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The effect of epigallocatechin 3-gallate on body weight and abdominal fat of white rats (*R. norvegicus*) exposed to monosodium glutamate

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Abstract: MSG can caused obesity that can affect metabolism in the body. The administration of EGCG can increase energy expenditure and metabolism. This study aimed to determine the effect of epigallocatechin 3-gallate (EGCG) on body weight and the percentage of abdominal fat of white rats (*R. norvegicus*) exposed to monosodium glutamate (MSG). Twenty-five rats were divided into five treatment groups. The C- group was given only aquadest and Sodium carboxymethyl cellulose (CMC-Na) 1%. The C+, T1, T2, and T3 groups were given MSG 120 mg/kg/BW and CMC-Na 1%, and EGCG at 4, 8, and 16 mg/kg/BW, respectively. All treatments were given orally for 28 days. The results showed that administration of MSG tends to be followed by an increase in body weight, except in group T2 where body weight was relatively stable. The administration of MSG 120 (to the C+ group) increased significantly ($p<0.05$) the percentage of epididymal fat and peritoneal fat. The administration of EGCG 8 (to the T2 group) significantly reduced ($p<0.05$) the percentage of retroperitoneal, epididymal, and peritoneal fat, compared to the group exposed to (C+ group). The percentage of retroperitoneal fat and epididymal fat was significantly lower ($p<0.05$), but the percentage of peritoneal fat was not significantly different ($p>0.05$) compared to normal mice (C-). The administration of EGCG 16 (to the T3 group) followed a significant increase ($p<0.05$) in retroperitoneal fat and epididymal fat, but the percentage of peritoneal fat was not significantly different ($p>0.05$), compared to (T2 group). It could be concluded that the administration of EGCG 8 mg/kg BW/day reduced the weight of retroperitoneal fat, epididymal fat, and peritoneal fat compared to mice given MSG alone. This research is expected to become the main reference for product processed from the substance EGCG which can reduce body weight and abdominal fat.
Keywords: cardiovascular disease, epididymal, obesity, peritoneal, retroperitoneal.

1. Introduction

Obesity is a condition with abnormal or excessive fat accumulation in adipose tissue (Panuganti et al., 2023). Obesity is associated with abnormal serum lipoprotein levels. In obese people, triglyceride levels in the blood are higher than in people who are not obese (Zou et al., 2022). One thing that is related to obesity is the consumption of food containing monosodium glutamate (MSG). MSG can improve the taste of food, increase food intake, affect the body's metabolism, and cause glucose intolerance and insulin resistance, thereby affecting energy balance and damaging the hypothalamic signaling cascade of leptin (Niaz et al, 2018; Kayode et al., 2023). Body fat, especially abdominal fat is strongly associated with risk factors for cardiovascular disease (Elffers et al., 2017).

Men have a higher tendency to accumulate abdominal visceral fat. Abdominal visceral fat accumulation in men is a strong independent predictor of mortality. Dietary fat is absorbed by enterocytes and transported into the circulation in the form of chylomicrons and very low-density lipoproteins. Chylomicrons in men are generally larger and in greater quantity than in women. For 1-2 hours after eating, these chylomicrons saturate the lamina propria and low-pressure lymphatic system, where they are hydrolyzed by lipoprotein lipase. The liberated fatty acids are then stored by nearby abdominal visceral adipocytes, leading to the accumulation of abdominal visceral fat (Nauli and Matin, 2019). Abdominal fat consists of retroperitoneal, epididymal, and peritoneal fat (Chusyd et al., 2016). MSG has effects on glucose tolerance, insulin sensitivity, and redox balance and is followed by obesity (Bautista et al., 2019).

Antioxidant compounds that have been studied in rats and have obesity-fighting activity are resveratrol, curcumin, quercetin, and anthocyanins (Khutami et al., 2022). The compound (-)-Epigallocatechin-3-gallate (EGCG) is the main polyphenol of green tea that has a variety of actions. EGCG acts as a powerful antioxidant that effectively scavenges reactive oxygen species (ROS), inhibits pro-oxidant enzymes including NADPH oxidase, activates antioxidant systems including superoxide dismutase, catalase, or glutathione, and reduces the production of abundant nitric oxide metabolites by inducing nitric oxide in human (Kim et al., 2014). Epigallocatechin 3-gallate (EGCG) is a nutraceutical that results in a decrease in body mass index, visceral fat levels, and body fat

