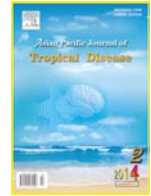


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The physiological response of obese rat model with rambutan peel extract treatment

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ABSTRACT

Objective: To determine body weight gain, expression of Igf-1 and Igf-1 receptor on obese rat model treated with rambutan peel extract (RPE) as a physiological response.

Methods: Normal and obese rat feed with normal and high calorie diet around 12 weeks and continued to treat with ellagic acid, RPE 15, 30 and 60 mg/kg body weight respectively. Physiological responses observed were weight gain and expression of Igf-1 with its receptor. Body weight of rat was weighed once per week. Expression of Igf-1 and igf-1R observed with fluorescence immunohistochemistry. The intensity of Igf-1 and Igf-1R expression was analysis using FSX-BSW software.

Results: The lowest weight gain was obtained on obese rat model treated with RPE 30 mg/kg body weight. The expression of Igf-1 and Igf-1R were reduced on obese rat model treated with RPE compared with obese rat model of non treatment ($P < 0.05$). The low expression of Igf-1 and Igf-1R was found on obese rat model treated with ellagic acid and RPE 30 mg/kg body weight.

Conclusions: The RPE was effecting to the physiological response on obese rat model. The RPE 30 mg/kg body weight inhibited body weight gain and decreased the expression of Igf-1 and Igf-1R of obese rat model.

1. Introduction

Obesity is increasing worldwide and rapidly becoming a health problem of epidemic proportions. The prevalence of overweight and obesity has increased sharply in both adults and children in two decade. It is predicted that worldwide prevalence of obesity showed a significant increase. Currently 1.6 billion adults worldwide are overweight and at least 400 million of them are obese. In 2015, an estimated of 2.3 billion adults will be overweight and 700 million of them will be obese[1].

This is believed to be related to the excessive consumption of food rich in calories related with worst eating habits such binge eating, night eating, high fructose corn syrup, alcohol abuse, smoking, *etc.* Good eating habits by healthy food and food rich in antioxidants may play a protective role against metabolic diseases. It is known that equilibrium between oxidants and antioxidants is crucial to the body. The advantage of antioxidant substances is one important factor to control individual healthy life that has a protective effect at level population[2].

Polyphenols are the most abundant antioxidants and their intake is 10 times higher than the intake of vitamin C and 100 times higher than that of vitamin E or carotenoids. Phenolic compounds act as capture electrons from reactive oxygen species and prevent elevation of its activity[3,4]. Furthermore, phenolic compounds such as flavonoid and tannin are particular that can capture metals like iron

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involved in free radical formation^[5]. Indirectly, polyphenols can interfere with the cellular detoxification systems, such as superoxide dismutase, catalase or glutathione peroxidases^[2]. Rambutan (*Nephelium lappaceum*) is a tropical fruit, which is widely grown in Indonesia. Its production reached 811 993 tons per year^[6]. On 1 g of rambutan fruit, as much as 0.4 g can be consumed while the rests are peels and seeds. Thus 487 195.8 tons of wastes are produced and not used even though rambutan peel contains compounds with strong antioxidant activity^[7,8]. Recently our study found that the main phenolic compounds are tannins and flavonoids^[9,10], which were identified as geraniin, coraligin, and ellagic acid (EA)^[8]. This study focused on physiological response with parameter body weight gain, expression of Igf-1 and Igf-1 receptor on obese rat model treated with rambutan peel extract (RPE).

2. Materials and methods

2.1. Ethical consideration

The study was approved by ethical review committee of Brawijaya University Research Ethics Committee as a member of National Research Ethics Committee of Republic Indonesia.

2.2. Material preparations

This research was used 12-week old normal and obese male rat model. Obesity was determined by Lee Index^[11]. The rambutan peel was extracted with ethanol and dried by rotary evaporator, then dissolved with corn oil and water. There were two prepared-control treatments, placebo as negative control and ellagic acid as positive control. The ellagic acid used in this study obtained from Sigma catalogue 14668.

