The effect of purple sweet potato (Ipomea batatas L) ethanol extract on Estrogen Receptor Alpha (ERα) and SOD mRNA expression in the menopause-liver animal model

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ABSTRACT

Background: Aging and estrogen deficiency in menopausal women increase susceptibility to liver diseases. In postmenopausal women, the change of ERα expressions and oxidative stress is related to liver diseases’ pathogenesis. Phytoestrogens have been shown to protect and repair the liver organ due to estrogen deficiency. Purple Sweet Potatoes contain anthocyanins, a subgroup of flavonoids that have been shown to have antioxidant and phytoestrogen activity. This study aims to evaluate the phytoestrogen and antioxidant activities of purple sweet potato in a liver animal model of menopause through Superoxide Dismutase (SOD) and ERα expressions.

Method: A true experimental study by randomized post-test-only control group design was conducted among 28 female Wistar rats-aged 10-12 weeks with a bodyweight of 180-220 gram, which underwent bilateral ovariectomies. These animals were divided into 4 groups, with 7 rats in each group. On day 7, Group T0, as control was given aquadest, ethanol extract of purple sweet potato was assigned to Group T1, T2, and T3 with doses 1, 2, and 4 mL/day, respectively, during 30 days. The SOD and ERα mRNA expressions were evaluated by qRT-PCR with specific primers. Data were analyzed using SPSS version 20 for Windows.

Results: The mRNA expressions of ERα were not significantly different among groups (p>0.05), while SOD expressions were significantly different among groups (p<0.05). The expression of SOD in Group T1 (504.39±231.4 pg/μL), T2 (2,147.92±417.1 pg/μL), and T3 (405.30±224.1 pg/μL) showed a significantly higher than Group T0 (15.34±15.27 pg/μL) (p<0.05)

Conclusion: Oxidative stress could be prevented by purple sweet potato in the liver through the SOD mRNA expression but not ERα.

Keywords: Menopause, Liver, Phytoestrogen, purple sweet potato, oxidative stress


INTRODUCTION

Women under 50 years old have a lower risk of liver disease than men due to estrogen’s protective effect. Estrogen deficiency in postmenopausal women increases liver diseases’ susceptibility, such as NAFLD (non-alcoholic fatty liver disease), liver fibrosis, and hepatocellular carcinoma. Estrogen receptor expression alteration and stress oxidative were correlated to cirrhosis and hepatocellular carcinoma in postmenopausal women. The liver is responsive to estrogen level alteration due to its estrogen receptor α (ERα) and β (ERβ) expressions. The estrogen receptor regulates various gene transcription and expression involved in the cell cycle, proliferation, apoptosis, and inflammation. The previous study showed an abnormality of ER expression in Liver tumors. The lack of expression of ERα was also found in hepatocyte of the rat model for liver fibrosis.

Estrogen has beneficial effects on the liver that may inhibit stellate cell proliferation and fibrogenesis. Estrogen also has an antioxidant effect. Therefore, lack of estrogen level in menopause leads to oxidative stress in the liver marked by decreasing SOD and glutathione S-transferase and increasing lipofuscin, which further causes susceptibility to liver diseases. Another consequence of the low estrogen level is immune system reduction. Pre-menopausal women have a stronger immune system than men, including dendritic cells, monocytes, macrophages activities, humoral, and cellular immune responses. While these protective effects are decreased in postmenopausal women.