in humans (Yoshitomi et al., 2021). Several studies show that EGCG can reduce weight compared to animals fed high-fat diets (HFDs) or genetically obese/diabetic animal models. EGCG decreased body weights and adipose tissue indices in both subcutaneous and epididymal adipose tissues in mice (Li et al., 2018). EGCG treatment improves glucose tolerance and causes weight loss in mice. Interestingly, EGCG activated autophagy and lipolysis in white adipose tissue in a diet-induced obesity mouse model, leading to a reduction in visceral adiposity, through an AMPK-mediated mechanism (Choi et al., 2020).

Research conducted by Mawarti, et al (2012), proved that giving EGCG at 8 mg/kg/BW was able to reduce the weight of visceral fat by 48.99% when compared to the positive control group of white rats which was close to the negative control group of white rats. The present research uses higher doses of MSG than previous research (Jubaidi et al., 2019) and it is aimed to determine the ability of EGCG as an anti-obesity agent in rats, which can reduce fat and body weight by suppressing fat/adipogenesis and absorption of fatty acids into adipose tissue by increasing the synthesis and oxidation of fat in the liver.

2. Material and Methods

The study was conducted in the experimental animal cages of the Faculty of Medicine, Universitas Airlangga, Surabaya, and the Laboratory of the Veterinary Pathology Division, of the Faculty of Veterinary Medicine, of Universitas Airlangga, Surabaya, from February to March 2024.

Ethical Approval

All procedures of this study received approval from the Research Ethics Committee, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, with Ethical Clearance Number: 1.KEH.024.02.2024.

Treatment of rats

This research used 25 male Wistar strain white rats (*R. norvegicus*) aged 2-3 months with an average body weight of 125-250 grams that were healthy, active, had no injuries, and had never been used for research. White rats were divided randomly into five treatment groups, with five mice in each group. Group C-: rats were given distilled water 0.5 ml/rat/day, and CMC Na 1% 0.5 ml/rat/day four hours later. Groups C+, T1, T2, and T3: mice were given MSG intake of 120 mg/kgBW/rat/day in 0.5 ml of distilled water (Kurtanty et al., 2019) and four hours later were given 0.5 ml of CMC Na 1%, and EGCG at 4, 8, and 16 mg/kg BW/day (Mawarti et al., 2012).

On the 29th day, rats were given an anesthesia procedure via injection with ketamine 7.5 mg/100gBW and xylazine 1 mg/100gBW intramuscularly. The next step is to rupture the aorta and harvest the organs for other research. Abdominal fat consists of retroperitoneal fat, epididymal, and peritoneal fat, taken and placed in a plastic medicine pot and weighed using a TN series digital mini scale with an accuracy of 0.01 gram. Abdominal fat units are expressed as % (W/W) of the rat's body weight.

Body weight is measured using a Harnic digital scale which was carried out at 09.00 a.m once a week before being given food. Abdominal fat weight is measured using a medicine pot containing each sample of retroperitoneal fat, epididymal fat, and peritoneal fat using a TN series digital mini scale with an accuracy of 0.01 grams after the anesthesia and surgery procedure.

Data analysis

Data analysis in this study used the one-way analysis of variance (ANOVA) test followed by Duncan's test at a significance level of 5%. Statistical analysis uses the Statistical Product and Service Solution (SPSS) version 23 application.

3. Results

In general, giving an MSG intake of 120 mg/KgBW/day for 28 days shows a trend of increasing body weight and abdominal fat percentage. Giving several doses of EGCG to rats fed 120 mg/KgBW/day of MSG for 28 days showed a relatively stable body weight trend and a decrease in the percentage of abdominal fat.

Body weight

The body weight of rats between groups and between weighing times for four weeks did not show significant differences ($p>0.05$). However, there was a trend of weight gain at five weights (before treatment, first, second, third, and fourth weeks after treatment) in the C+ and T1 groups, and tended to be stable in the C- and T2 groups (Figure 1).