RPE solution was prepared by weighed the 0.1 g RPE added 2 drops of corn oil stirred until homogeneous and sterile aquadest distilled water was added to the stock of RPE obtain of the concentration of 1%. Then RPE treatment dosage 15, 30 and 60 mg/kg body weight was devised by picking up 15, 30 and 60 mL of stock solution plus 85, 70 and 40 mL distilled water respectively. Standard volume of given was body weight of rat multiplying by % concentration.

2.3. Subject

The rats obtained from Wistar Laboratory, Bandung-

Indonesia, were divided into six treatment groups: non-treatment (NT), placebo (P), EA 15 µg/kg body weight (relation water/corn oil), and three doses of RPE (15, 30, 60 mg/kg body weight). The animals were kept in standard rat cages at Biosains Laboratory. All normal rats were fed with normal diet whereas obese rats were fed with phokphan 551 as a high-calorie source of diet. The RPE treatment was given using oral administration method every two days for 12 weeks. The group of obese rats was continuously fed *ad libitum* with high calorie diet while the normal rats received standard diet without physical exercise. The rat body weight gain, food intake, and amount of feces were measured once per week. After 12 weeks, the rats were sacrificed based on standard protocol from university research ethics. Visceral fat was taken from the posterior caudal and 0.5 g visceral fat was fixed by 4% paraformaldehyde hereafter embedded with liquid paraffin. Paraffin blocks were sliced to get microanatomy slide. The histology slides were stained by Hematoxylin-Eosin to measure adipocyte. The expression of Igf-1 receptor and ligand was determined by immunofluorescence with specific antibody.

2.4. Measurement of weight gain

Body weight of rat was weighed with triple beam equipment once per week until 12 weeks. Food consumptions and feces production of rats at metabolic cages were measured on Week 1, 4, 10 and 12 respectively.

2.5. Immunofluorescence analysis

The expression of Igf-1 and Igf-1R observed by immunofluorescence double staining used standard protocol method of Bancroft and Gamble^[12]. We used primary antibodies IgG mouse for Igf-1 and IgG rat to receptor (Lifespan Bioscience). The primary antibody was dissolved in 2% bovine serum albumin (1:1500) then incubated at room temperature for 1 h. The secondary antibody used goat-anti-mouse IgG-FITC and goat-anti-rabbit IgG-Rhod (Santa Cruz Biotechnology) which was dissolved in 2% bovine serum albumin (1:2000). The slides were incubated for 1 h at room temperature and washed with phosphate buffer solution three times for 10 min. Once dried, the immunofluorescence visualization could be performed. Result of immunofluorescence was visualized by Olympus FSX100 microscope and analyzed using FSX-BSW program to determine Igf-1 and Igf-1R expression in visceral adipocyte.

2.6. Statistical analysis

One-way analysis of variance (ANOVA) was used for statistical analysis of data. The regression analysis was showed correlation between the amount of feed consumed and the amount of feces produced to the body weight gain. *T*-test was performed to analyze the different of food calorie intake between groups. Duncan's multiple range tests was used for determining the significance. A probably value of $P < 0.05$ was considered as a significant.

3. Results

RPE treatment caused a reduction in weight gain compared to non-treatment. The results of the *t*-test showed that there was significantly different between calorie intake and weight gain on normal and obese rats. There was a correlation between treatment and body weight gain. Treatment with RPE reduced body weight gain significantly at all concentrations. At 30 mg/kg body weight, the gain was reduced by about 25%. Similar to 15 µg/kg body weight of EA, 15 mg/kg body weight of RPE had a smaller effect and, surprisingly, 60 mg/kg body weight of RPE also had a smaller effect compared to non-treated animals and to the placebo control ($P < 0.05$). Obese rats consumed significantly less food, but considering the higher calorie content, this difference was reversed in that the obese rats consumed 30% more calories. Food consumption was not significantly affected by any of the treatments. Obese rats produced approximately 60% fewer feces which may be partially explained by the lower consumption in grams, however there was no correlation with increasing body weight gain.