Previous experimental study showed that estrogen could recover antioxidant enzyme activity and decrease lipid peroxidation and lipofuscin in the aging rat’s liver tissues. Estrogen also increased antioxidant and anti-apoptosis activity...
and fibrogenesis in the rat model of liver fibrosis.\textsuperscript{7,8}\n
On the animal models of menopause, estrogen prevented fat accumulation in the liver and induced liver cell regeneration through ER\textalpha.\textsuperscript{7,8}\n
Phytoestrogen is promoted as an alternative to estrogen replacement since these natural compounds have a similar estrogen structure. It has estrogenic activity and no harmful side effects.\textsuperscript{9} In addition, phytoestrogens have an antioxidant effect through free radicals scavengers and regulate Manganese-Superoxide Dismutase (MnSOD) and catalase expressions.\textsuperscript{9} Flavonoids are the best known of phytoestrogen that has crucial effects on NAFLD’s pathogenesis, including lipid metabolism, insulin resistance, inflammation, and oxidative stress.\textsuperscript{10,11}\n
Anthocyanin is a subgroup of flavonoids which is the largest and most important group of natural water-soluble pigments.\textsuperscript{12} It has a similar structure with estrogen and exerts phytoestrogen activity in in vitro and in vivo studies.\textsuperscript{13} Previous study showed that anthocyanin was potent antioxidants that could protect cells due to oxidative stress.\textsuperscript{14} Anthocyanin effectively protected liver aging of rats induced by D-galactose. It could decrease liver tissue injury, fibrosis, and inflammatory factors (IL-1, IL-6, and TNF-\textalpha). It also maintains the stability of the plasma redox system (GSH-PX, T-SOD, and MDA).\textsuperscript{15} Besides, liver dysfunction was prevented by anthocyanin-rich rice berry bran extract due to oxidative stress inhibition, inflammation, and apoptosis.\textsuperscript{16}\n
Purple sweet potato cultivated in Bali has a high anthocyanin content that has antioxidant activity.\textsuperscript{14} The previous study indicated that purple sweet potato had phytoestrogen activity in the vagina animal model of menopause.\textsuperscript{17} Also, the purple sweet potato could decrease fatty degeneration in the hepatocytes animal model of menopause that was predicted due to its antioxidant and phytoestrogen activity.\textsuperscript{16} Based on those mentioned above, this research examined ethanol extract of purple sweet potato effect on the expression of mRNA ER\textalpha and SOD in estrogen deficiency of female rats by bilateral ovariectomy.

METHODS

This experimental study used a randomized post-test-only control group design and has been approved by the Ethics Committee Faculty of Medicine, Udayana University. Ethanol extract of purple sweet potato was made according to Jawi et al. research protocol.\textsuperscript{14}\n
Experimental animals were obtained from the animal laboratory unit, Department of Pharmacology, Faculty of Medicine, Udayana University, Bali. This study used 28 female Wistar rats aged 10-12 weeks with a bodyweight of 180-220 grams. Maintenance and ovariectomy procedure was carried out according to a study by Paramitha et al.\textsuperscript{18} On day 7 after bilateral ovariectomy, those animals were divided into 4 groups by random allocation. Group T0 received saline 1 mL/day and Group T1, T2, T3 received ethanol extract of purple sweet potato per oral every day with dosage 1, 2, 4 mL/day, respectively, during 30 days. Then, rats were sacrificed by Ketamine HCl, and the liver was collected for qRT-PCR analysis.

RT-PCR evaluated the gene expression of ER\textalpha and SOD. Total RNA was extracted from 20 mg of liver according to the protocol of the RNeasy Protect Mini Kit, Hildenberg, Germany. Real-time PCR MyGo Mini (IT-IS Life Science, UK) was carried out using Kappa Sybr fast one-step qRT-PCR kit (Kappa Biosystems, USA). Absolute levels of the mRNAs were calculated using the standard curve method. Standard DNA fragments of ER\textalpha and SOD were synthesized by amplification using a specific primer of its target. The threshold cycle (Ct) value of each target was converted to a concentration using the appropriate standard curve. The real-time PCR method was done according to Bustin et al.\textsuperscript{19} The primers used for the amplification of SOD1 was AATGTGTCCATTGAAGATCGTGTGA (Forward) and GCCCTCCAGCATTCTCCTGTT (Reverse). The primers used for the amplification of ESR1 were GCTTACTGACCAACCTGGCAGA (Forward) and GGATCTCTAGCCAGGCACATTC (Reverse). Both primers have an annealing temperature of about 60°C. The ER\textalpha and SOD expression levels were calculated using a standard curve from the cycle of the threshold of RT-PCR. One-Way ANOVA analyzed estrogen receptor \textalpha mRNA data and the Kruskal-Wallis test of ranks analyzed superoxide dismutase mRNA data. Significance was set at p<0.05 for all analyses by SPSS version 20 for Windows.

RESULTS

Estrogen receptor \textalpha mRNA expression

Estrogen receptor \textalpha mRNA expression of control and treated group was showed in Table 1. Based on Table 1, the mRNA expression of Era in T0 group was 21,351.09±7,953 pg/\muL, followed by 23,013.35±5,363 pg/\muL (T1), 19,874.95±4,390 pg/\muL (T2), and 22,674.78±5,715 pg/\muL in T3 group (Table 1). There was a slight difference in Era mRNA expression between the control group and the treatment group. Estrogen receptor expression was highest in the T1 group and lowest in the T2 group (Table 1). One-way ANOVA analysis results
in p-value = 0.0748 indicate that there were no statistically significant differences in ER α mRNA expression among groups (p<0.05) (Table 1).

