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

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

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

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. 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.

In 1993, dry extract of rhubarb (Rheum rhaponticum L.; Dahuang) was first used to treat symptoms of menopause. Rheum root dry extract mainly contains rhaponticin which has a stilbene backbone. Tamoxifen and other SERMs derive compounds have stilbene’s backbone. 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
The dried powder of Rheum roots was extracted according to the method of Bahtiar et al.

Chromatographic and mass conditions
Chromatographic analysis and mass spectrometry detection were carried out according to the method of Zhou et al. 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.

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.

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 rapienic tin 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, rapienic tin, rapienic genin, and desoxyrapienic tin. Rapienic tin 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.
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 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           |

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 to tamoxifen; therefore, R. officinale extract has a similar result with tamoxifen.[21]

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.[16] 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.

**REFERENCES**

1. Emam MN, Abo El Gheit RE. New treatment paradigm of combined raloxifene and conjugated estrogen for postmenopausal symptoms in VCD-induced menopausal rats. Alexandria J Med 2017;53:227-36.
2. Ushiroyama T, Ikeda A, Sakai M, Higashiyama T, Ueki M. Prevention of postmenopausal bone loss with exchange for short-term HRT for 1α-hydroxycholecalciferol. Maturitas 2003;45:119-27.
3. Saito N, Kawase K, Yamashita N, Tang Y, Wang Y, Wang J, et al. Identification of 10-dehydroxyglycyrrhetic acid as a potential human estrogen receptor alpha partial agonist. Bioorg Chem 2019;88:102977.
4. Sandberg K. HRT and SERMs: The good, the bad … and the lovely? Trends Endocrinol Metab 2002;13:317-8.
5. Müller ST, Pählig S, Merabet A, Abdelsamie AS, van Koppen CJ, Keiler AM, Papke A, Kretzschmar G, Zierau O, Vollmer G. Functional analysis of estrogen-related receptors and therapeutic potential. Phytochem Rev 2020; 21:1-19.
6. Zhang RX, Li MX, Jia ZP. Rehmannia glutinosa: Review of botany, chemistry and pharmacology. J Ethnopharmacol 2008;117:199-214.
7. Keiler AM, Papke A, Kretzschmar G, Zierau O, Vollmer G. Substrate specificity in vivo and in vitro in the formation of stilbenes. Biosynthesis of rhaponticin. Arch Biochem Biophys 1980;200:72-8.
8. Ruprich N, Hildebrand H, Kindl H. Substrate specificity in vivo and in vitro in the formation of stilbenes. Biosynthesis of rhaponticin. Arch Biochem Biophys 1980;200:72-8.
9. Saito N, Kawase K, Yamashita N, Tang Y, Wang Y, Wang J, et al. Identification of 10-dehydroxyglycyrrhetic acid as a potential human estrogen receptor alpha partial agonist. Bioorg Chem 2019;88:102977.