The results of the ANOVA showed that there was a significant difference between the amount of feed consumed and the amount of feces produced on normal

and obese rats. The results of the regression analysis showed that there was no correlation between the amount of feed consumed and the amount of feces produced to the body weight gain. There was tendency that the less calories intake which was consumed made decreased body weight gain (Figure 1). As expected, the microanatomy slides of visceral fats showed that the adipocytes in obese rats model were significantly larger. After RPE treatment, adipocytes appeared smaller in obese rats (Figure 2).

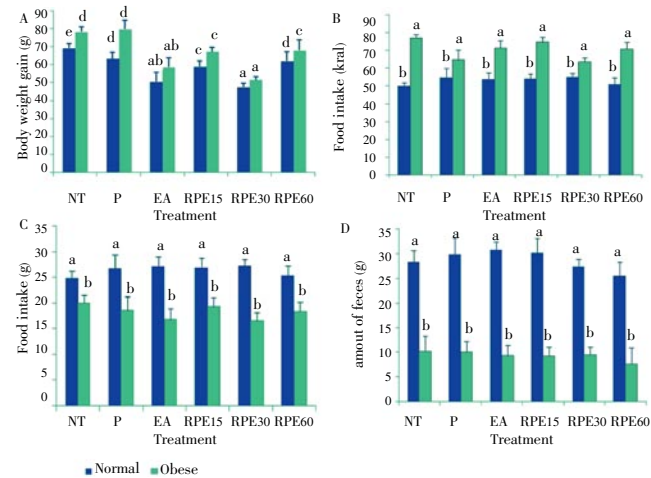


Figure 1. Differences between food calorie intake and body weight gain.

A: Average of body weight gain after 12 week treated RPE; B: Average of food intake in calorie after conversion on gram daily; C: Average of food intake on gram daily; D: Average amount of feces daily.

Igf-1-labeled FITC and Igf-1R-labeled Rhod were monitored in visceral fat tissue (Figures 3 and 4). While staining was strong in non-treated obese rats, there was a significant reduction of both Igf-1 and Igf-1R in tissues of rats treated with 30 mg/kg body weight RPE or EA. At the same time, the size of adipocytes appeared to be smaller. The analysis of variance showed that the RPE 30 mg/kg body weight treatment decreased the Igf-1 expression by approx. 44% compared to the other treatments ($P < 0.05$). There was a significant difference on Igf-1R expression among all the treated rats. The RPE treatment showed decreased around 40% the Igf-1R expression compared to

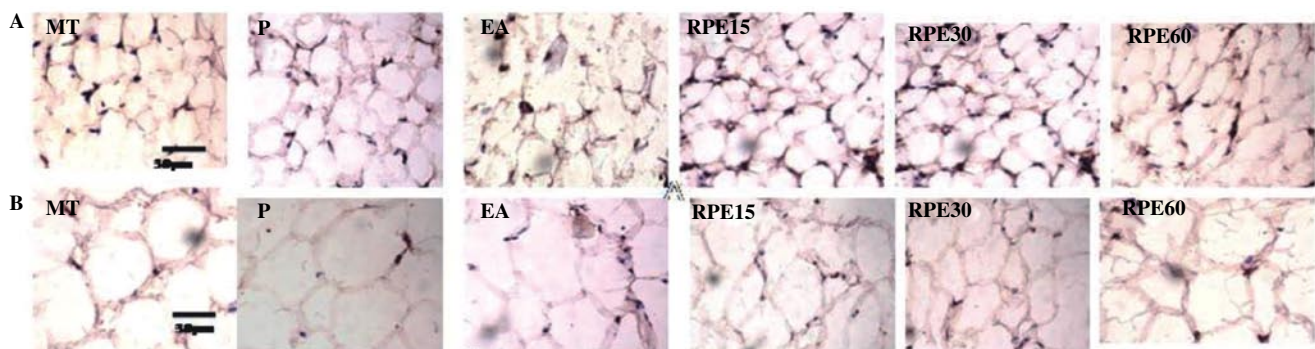


Figure 2. The comparative size of adipocyte on normal and obese rats treated with RPE.

A: adipocyte on normal rats; B: adipocyte on obese rats. NT: non-treatment; P: placebo; EA: ellagic acid 15 µg/kg body weight; RPE15: dosage 15 mg/kg body weight of RPE; RPE30: dosage 30 mg/kg body weight of RPE; RPE60: dosage 60 mg/kg body weight of RPE (Scale: 30 µm, magnification : 400×).