Abdominal fat

Administration of MSG 120 mg/KgBW/day for 28 days (C+ group) had no effect ($p>0.05$) on the percentage of retroperitoneal fat, but increased ($p<0.05$) the percentage of epididymal fat and peritoneal fat. Administration of EGCG 8 mg/kg BW/day for 28 days (T2 group) reduced ($p<0.05$) the percentage of retroperitoneal, epididymal, and peritoneal fat compared to the group exposed to MSG alone (C+ group). The percentage of retroperitoneal fat and epididymal fat was lower ($p<0.05$), but the percentage of peritoneal fat was not significantly different ($p>0.05$) compared to normal mice (C-). Administration of EGCG 16 mg/kg BW/day for 28 days (T3 group) followed an increase ($p<0.05$) in retroperitoneal fat and epididymal fat, still the percentage of peritoneal fat was not significantly different ($p>0.05$) compared to the administration of EGCG 8 (T2 group) (Table 1).

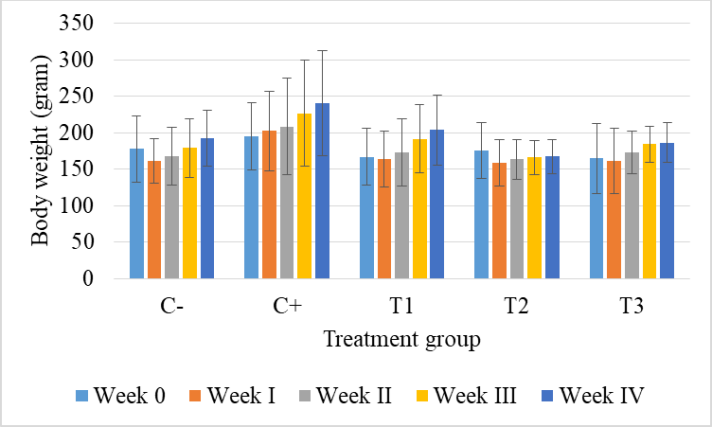

Figure 1 – Rat body weight (grams) by adding MSG and EGCG to rat feed at weeks 0 (before treatment), I, II, III, and IV. K(-): Rats given distilled water and CMC Na 1%; K(+): Rats given MSG 120 mg/KgBW; T1, T2, T3: Rats given MSG 120 mg/KgBW and EGCG at 4, 8, and 16 mg/KgBW.

Group	Fat (%)		
	R	E	P
C-	$3,26^{c}\pm0,88$	$1,69^{b}\pm1,06$	$0,66^{a}\pm0,36$
C+	$2,84^{bc}\pm0,77$	$3,15^{c}\pm1,16$	$1,65^{b}\pm0,69$
T1	$2,30^{b}\pm0,45$	$3,40^{c}\pm0,59$	$1,04^{a}\pm0,65$
T2	$1,20^{a}\pm0,21$	$0,29^{a}\pm0,22$	$0,82^{a}\pm0,23$
T3	$2,24^{b}\pm0,78$	$1,65^{b}\pm0,78$	$0,96^{a}\pm0,68$

Table 1 – Percentage (W/W) of retroperitoneal, epididymal, and peritoneal fat to live weight of white rats after administration of MSG and EGCG in rat feed.

4. Discussion

In this study, giving MSG in feed tended to be followed by an increase in body weight. Previous reports stated that administering monosodium glutamate at a dose of 4 mg/gBW orally for 2-4 weeks in rats caused an increase in body weight (Bayram et al., 2022). The administration of monosodium glutamate at a dose of 60 mg/kgBW orally for 21 days of treatment in rats caused an increase in body weight (Tsuneyama et al., 2014). Monosodium glutamate can affect energy balance by making food tastier and disrupting the performance of leptin signaling in the hypothalamus (Kayode et al., 2023). Administration of MSG causes the production and release of NO and serotonin which will indirectly activate the vagus nerve (from the stomach and liver branches) and provide the first effect of the MSG dose into the gastrointestinal lumen. Through autonomic innervation and the function of the gastrointestinal tract itself, these stimuli influence adipocyte fat metabolism (Kayode et al., 2023).

