Efficacy Of Temulawak Extract (*Curcuma xanthorrhiza Roxb*) in Reducing Ureum Levels of White Rats (Rattus Norvegicus) on Maximum Physical Activity: Pure Experimental Research

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Abstract. Maximum physical activity triggers a transformation of renal hemodynamics and increased protein catabolism, causing urea levels to rise. One natural source of antioxidants is ginger, which can reduce uranium levels. This aims to analyze the effect of ginger extract on urea levels during maximum physical activity. Using a pretest-posttest with control group (K) design, a total of 28 male rats were divided into four groups: control and three intervention groups provided with ginger extracts at various doses (P1: 6.75 mg/day; P2: 13.5 mg/day; P3: 20.25 mg/day) after performing maximum physical activity by swimming. Blood urea levels were measured afterwards and later analyzed using One Way Anova followed with LSD test. The results found increased urea (5.66 mg/dl) in group K, while all intervention groups showed lower levels, with group P3 having the highest difference (P1: 12.66 mg/dl; P2: 21.83 mg/dl; P3: 25.16 mg/dl). Statistical test revealed that low, medium, and high doses of ginger extract significantly affected urea levels in mice with maximum physical activity. Further post hoc test revealed significant difference between control and intervention groups. In conclusion, administering ginger extract at low, medium, and high doses can significantly reduce blood urea levels in mice with maximum physical activity.

Keywords: Ginger Extract, Urea Levels, Maximum Physical Activity, Temulawak

1 INTRODUCTION

Excessive physical activity can increase the formation of free radicals from increased oxygen intake. Increased oxygen intake, especially in contracting muscles, causes ROS or reactive oxygen species to occur due to the release of superoxide electrons from mitochondria [1]. Reactive oxygen species (ROS) play an essential role in causing oxidative stress in various biological systems [2].

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Excessive physical activity can have varying effects on kidney tissue depending on the intensity, duration, and type of exercise. Research indicates that high-intensity exercise can transiently and adversely affect the kidneys [3]. Studies have shown that high-intensity exercise can lead to acute kidney injury and renal damage [4-5]. Additionally, high-intensity exercise has been associated with renal medullary carcinoma in individuals with sickle cell trait [6]. On the other hand, moderate-intensity exercise has been found to improve renal function and reduce mortality in chronic kidney disease patients [7]. Lifelong aerobic exercise has been shown to offset age-related kidney damage [8].

The influence of exercise on renal function is determined by various factors such as dehydration, hormonal variability, and renal blood flow changes during physical exertion [9-10]. Prolonged exercise combined with dehydration can stimulate the release of certain hormones affecting kidney function [9]. Hormonal variability, particularly in women, may play a role in protecting against muscle damage induced by exercise [11].

Furthermore, exercise has been found to protect the kidneys by inhibiting renal fibrosis and reducing inflammation levels, thereby potentially preventing renal damage [12-13] However, the specific impact of exercise on kidney health greatly depends on the individual's fitness level and the type of exercise performed [14-15]. While exercise can help preserve and enhance kidney health, it is crucial to consider the intensity and duration of exercise to minimize the risk of kidney damage [16].

The body can neutralize small amounts of free radicals through endogenous antioxidants, producing non-toxic compounds. The increase in free radicals causes endogenous antioxidants to be quickly depleted because their use is more significant than their regeneration [17]. Alternative exogenous antioxidants that are safe and relatively cheap can be obtained from various types of plants. Temulawak (Curcuma et al.) has properties, one of which is as an antioxidant [17].

Temulawak contains curcuminoids with three compounds: demethoxycurcumin, bisdemethoxycurcumin, and curcumin [18]. Curcumin consists of a phenolic hydroxy group and a β diketone group in its chemical structure, resulting in strong antioxidant properties. The structure of the curcumin compound has two phenolic groups, where 1 curcumin molecule is able to ward off two free radicals [19-20]. Research [21] shows that curcumin supplementation at a dose of 100 mg can significantly reduce urea levels by inhibiting the production of reactive oxygen in mice that experience stress due to swimming. Curcumin functions as an antioxidant that is able to repair kidney damage caused by ROS by increasing Nrf2 translocation, inhibiting oxidative stress, and increasing antioxidant enzymes [22]

The potential of ginger as an antioxidant and nephroprotector against kidney damage due to free radicals is expected to reduce blood urea levels after maximum physical activity. This study aims to examine the effect of ginger on urea levels in mice.

2 METHOD

This study was a pretest-posttest with control group experiment using 28 male rats as subjects. The test animals were divided into four groups, consisting of a control group (K), which was given maximum physical activity without giving temulawak extract, an intervention group (P1, P2, and P3) given maximum physical activity and temulawak extract with different doses (6.75 mg, 13.5 mg, and 20.25 mg/day) for 28 days. Measurement of maximum physical activity ability was carried out by swimming using the forced swim test method. Blood samples were collected immediately after tiring swimming on the last day of treatment. Measurement of urea levels using a spectrophotometer with the calorimetry method. All data analysis used SPSS 24 software to perform One Way Anova test and followed with the Post Hoc LSD test. The body weight and feed intake were tested using the Anova Repeated Measure test.