**Superoxide Dismutase mRNA Expression**

Table 1 also showed the SOD mRNA expression in each group such as 15.34±15.27 pg/μL in T0 group, followed by 504.39±231.4 pg/μL (T1); 2,147.92±417.1 pg/μL (T2); 405.30±224.1 pg/μL (T3). The highest SOD mRNA expression in liver tissues was in group T2 that received 2 mL/day of purple sweet potato and lowest in group T0 (control group) (Table 1). Statistical analysis indicated that SOD mRNA expression in the purple sweet potato group (T1, T2, and T3) was significantly higher than the control group (T0) (p=0.001) (Table 1).

**DISCUSSION**

The protective role of estrogen in hepatocellular carcinoma (HCC) was discovered based on clinical data that showed its morbidity and mortality is higher in men than women. Several further studies confirmed the anti-cancer effects of estrogen on the liver. Even though both estrogen receptor is expressed on liver tissue, this study examines the only ERα due to the previous study found differences in ERα mRNA expression between HCC and healthy liver, men and women as well as between postmenopausal and pre-menopausal women. Hormone replacement therapy for menopause women decreased the risk of HCC and increased their survival times. These protective roles of estrogen provide the possibility that phytoestrogen acts like estrogen in liver disease pathogenesis.

Ethanol extract of purple sweet potato contains high anthocyanin and acts as a phytoestrogen. The previous study found purple sweet potato prevented fat degeneration in an animal model of menopause and probably due to its phytoestrogen effect. This study found the expression of ERα in the liver of all animal models. These results were similar to other research that found estrogen receptor expressed in the liver. However, this study found no significant difference among groups. This result could be due to the non-genomic pathway by phytoestrogen of purple sweet potato in liver disease since the expression of ERα did not increase in treatment groups compared to the control. Furthermore, estrogen receptor isoform should be elucidated, which is the most expressed in liver disease due to its controversy about liver diseases.

Oxidative stress plays a role in the pathogenesis of liver disease in postmenopausal women due to aging and estrogen deficiency. The previous study found that SOD, catalase, and nitric oxide level decreased in aged liver rats, while oxidative products increased. These facts provide reasons for using antioxidants to prevent and treat liver diseases in old age and menopause. This study showed that SOD expressions increased in groups treated with ethanol extract of purple sweet potato compared to the control. These findings agree with previous studies showing the antioxidant properties of purple sweet potato to prevent stress oxidative on liver tissue. Another study also found that purple sweet potato decreased fat degeneration in hepatocytes animal model of menopause.

This study revealed that the highest SOD expression was in Group 2 mL/day, while its expression in a higher dose (4 mL/day) was lower than that of dose 1 mL/day and 2 mL/day. This result due to the pro-oxidant effect of anthocyanin. The previous study also found the antioxidant and pro-oxidant activity of anthocyanin-enriched tea. Antioxidant flavonoids, including anthocyanin, could have pro-oxidant activity in a higher dose as described from previous study.

**CONCLUSION**

Purple sweet potato prevents oxidative stress-induced estrogen deficiency due to bilateral ovariectomy in liver rats at an optimal dose of 2 mL/day. It has a pro-oxidant effect in higher doses. In contrast, ERα expression could not be significantly increased.

**CONFLICT OF INTEREST**

The author declares no conflict of interest related to the material presented in this article.

**ETHICS CONSIDERATION**

Ethics approval has been obtained by the Ethics Committee, Faculty of Medicine, Universitas Udayana, Bali, Indonesia, prior to the study.

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**Table 1. The mRNA expression of ERα and SOD by One-Way ANOVA analysis**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean±SD</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Estrogen Receptor α (ERα) (pg/μL)</td>
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<tr>
<td>Group T0</td>
<td>21,351.09±7,953</td>
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<tr>
<td>Group T1</td>
<td>23,013.35±5,363</td>
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<tr>
<td>Group T2</td>
<td>19,874.95±4,390</td>
<td></td>
</tr>
<tr>
<td>Group T3</td>
<td>22,674.78±5,715</td>
<td></td>
</tr>
<tr>
<td>Superoxide Dismutase (SOD) (pg/μL)</td>
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<td></td>
</tr>
<tr>
<td>Group T0</td>
<td>15.34±15.27</td>
<td>0.001</td>
</tr>
<tr>
<td>Group T1</td>
<td>504.39±231.4</td>
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</tr>
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<td>Group T2</td>
<td>2,147.92±417.1</td>
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<tr>
<td>Group T3</td>
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ORIGINAL ARTICLE

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AUTHOR CONTRIBUTION
All of the authors equally contribute to the study from the conceptual framework, data gathering, and data analysis until reporting the study results through publication.

REFERENCES