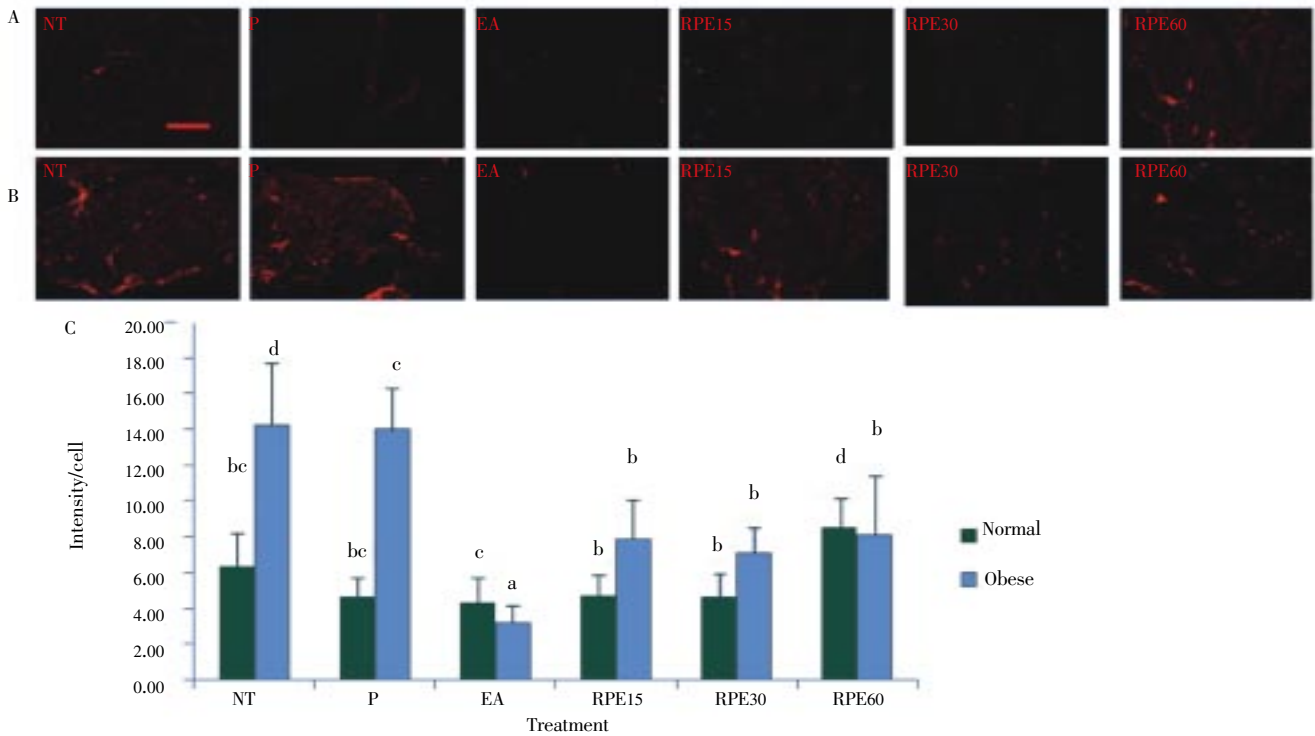


Figure 4. High Igf-1R expression in non-treated and placebo on visceral fat and low expression Igf-1R in control positive and treated on visceral rats exposed by immunofluorescence with of Igf-1R-Rhod on normal and obese rats. A: Normal rats; B: Obese rats. NT: Non-treatment; P: placebo; EA: ellagic acid 15 µg/kg body weight; RPE15: dosage 15 mg/kg body weight of RPE; RPE30: dosage 30 mg/kg body weight of RPE; RPE60: dosage 60 mg/kg body weight of RPE. (Scale: 2 µm, magnification: 100×). C: Bar scale of expression Igf-1R on normal and obese rats.

