Preventive Effect of Ethanol Extract of Red Spinach (*Amaranthus tricolor* L.) on Diet-induced Obese Zebrafish

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Authors' contributions

This work was carried out in collaboration among all authors. Author AY designed the study and wrote the first draft of the manuscript. Author AZP performed the data of in vivo, statistical analysis and wrote the protocol. Authors NS and IKA managed the analyses of the study. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background: Nowadays obesity recognized as chronic or non-communicable disease. Pathophysiology of obesity caused by an imbalance between energy intake and expenditure. Obesity was known to be a risk factor for the development of metabolic syndrome. The aim of this study is to evaluate activity of ethanol extract of red spinach (EERS) to prevent obesity in diet-induced zebrafish.

Materials and Methods: Acclimatization period for zebrafish was carried out for 2 weeks. After the acclimatization, zebrafish were divided into 6 groups (n = 10 in each group) such as normal group (negative control); obese group (positive control); standard drug (orlistat 4,5 µg/ml); EERS group (50 µg/ml); EERS group (100 µg/ml); and EERS group (200 µg/ml). During a period of 4 weeks, normal group received a standard diet and didn't received EERS administration. Positive control

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group received Artemia. Treated group received *Artemia* and were combined by administration of EERS. To determine obesity criteria we calculated of zebrafish BMI.

Results: Based on BMI calculation, EERS 50 µg/ml, 100 µg/ml and 200 µg/ml showed a preventive effect on obesity compared to the positive control group. In addition, EERS 50 µg/ml was able to reduce BMI lower than the other extract groups.

Conclusion: It can be concluded that EERS 50 µg/ml has strength preventive effect on diet-induced obese zebrafish. This effect might be influenced by the presence of phytochemical compounds of extract such as flavonoid, saponins, and tannins.

1. INTRODUCTION

The prevalence of obesity in the worldwide still increased every year. Increased prevalence of obesity has contribution against increased morbidity and mortality [1,2]. Obesity was known as a complex disorder or non-communicable disease, characterized by excessive fat storage in adipose tissue caused by imbalance between energy intake and its expenditure [3,4]. Obesity has association with metabolic syndrome, such as type two diabetes mellitus (T2DM), hypertension, dyslipidemia, cardiovascular diseases, cancer, and non-alcoholic liver disease (NAFLD) [5,6].

Several studies in human and animal (i.e. rodents) explained that obesity has relationship with excessive diet intake. Excessive diet intake both high carbohydrate diet and high-fat diet influence excessive fat accumulation in adipose tissue [7-11]. In addition, excessive fat accumulation in adipose tissue released pro-inflammatory cytokines which responsible against the presence of metabolic syndrome [12]. Therefore, animal model study especially obesity became important for depth explored and to obtain effective treatments against obesity [13,14].

Obesity and its treatment have been studied in several animal model. Currently, animal model for obesity could developed using zebrafish (*Danio rerio*). Zebrafish is a good model system for developmental biology, genetic studies, and biomedical research [15,16]. Zebrafish has the similarities gene, physiological, neural and endocrine signals as humans, so zebrafish could be considered as established model for obesity [17,18].

Red spinach (*Amaranthus tricolor* L.) is purple red color vegetable with several pharmacological activities. The pharmacological activities of red spinach including antioxidant, antibacterial, hepatoprotective, diuretic, and hypolipidemic [19]. Red spinach use in society to reduce body weight gain have been reported. Therefore, the aim of this study is to evaluate of red spinach activity to prevent obesity in diet-induced obese zebrafish.

2. MATERIALS AND METHODS

2.1 Plant Material and Identification

Red spinach leaves were obtained from Balai Penelitian Tanaman Rempah dan Obat (BALITTRO), Bogor, West Java, Indonesia. Identification of red spinach leaves was carried out at Lembaga Ilmu Pengetahuan Indonesia (LIPI), Jakarta, Indonesia.

2.2 Extraction Process

Red spinach leaves were dried, cutted, and grounded to be soft. Furthermore, red spinach leaves was extracted with 96% ethanol by maceration and evaporated through rotary evaporator at 50^0C, speed of 50 rpm. Extract were used for *in vivo* study using zebrafish and phytochemical screening.

2.3 Phytochemical Screening of Extract of Red Spinach

Phytochemical screening of extract of red spinach leaves were performed to observe the presence of phytochemical constituents such as alkaloids, flavonoids, saponins, tannins, quinones, and steroids/triterpenoids.

