ORIGINAL ARTICLE

Rhaponticin contained *Rheum officinale* root extract improved Postmenopause symptom of Ovariectomized Rat

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J. Adv. Pharm. Technol. Res.

ABSTRACT

Postmenopausal women have decreased levels of the hormone estrogen. Reduced estrogen levels will often involve many symptoms that reduced quality of life. This research aims to analyze the effects of *Rheum officinale* root extract on postmenopausal model rats. To this end, thirty rats underwent ovariectomy (OVX) surgery and six rats were operated without having their ovaries removed. The OVX was confirmed by body weight–uterus weight ratio and a vaginal swab. Six groups of the rats were performed: SHAM group and negative control groups are given vehicle; the positive control was assigned tamoxifen; and the extract has been given three doses 7, 35, and 175 mg/200 g BW, respectively, for 30 days. The calcium content of bone ash was measured using atomic absorption spectrophotometer. Blood pressure was evaluated using CODA®, and the metabolites in the blood were assessed using gas chromatography–mass spectrometry (MS) and high-performance liquid chromatography. As a result, using ultra-performance liquid chromatography (UPLC)-MS, we found that the extract's major component was rhaponticin and its metabolites. The bone calcium levels increased with increasing doses of the extract. In the OVX group, the bone calcium content was decreased significantly 51.56% ± 8.9% g compared with the SHAM group 62.97% ±5.6% g, and the administration of Rheum extract could restore the calcium content of the bone to become 69.27% ± 3.8% g. From the above data, we concluded that Rheum root extracts contain astrigin, rhaponticin, rhapontigenin, and desoxyrhaponticin. Rheum root extract could improve calcium content and lipid profiles of OVX rats by stimulation osteoblastogenesis. Rheum root extracts could control the blood pressure of OVX rats by reducing lipid profiles.

Key words: Calcium, hypertension, Kalembak, lipid profiles, osteoporosis, postmenopausal, rhaponticin, *Rheum officinale*

INTRODUCTION

Postmenopausal women have decreased levels of the hormone estrogen. Reduced estrogen levels will increase bone remodeling and lead to imbalanced activity between osteoclasts and osteoblasts, as osteoblasts activity can not compensate for osteoclast activity, thereby decreasing bone mass and made osteoporotic.[1] Hormone replacement

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Submitted: 15-Dec-2020 **Revised:** 16-Feb-2021
Accepted: 17-Mar-2021 **Published:** 27-Apr-2021

Access this article online

Quick Response Code:

Website: www.japtr.org

DOI: 10.4103/japtr.JAPTR_324_20

How to cite this article: Bahtiar A, Setyowati HT, Mahanani RR, Wati A, Arsianti A, Fadilah F. Rhaponticin contained *Rheum officinale* root extract improved Postmenopause symptom of Ovariectomized Rat. J Adv Pharm Technol Res 2021;12:175-9.

therapy (HRT, a combination of estrogen and progestin) has been used for many years as the gold standard to deal with the symptoms of menopause. However, long-term use may increase breast cancer risk, endometrial cancer, and thrombosis.[2,3] There are several alternatives to HRT; one of them is selective estrogen receptor modulators (SERMs). SERMs are compounds that do not have a steroid structure such as estrogen but have a tertiary structure that can bind to ERα and ERβ.[3-5]

In 1993, dry extract of rhubarb (*Rheum rhaponticum* L.; Dahuang) was first used to treat symptoms of menopause.[6] Rheum root dry extract mainly contains rhaponticin which has a stilbene backbone.[7] Tamoxifen and other SERMS derivate compounds have stilbene's backbone.[8,9] Rheum species in Indonesia are rhubarb, *Rheum officinale*. The present research wants to evaluate rhubarb extract's function on the animal model's bone, cardiovascular, and lipid profiles with the background described above.

MATERIALS AND METHODS

Chemicals and reagents

All chemical reagents were purchased from Sigma-Aldrich. PT offers tamoxifen, Kalbe Farma, Indonesia. The Ketalar injection was purchased from PT, Pfizer, Indonesia. Carboxymethyl cellulose was purchased from Brataco, Indonesia.