The body weight of rats exposed to MSG and then given EGCG 8 mg/kgBW/day tended to be stable during the four weeks of treatment. EGCG is the main antioxidant component in green tea. Studies using green tea extract reported significantly reducing the average body weight and body mass index in obese groups (Ohishi et al., 2021; Wang et al., 2023). The mechanism by which EGCG influences body weight and body composition includes thermogenesis and substrate oxidation mediated by the sympathetic nervous system. Energy status in the liver, especially ATP production, can trigger signals to the appetite control center in the brain via vagal sensory neurons. Therefore, decreased fatty acid oxidation in the liver and a concomitant decrease in ATP levels increase appetite (Kurogi et al., 2015; Saito et al., 2020). EGCG decreases intestinal absorption of lipids and proteins, thereby reducing calorie intake. In addition, EGCG activates Adenosine monophosphate-activated protein kinase (AMPK) which is in the liver, skeletal muscle, and adipose tissue. Activated AMPK will reduce gluconeogenesis and fatty acid synthesis and increase catabolism, thereby causing weight loss (Yang et al., 2016). Administration of high doses of EGCG in obesity causes a decrease in ghrelin secretion and causes an increase in adiponectin levels which has the function of increasing insulin sensitization (Chen et al., 2016). Supplementation with green tea extract (GTE) reduces the weight and size of adipocytes in subcutaneous adipose tissue (Macêdo et al., 2019).

Based on the results of this study, it appears that the administration of MSG does not increase the weight of retroperitoneal fat and increases the weight of epididymal and peritoneal fat in white rats (*R. norvegicus*) compared to treatment without administration of MSG and EGCG. Administration of MSG causes an increase in blood glucose levels. Higher doses of parenteral MSG caused insulin resistance in rats as evidenced by a significant increase in plasma glucose in the oral glucose tolerance test and accumulation of visceral fat (Rene et al., 2019). Giving monosodium glutamate to rats at a dose of 4g/kg BW for 21 days caused obesity in experimental animals, increasing fat mass by 66% in retroperitoneal fat, 80% in epididymal fat, and 134% in visceral fat (Miranda

et al., 2014). Obese male white rats show signs of phagocytic shift in peritoneal fat experiencing systemic inflammation as indicated by higher adiposity and predominant adipose tissue inflammation (Rudyk et al., 2023). The mechanism of EGCG found in camellia sinensis tea is to inhibit the pancreatic lipase enzyme in the intestinal lumen so that the intestine experiences hydrolysis of phospholipids and a decrease in triacylglycerol, and cholesterol absorption in the intestine decreases (Luo et al., 2019). Reducing cholesterol in the intestine causes a decrease in LDL levels in the body (Cohn et al., 2010). In addition, epigallocatechin 3-gallate (EGCG) can mimic the way insulin works and inhibit acetyl CoA in the mitochondrial matrix which will help increase fat oxidation (Tang et al., 2021).

Based on the research results, administration of MSG and EGCG at a dose of 4 mg/KgBW reduces the weight of retroperitoneal and peritoneal fat and does not reduce the weight of epididymal fat in white rats (*R. norvegicus*) compared to treatment with MSG. Giving MSG and EGCG at a dose of 8 mg/KgBW and MSG and EGCG at a dose of 16 mg/KgBW show that it could reduce the weight of retroperitoneal, epididymal, and peritoneal fat in white rats (*R. norvegicus*) compared to treatment with MSG. Giving MSG and EGCG at a dose of 8 mg/KgBW reduces the weight of retroperitoneal, epididymal, and peritoneal fat in white rats (*R. norvegicus*) compared to treatment giving MSG and EGCG 4 mg/KgBW and treatment giving MSG and EGCG 16 mg/KgBW. Giving MSG and EGCG at a dose of 16 mg/KgBW reduces the weight of retroperitoneal fat and does not reduce the weight of epididymal and peritoneal fat in white rats (*R. norvegicus*) compared to treatment without MSG and EGCG.

The treatment group was given a high-fat diet and epigallocatechin 3-gallate at a dose of 50 mg/KgBW can reduce retroperitoneal and epididymal fat in rats for 8 weeks (Santana et al., 2015). Administration of epigallocatechin 3-gallate at a dose of 50 mg/KgBW and 100 mg/kgBW can reduce epididymal fat accumulation in rats (Li et al., 2020). EGCG effectively increases lipid catabolism (Xu et al., 2023), and decreases plasma triglycerides in obese humans (Chatree et al., 2021).

5. Conclusion

The consumption of monosodium glutamate 120 mg/kgBW/day for 28 days in rats (*R. norvegicus*) increased the weight of epididymal fat and peritoneal fat. The administration of epigallocatechin 3-gallate 8 mg/kgBW/day reduced the weight of retroperitoneal fat, epididymal fat, and peritoneal fat, compared to rats given monosodium glutamate.

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