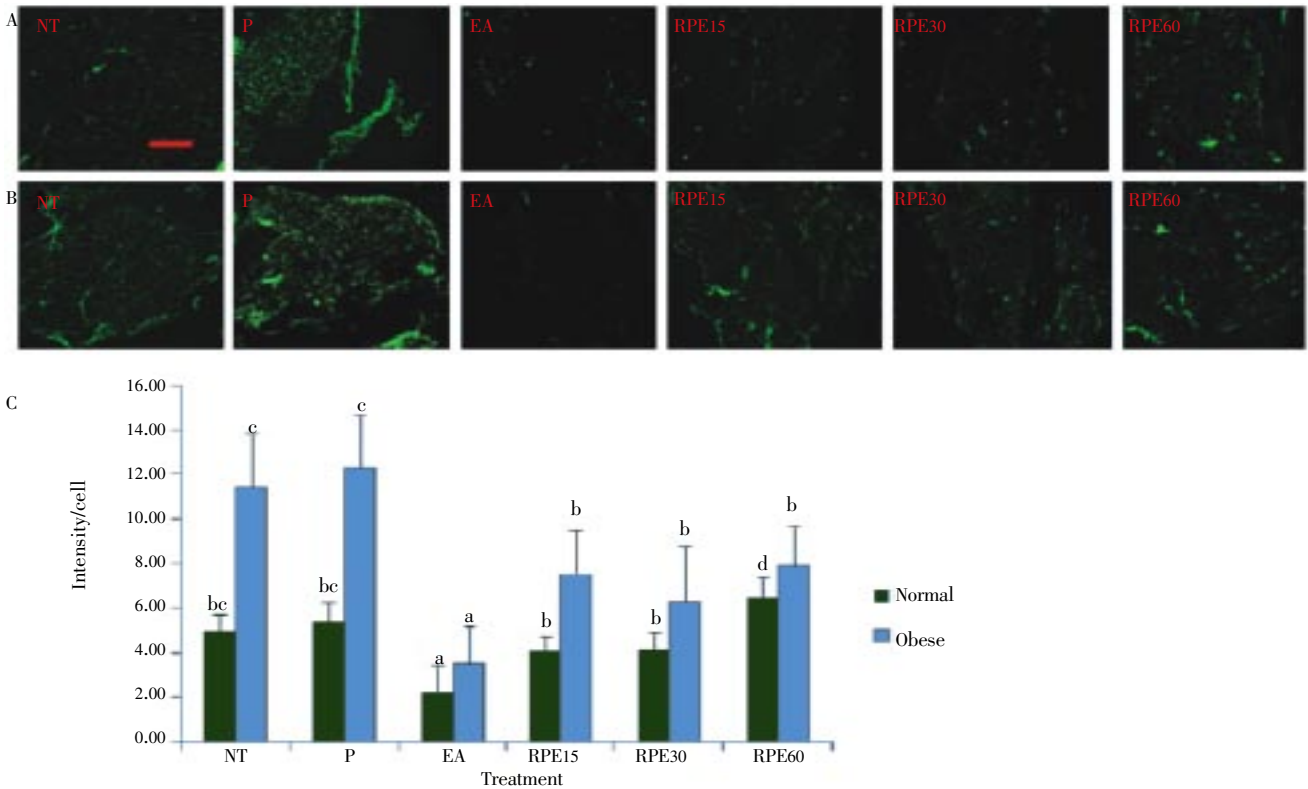


Figure 3. The high of Igf-1 expression of non-treated and placebo on visceral fat and low expression Igf-1 in control positive and treated on visceral rats exposed by immunofluorescence with Igf-1-FITC on normal and obese rats. A: Normal rats; B: Obese rats. NT: Non-treatment; P: Placebo; EA: Ellagic acid 15 µg/kg body weight; RPE15: dosage 15 mg/kg body weight of RPE; RPE30: dosage 30 mg/kg body weight of RPE; RPE60: dosage 60 mg/kg body weight of RPE. (Scale: 2 µm, magnification: 100×). C: Bar scale of expression Igf-1 on normal and obese rats.

the obese rats. The micrographs suggested a decrease in Igf-1 and Igf-1R with EA and 30 mg/kg RPE treatment, and

that is much more pronounced in the obese rats compared to the normal rats.