2.4 Experimental Animal

Adult male zebrafish (*Danio rerio*) were obtained from Bandung, West Java, Indonesia. All zebrafish in this study were maintained in a controlled environment (involves 12:12 light: dark cycle, room temperature at 26^0C, pH 7,5). Water quality maintained according to zebrafish

guidelines. Animal study was conducted on Laboratory of Pharmacology, Faculty of Pharmacy, Bhakti Kencana University.

2.5 Experimental Design

Acclimatization for zebrafish was carried out for 2 weeks. During acclimatization, all zebrafish received standard diet (*Tetrabite*). After the acclimatization, zebrafish were divided into 6 groups (n = 10 in each group) such as normal group (negative control group); obese group (positive control group); standard drug (orlistat 4,5 µg/ml); EERS group (50 µg/ml); EERS group (100 µg/ml) ; and EERS group (200 µg/ml). For a period of 4 weeks, normal group received a standard diet and didn't received EERS administration. Positive control group received *Artemia*. Treated group received *Artemia* and were combined by administration of red spinach (preventive method). The obese group and extract-treated group was given 60 mg/group/fish in experimental diet. In this study, we use Artemia to induce obesity in zebrafish. Overfed using *Artemia* based on the previous study [17].

2.6 Determination of Body Mass Index (BMI)

Zebrafish weight and length were measured every week (2 times/week). Body length of fish was measured from head to end of the body. Zebrafish body weight and length were used for BMI measurement. BMI measurement needed to determine of obesity criteria. BMI of fish was calculated by weight (g) divided with the square of body length (cm). Obesity criteria on male zebrafish (BMI > 1.1 fold) and female zebrafish (BMI > 1.3 fold) [17].

2.7 Statistical Analysis

Data were expressed as mean $\pm$ standard deviation (SD). Statistical analysis was performed using One Way ANOVA coupled by post hoc test Tukey HSD with significance difference ($p<0.05$).

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening of Extract of Red Spinach

The result of phytochemical screening showed that extract of red spinach contained the presence of flavonoids, tannins, and saponins (Table 1). Based on the previous study were performed by Iswantini et al. [20]. explained that chemical components in plants such as flavonoids, saponins, alkaloids, tannins, and steroids/triterpenoids have potential effect as anti-obesity.

3.2 Effect of EERS on Body Weight and BMI

As expected, at the first, second, third, and fourth week of the experiment, the positive control group had significant gains in body weight and BMI after administration of *Artemia* 60 mg/group/fish. Body weight of positive control group at the first, second, third, and fourth week such as 0,248±0,053; 0,385 ± 0,007; 0,375 ± 0,05; 0,376 ± 0,02, respectively (Fig. 1).

In particular, as shown in Fig. 1, at the fourth week, the body weight decreased in EERS dose 50 µg/ml compared to the positive control group (0,265 ± 0,006; $p < 0,05$). The body weight of positive control group at the fourth week such as 0,376 ± 0,022. Furthermore, EERS 100 µg/ml and 200 µg/ml showed modest decrease in body weight

In this study, BMI showed the same pattern with the body weight. Fig. 2 showed EERS 50 µg/ml, 100 µg/ml and 200 µg/ml showed a preventive effect on obesity during treatment. Furthermore, EERS 50 ppm was able to reduce BMI lower than other EERS group. EERS 50 µg/ml showed strong effect by statistic at fourth week compared to the positive control group (0,026 ± 0,05). In addition, orlistat as the standard drug in this study, also showed its effect to decrease body weight and BMI at 4,5 µg/ml.

Table 1. Result of phytochemical screening of EERS

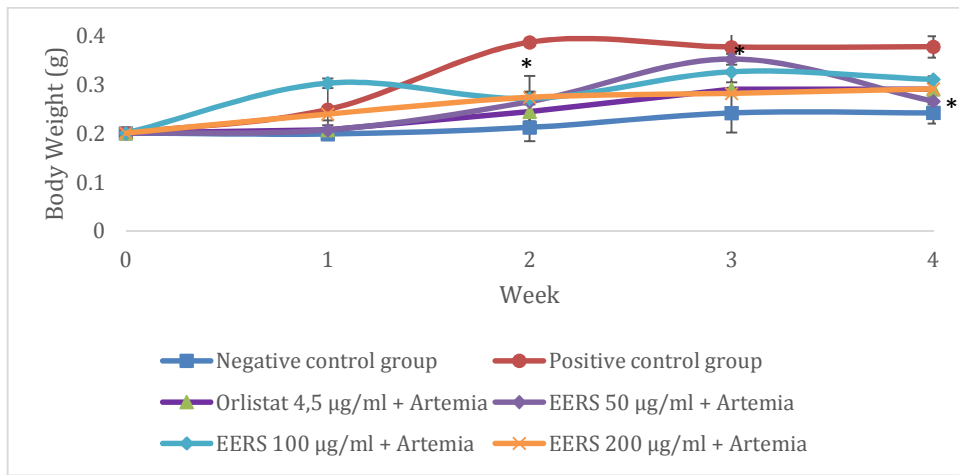| Compounds	Results
Alkaloids	-
Flavonoids	+
Saponins	+
Tannins	+
Quinones	-
Steroid/Triterpenoid	-

