection at a dosage of 60 mg/kg BW/day was administered as post-operative treatment for three days, and waited for 14 days to generate hypoestrogenic rats.

Kudzu Root isoflavone extract

Kudzu roots were obtained from Kangean Island, Sumenep, East Java, Indonesia. Kudzu roots were extracted using 80% methanol as a solvent, and then the paste extract was analyzed for the isoflavone genistein daidzein content in the sample using High-Performance Liquid Chromatography (HPLC). The findings revealed that the total isoflavone content was 5637.5 ppm, with genistein levels of 1970 ppm and levels of daidzein 3667.5 ppm. Thus 1 ml of Kudzu roots extract is equivalent to 5.6375 mg (with a ratio of G:D = 0.6304 : 1.1736 mg/kg BW/day). Kudzu roots extract was administered orally for 21 days (sub-chronic) in the OVX+IGD1, OVX+IGD2, and OVX+IGD3 groups with doses of isoflavones 15, 30, and 60 mg/kg BW/day, respectively. Hypoestrogenic were characterized by E2 levels less than 9 pg/ml, vaginal smears dominated by parabasal and intermediate epithelial cells similar to those of diestrus, and atrophic uterus.

Measurement of MDA plasma levels

Reliable MDA levels were found in plasma/serum MDA [8]. A total of 100 ul of plasma was added with 0.9 ml of aquabidest to the sample, then 0.5 ml of TBA reagent was added. The solution-containing tube was then heated in a water bath at 95ºC for one hour. The sample was then centrifuged for 10 minutes at 7000 rpm. The obtained supernatant was measured for absorbance using a spectrophotometer to measure level of plasma MDA.

Ethics

The Health Research Ethics Committee, Faculty of Medicine, Brawijaya University, Malang, Indonesia, authorized all procedures used in this study, which were carried out in accordance with the required instructions and regulations.

Statistical analysis

SPSS 24.0 for Windows software was used to analyze the data. One-way ANOVA was used, followed by the Tukey Honestly Significant Difference test. $P<0.05$ was used to denote statistical significance. The data show the mean and standard deviation (SD).

RESULT

A total When compared to the group I (control group), MDA plasma levels increased significantly in the group II (OVX group). Figure 1 depicts the concentration of

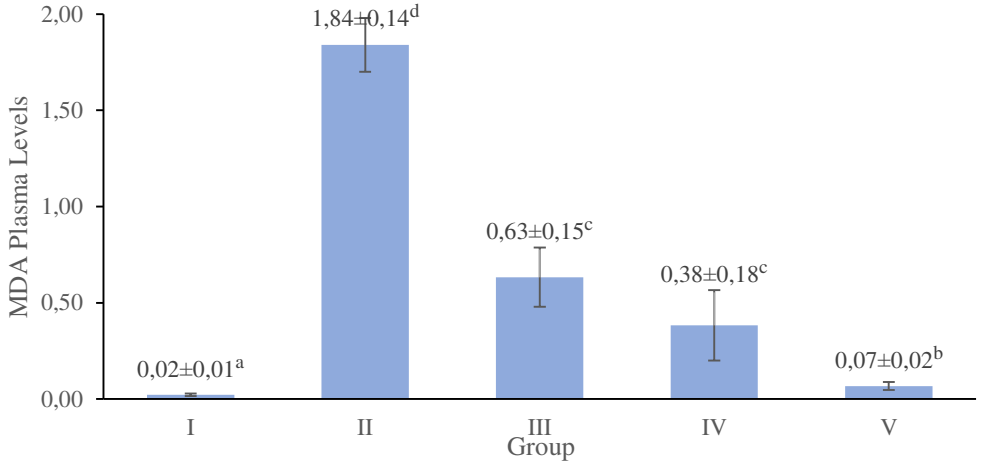

Figure 1. Malondialdehyde plasma levels in five groups treatment. Group I, control group (untreated and not underwent OVX); group II, OVX group (rats underwent OVX); groups III, rats underwent OVX and given IGD at dose of 15 mg/kg BW; group IV, OVX rats with administration of IGD at 30 mg/kg BW; and group V, OVX rats given IGD at dose of 60 mg/kg BW. P<0.05 was used to denote statistical significance.

MDA plasma in III, IV, and V groups were significantly declined than in the group II.