Plant materials

Rhubarb root (*R. officinale* Baill.) obtained from Tawangmangu, Solo, Central Java. The plant was determined by the Center for Research and Development of Medicinal Plants and Traditional Medicine (certificate of determination No. 125/Dec/2013).

Preparation of Rheum roots extracts[10]

The dried powder of Rheum roots was extracted according to the method of Bahtiar *et al*.[10]

Chromatographic and mass conditions

Chromatographic analysis and mass spectrometry detection were carried out according to the method of Zhou *et al*.[11] using Waters Acquity UPLC system (Waters Corp., Milford, MA, USA) at the Biocenter, Gyeonggido Business and Science Accelerator (GBSA), Suwon, Korea.

Animals

This research had certified by the Ethical Committee of Faculty of Medicine, University of Indonesia (UIFM No. 164b/H2.F1/Ethics). Thirty rats underwent ovariectomy (OVX) surgery, and six rats were operated without having their ovaries removed. The OVX was confirmed by uterus weight–body weight ratio and a vaginal swab. Six groups of the rats were performed: SHAM group and negative control groups are given vehicle; the positive control was assigned tamoxifen; and the extract has been given three doses 7, 35, and 175 mg/200 g BW, respectively, for 30 days.[12]

The calcium content of bone

The calcium content of the bone was analyzed using femurs as calcium source according to the methods of Bahtiar *et al*., by atomic absorption spectrophotometry Shimadzu AA-700.[12]

Determination of the lipid profile of serum

Lipid profiles had determined according to kits' procedure by an enzymatic colorimetric method (DiaSys, Germany).

Measurement of blood pressure

A noninvasive blood pressure gauge CODA® (Kent Scientific Corporation, USA) was used to measure blood pressure. The systolic and diastolic pressures were analyzed four times. They were as follows: before OVX, 21 days after OVX, and after 28 days of extract treatment.

Analysis of amino acids

Serum samples were measured and evaluated using gas chromatography (GC)-MSD 5975C, Agilent Technologies (USA)

RESULTS

Identification of rhaponticin in Rheum extract

Figure 1 shows the identification of the components of the extract using high resolution of MS. There are four significant peaks detected in the extract. Four components of Rheum extract were identified as astringin, rhapontin, rhapontigenin, and desoxyrhaponticin. Rhaponticin has a retention time of 10.25 min.

Effects of Rheum extract on the calcium content of bone

Table 1 shows that OVX increased rats' body weight but decreased the uterine index and calcium content of the OVX rats. The administration of tamoxifen reduced OVX rats' body weight and increased the uterine index and calcium content of OVX rats' bone. The administration of Rheum extract showed a reduced body weight gain of OVX rats but no effect on the uterine index. At a high dose, Rheum extract could increase the calcium content of the bone.

Effects of Rheum extract on the lipid profile of ovariectomy rats

Table 2 shows that OVX increased cholesterol, triglycerides, and low-density lipoprotein (LDL) but decreased high-density lipoprotein (HDL) in animal models. The tamoxifen administration could reduce high cholesterol, triglycerides, and LDL of OVX rats with increased HDL. The administration of Rheum extracts was similar to the tamoxifen effect.