(+) : detected

Fig. 1. Effect of EERS on body weight. (*) Results as expressed as mean ± SD from 3 zebrafish, significantly difference with positive control group, *p* < 0,05
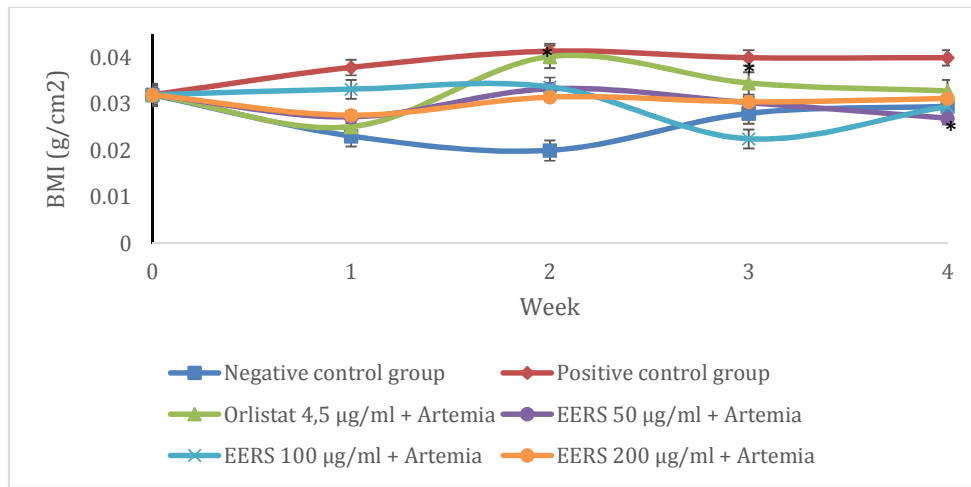

Fig. 2. Effect of EERS on BMI. (*) Results as expressed as mean ± SD from 10 zebrafish, significantly difference with positive control group, *P* < 0,05
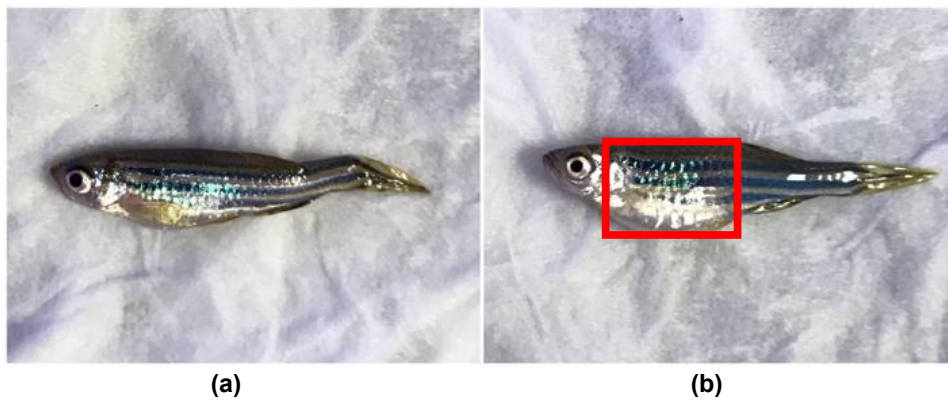

| (a) | (b) |

Fig. 3. (a) Normal male zebrafish and (b) obese male zebrafish. Red box indicated excessive fat accumulation in abdominal area

4. CONCLUSION

Based on the results of study, it can be concluded that EERS 50 µg/ml has strength preventive effect on diet-induced obese zebrafish. Therefore, red spinach use in society should be considered, because its great effect to help reduce body weight, especially in obesity condition.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All procedures in this study has been accepted by Animal Ethic Committee Universitas Padjajaran, Bandung, Indonesia (No. 561/UN6.KEP/EC/2020).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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