DISCUSSIONS

Menopause is characterized by the end of the ovarian cycle and cyclical synthesis of progesterone and estrogen, sex steroid hormones with several anti-degenerative disease effects, and antioxidant activity [14,15]. Estrogen promotes antioxidant activity by influencing the expression of antioxidant enzymes, which regulate the amounts of biological reducing agents and potent anti-inflammatory substances [1,14]. The findings of this study revealed that MDA plasma levels increased significantly in the OVX group; MDA levels were indicators of oxidative conditions. It is suggested that OVX leads to a hypoestrogenic condition that causes oxidative stress. Other studies have revealed that Rats that underwent OVX have increased MDA levels in the brain and serum [16]. Hypoestrogenism in menopausal women reduces estrogen's antioxidant action and promotes the production of free radical molecules, often known as ROS. ROS generation results in oxidative stress, characterized by increased MDA levels, inducing lipid peroxidation [17,18]. Oxidative stress leads to an accumulation of oxidative products, which damage lipids, proteins, and DNA [4,5], which will lead to apoptosis [7]. Postmenopausal women are about three times more likely than premenopausal women to have excessive lipid peroxidation. Menopausal symptoms are caused by E2 deficiency, and oxidative stress worsens the symptoms. The link between lipid peroxidation and menopausal symptoms suggests that menopause might increase oxidative stress [19]. Notably, estrogen has a beneficial antioxidant effect at high doses by blocking the 8-hydroxylation of guanine DNA bases. However, at low concentrations, this hormone exhibits pro-oxidant properties, especially when it includes catechol. Breaks in genetic material, the production of DNA adducts, and base oxidation are all examples of these impacts [2].

Antioxidants act as cleaners, removing excess ROS and assisting in maintaining the body's oxidative/antioxidant equilibrium [3,20]. The antioxidant system's function is not to completely eliminate oxidants, but to maintain them at a regulated level [4]. The findings elucidated that administration of IGD could decrease MDA plasma levels significantly. This finding is consistent with previous studies showing that isoflavones can reduce MDA levels in vaginal tissue, brain, and serum [16,17]. Isoflavones have a similar structure to estradiol, bind to the estrogen receptors (ERα and ERβ), and mimicking estrogenic action [21]. Isoflavones have good antioxidant activity for preventing oxidative stress and reducing oxidation rates [22]. Kudzu root has a high concentration of polyphenols, including isoflavones, that are essential phytochemicals. Among the tested substances in Kudzu extract, genistein had the most significant estrogenic activity, followed by daidzein [11]. Along with its chemical structure is related to estradiol, daidzein and genistein potentially bind to the estrogen receptor and exhibit mild estrogenic and/or antiestrogenic actions [11,23]. Genistein has been shown to reduce menopausal symptoms and other endocrine diseases [24].

Another study exhibited that MDA levels steadily rose post-ovariectomy, which might be due to the loss of estrogen's antioxidant properties. This shows that a

reduction of estrogen may aggravate oxidative stress [25]. Estrogen is a potent antioxidant that reduces oxidative physiological stress, whereas estrogen deficiency has been shown to accelerate the aging process in women [17]. The conventional estrogen and phytoestrogen routes include intracellular estrogen receptors mediated by transcriptional activation of estrogen-responsive genes (including target genes MnSOD, eNOS, and cytochrome-o-oxidase). The hormone-receptor complex attaches to a particular estrogen response element (ERE) in the target gene's promoter region, leading in transcriptional activity [8]. Isoflavones boost antioxidant capacity by increasing the production of the antioxidant enzymes manganese superoxide dismutase (MnSOD) and catalase, lowering ROS, inducible nitric oxide synthase (iNOS), and endothelial NOS (eNOS) [26]. Groups III, IV, and V significantly prevented increased plasma MDA levels in ovariectomized female rats. The dose closest to the condition of healthy mice or the negative control group (group I) was in group V with a dose of 60mg/kg BW.

CONCLUSION

Menopausal estrogen deficiency may result in oxidative stress. However, isoflavone genistein daidzein in kudzu roots extract has the potential to be a protective agent against oxidative stress. The protective effect of IGD was evidenced by the decline of MDA plasma levels in ovariectomy rats. As a result, it is possible that phytoestrogen treatment counteracts oxidative stress and thus enhances the quality of life in menopausal women and prevents illnesses associated with oxidative events.

ACKNOWLEDGMENTS

We would like to thank everyone who supported to this research.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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