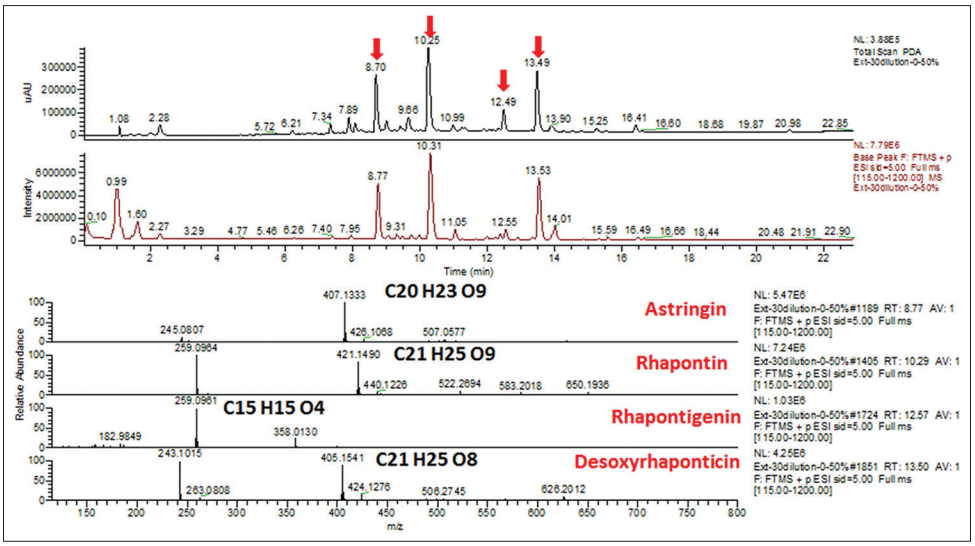

Figure 1: Identification of extract component using ultra-performance liquid chromatography–mass spectrometry. Four components of Rheum extract identified as astringin, rhapontin, rhapontigenin, and desoxyrhaponticin

Table 1: Bodyweight gain, uterine index, and calcium content of OVX Rats

	SHAM	OVX	Tamoxifen	Rheum Extract		
				Dose I	Dose 2	Dose 3
Body weight gain (g)	72.63±11.80	104.2±26.5*	66.75±11.8**	78.23±6.68**	88.35±23.3**	80.88±28.4**
Uterine index	2.03±0.55	0.89±0.47*	1.15±0.37***	0.89±0.53*	0.82±0.30*	0.92±0.12*
Calcium content (g)	62.96±5.56	51.56±8.88*	60.31±3.77**	56.56±4.04*	60.96±6.53**	69.27±3.85**

*Significantly different with SHAM ($P<0.05$). **Significantly different with OVX ($P<0.05$)

Table 2: Lipid profiles of OVX Rats

	Sham	OVX	Tamoxifen	Rheum Extract		
				Dose I	Dose 2	Dose 3
Cholesterol	76.95±6.90	101.4±12.39*	78.82±16.14**	70.24±8.40**	74.44±11.37**	74.06±17.38**
Triglyceride	48.02±11.01	78.66±26.73*	65.81±10.62**	48.41±6.89**	57.33±21.67**	67.74±20.91**
HDL	49.10±8.01	39.22±8.39	50.79±12.96**	51.92±7.26**	55.07±11.13**	48.45±7.64**
LDL	18.24±10.85	46.42±22.15*	14.88±10.66**	8.63±7.80**	7.91±4.95**	12.06±11.99**

*Significantly different with SHAM ($P<0.05$). **Significantly different with OVX ($P<0.05$)

Effects of Rheum extract on blood pressure of ovariectomy rats

Table 3 shows that OVX increased systole and diastole of OVX rats but can be reduced by tamoxifen administration. The administration of Rheum extracts reduced systole and diastole.

Effects of Rheum extract on 4-methylproline and l-proline

Table 4 shows that OVX increased 4-methylproline and l-proline in model rats. Tamoxifen and Rheum extracts could reduce 4-methylproline and l-proline in OVX rats.

DISCUSSION

Four components of Rheum extract were identified as astringin, rhapontin, rhapontigenin, and desoxyrhaponticin with retention time 8.70, 10.25, 12.49, and 13.49 minutes, respectively, as shown in Figure 1. Rhaponticin shows the highest peak compared with other components. This indicated that the extract's effect was dictated by the high components of the extracts, as shown in another experiment that found a positive correlation between the content and the effects.[13-15]

Table 1 is shown that OVX increased body weight compared with SHAM rats. These results are similar to Iwasa *et al*.; they found that OVX-treated rats showed higher serum leptin levels.[16] The administration of tamoxifen and Rheum extracts does not affect the body weight of OVX rats. Some studies found that the estrogen effects are mediated through ERα and Erβ receptors, and lack of ERα in hypothalamic proopiomelanocortin neurons leads to overeating. There is a balanced ratio of these two receptors before menopause in women, but after menopause, this ratio is disrupted and is associated with increased ERβ signaling. Thus, it has been