4. Discussion

Many researchers recently report that bioactive compound of herbal is one alternative of anti-obesity in animal's model. The black tea treatment could decrease body weight in rats^[13–17]. The previous findings confirmed that the catechins are more than caffeine at clinically appropriate doses, affecting lipid metabolism in non-obese and obese subjects^[15,18,19].

The putative active phytochemical contents of RPE are flavonoid and tannins *i.e.* ellagic acid may play an important role in affecting body weight gain. Our research showed that RPE reduced of body weight gain on obese rat model. In previous study, RPE was reduced body weight gain on normal rats^[9]. Tannins group has specific function to recognize carbonyl of some peptides that mainly exert their effects on proteins and carbohydrates^[20,21]. They may cause substrate deprivation and/or enzyme inhibition^[21]. Further, the formation of complexes with proteins and carbohydrates renders nutrients inaccessible to microorganisms. Tannins are also chelating agents, and this could reduce the availability of certain metallic ions necessary for the metabolism of intestinal microorganisms^[20]. Furthermore, tannic acid may exert anti-nutritional effects by binding to proteins of the gut wall and interfering with gut function rather than by inhibition of dietary protein digestion^[22].

Reducing weight gain as the result of RPE 30 mg/kg body weight treatment was presumed to cause an inhibition on Igf-1 expression. Igf-1 is a hormone that plays a role in stimulating of the transcription factor of pre-adipocytes into mature adipocytes. Feeding high-calorie and high-fat triggers the expression of Igf-1^[13,23,24]. The inhibition on Igf-1R due to the provision of polyphenols causes weight loss in mice fed a high-fat diet^[13]. This study showed decreasing Igf-1 and Igf-1R expression in rats treated with RPE 30 mg/kg body weight. This is thought to inhibit the activation of MAP Kinase which results in adipogenesis inhibition.

Since obesity is an essential component of metabolic syndrome, it is important to maintain optimal body weight and to avoid the onset of obesity. Weight loss strategies include dietary therapy, physical therapy, pharmacotherapy, and surgery. All these therapies are costly, time consuming and tend to be non-sustainable. RPE may prevent the onset of obesity by efficiently reducing body weight gain before an extensive therapy is required or to prevent regain of body weight after a weight loss therapy. RPE could be a promising food ingredient to suppress weight gain and its long-term consumption could contribute to the maintenance of optimal weight.

There was no appreciable change in calorie intake depending on the treatment. However, the treatment seemed to affect the weight gain of rats, especially with 30 mg/kg

body weight of RPE, whereas 15 mg/kg body weight and 60 mg/kg body weight had a smaller effect. The lowest weight gain was observed with 15 µg/kg body weight of pure EA as has been previously described^[25]. The reduced weight gain observed with ellagic acid and RPE may be caused by the suppression of lipid absorption, reduction in biosynthesis of fatty acid, or enhancement of fatty acid oxidation. Clearly, the intake of calories was not changed with EA or RPE treatment.

We also investigated the accumulation of Igf-1 and Igf-1R expression in the treated and non-treated animals because these proteins are involved in the adipogenesis process. It appears that RPE function is upstream from Igf-1 and Igf-1R expression because, especially in obese rats, we observed a pronounced reduction of the proteins in EA and RPE treated animals. Consequently, the size of adipocytes was also reduced after treatment.

Based on the literature, EA was considered the main active ingredient for reduction of body weight gain^[26–28]. The comparison of RPE and pure EA treatment revealed that there was apparently a combination effect of EA and other compounds in RPE since the calculated dosage of EA was much higher than described as effective in the literature^[29]. This is a new finding that conducted RPE can use as a basic of further research about anti-obesity therapy. Further experiments are required to elucidate the mechanism of RPE action and to understand if the activity is caused by EA alone or rather by a combination of bioactive substances in the RPE.

In summary, our results showed that RPE can inhibit body weight gain and also suppressed the expression of Igf-1 and Igf-1R in obese rat model.

Conflict of interest statement

We declare that we have no conflict of interest.

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