Table 3: Blood pressure of OVX rats

	Sham	OVX	Tamoxifen	Rheum Extract		
				Dose 1	Dose 2	Dose 3
Systole (mmHg)	133.98±7.55	147.72±5.70*	133.25±3.64**	140.05±7.24	128.37±20.0**	138.5±9.46
Diastole (mmHg)	99.37±9.15	113.67±5.28*	97.67±6.53**	97.63±14.71**	94.72±18.91**	100.15±12.84

*Significantly different with SHAM ($P<0.05$). **Significantly different with OVX ($P<0.05$)

Table 4: 4-Methylproline and l-proline of OVX rats

	Sham	OVX	Tamoxifen	Rheum extracts
4-Methylproline (%)	7.02	24.31	10.11	10.88
L-Proline (%)	4.05	8.23	2.85	2.08

concluded that ERα signaling is involved in regulating food intake.[17-19]

Tamoxifen has both antagonistic and agonistic effects on the ER. It acts as a complete antagonist in the mammary glands, a partial agonist in the uterus, and a complete agonist in the bone and cholesterol metabolism. Tamoxifen reduces fat mass through boosting ROS.[20] *R. officinale* root extracts have no effects on body weight since *R. officinale* contained rhaponticin, a stilbene structure similar to tamoxifen. Therefore, *R. officinale* extract has a similar result with tamoxifen.[21]

Moreover, OVX made uterine atrophy; decreasing in estrogen lead the uterus to become small and fibrotic.[22] Tamoxifen could prevent uterine atrophy but not for *R. officinale* root extract-treated rats.[23] This result indicated that the *R. officinale* root extracts showed a different mechanism of action from tamoxifen on uterine cell proliferation.

Table 2 shows that the calcium content of the OVX rats decreases, and the administration of tamoxifen and *R. officinale* root extract could recover the calcium content. Tamoxifen prevented OVX by increasing urinary hydroxyproline or Ca and conserved bone.[24] The mechanism of Rheum extract in bone calcium has not been elucidated yet, but rhaponticin that contained in Rheum extract has a similar molecular structure with tamoxifen; they have a stilbenoid group.[15,25]

OVX increased blood pressure both systole and diastole; as shown in Table 3, plasma levels of NO metabolites, nitrites, and nitrates were reduced by the OVX.[26] The administration of tamoxifen and Rheum extract could recover blood pressure to normal value.[27]

The GC-MS result shows in Table 4; methyl-proline and L-proline were increased during OVX and recovered after administration of tamoxifen and *R. officinale* root extract. This result was similar to that of other researchers;[28,29] these amino acids increased, indicating that bone collagen type 1 synthesis increases the density of bone.

In our previous study, Rheum extract could stimulate osteoblastogenesis by increasing RUNX2, BMP2, and alkaline phosphatase.[17,18] This current *in vivo* results confirmed the previous work: the bone density of osteoporotic rats could increase by the administration of *R. officinale* root extract.

We suspected that substances in the extracts that similar to tamoxifen could stimulate osteoblastogenesis and increase bone density.[6] Tamoxifen contains a stilbene group which believes that it has selectivity when the acts on ERs.

We then evaluated the Rheum extract composition using ultra-performance liquid chromatography (UPLC)-MS [Figure 1] and found that the Rheum extract consists of astrigin, rhaponticin, rhapontigenin, and desoxyrhaponticin. All components have stilbene groups.

This result indicated that rhaponticin and derivatives in this extract could recover the bone density of OVX rats.

CONCLUSIONS

Rheum root extracts contain astrigin, rhaponticin, rhapontigenin, and desoxyrhaponticin. Rheum root extract could improve calcium content and lipid profiles of OVX rats by stimulation osteoblastogenesis. Rheum root extracts could control blood pressure of OVX rats by reducing lipid profiles.

Acknowledgment

We thank Prof. Jaehong Han and Mihyang Kim, Ph.D., from Chung-Ang University as host in Korea. We thank Jong-Suk Lee, Ph.D., from Biocenter, Gyeonggido Business and Science Accelerator, who evaluated the content of the extract by UPLC.

Financial support and sponsorship

This research was funded by International Collaboration Based Research Grant from Universitas Indonesia to AB.

Conflicts of interest

There are no conflicts of interest.

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