3 RESULTS AND DISCUSSION

3.1 Rat Body Weight

Body weight measurements were carried out every day during the intervention to review changes in the weight of the mice during the study. The average weight development of mice in groups K, P1, P2, and P3 during the study can be seen in Figure 1.

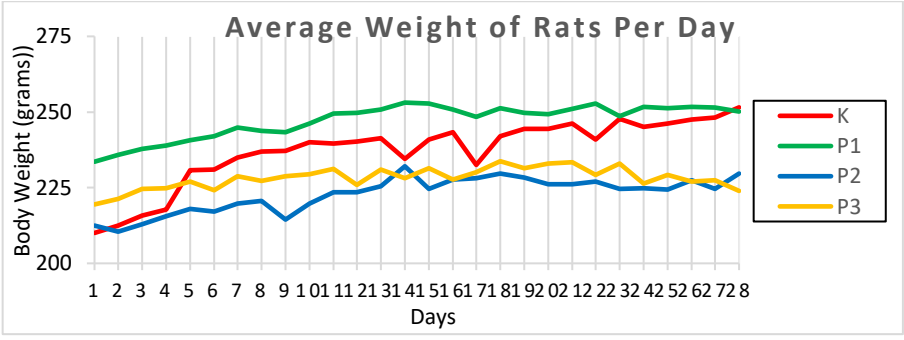

Fig. 1. Development of Rat Body Weight

Based on Figure 1, the final body weight of the mice in the four groups tended to increase compared to the initial body weight. However, based on the Repeated Measures Anova test, a p-value of 0.487 (p> 0.05) was obtained, indicating no significant difference in average body weight over time between groups K, P1, P2, and P3. This shows that the body weight of the four groups is relatively the same.

Weight gain can be caused by increased muscle mass. Mice have carried out regular physical activity accompanied by a gradual increase in activity time within a specified period. Both of these things will significantly impact the muscle enlargement process. Muscle enlargement occurs due to elevated size of muscle fiber myofibrils, myosin,

and actin filaments that are active during solid muscle contractions and an increase in the number and strength of connective tissue, ligaments, and tendons. The increase in muscle size can reach two to three times by intensive physical activity. The increase in muscle mass can be influenced by many factors, such as genetics, where each individual's body response to physical activity is different, and each individual's body metabolism is also different [23-24].

3.2 Rat Feed Intake

The feed given to each group per day is 40 grams and the remaining feed is weighed daily. The average feed intake of rats in groups K, P1, P2, and P3 during the study can be seen in Figure 2.

Based on Figure 2, the final feed intake of mice in the four groups tended to decrease compared to the initial feed intake. However, statistical analysis using the Repeated Measures ANOVA test showed p-value 0.978 ($p>0.05$). In other words, there was no significant difference in the average feed consumption of mice between groups K, P1, P2, and P3 over time. This shows that the feed consumption of mice in the four groups is relatively the same.

Decreased feed intake in mice can be caused by stress due to maximum physical activity. Excessive activity can reduce ghrelin levels in the brain while increasing leptin concentrations in mice [25]. Leptin and ghrelin are two main hormones involved in regulating appetite. Leptin, a cytokine derived from adipocytes, suppresses appetite and increases energy expenditure [26]. Conversely, ghrelin or commonly known as the "hunger hormone", can stimulate appetite, increase food intake, and accumulate fat storage [27]. The higher the intensity of the exercise and the longer the duration, the more likely the exercise is to cause loss of appetite and changes in appetite hormones [29].

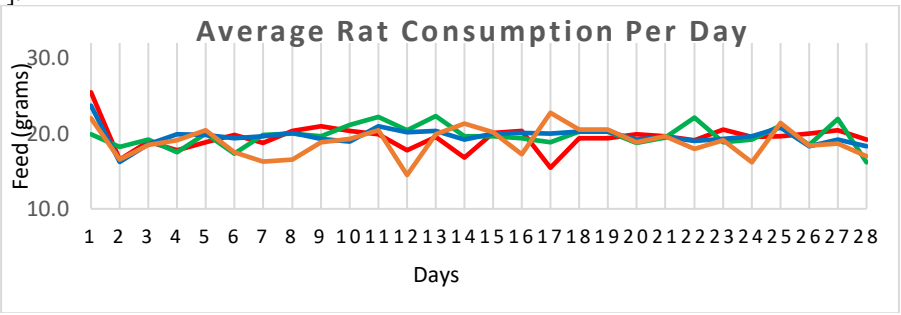

Fig. 2. Development of Rat Feed Intake

3.3 Effect of Curcuma Extract (Curcuma et al.) on Reducing Urea Levels in White Rats (Rattus Norvegicus)

The difference in average urea levels is obtained from the urea levels before treatment minus the urea levels after treatment. Based on Table 1, the control displayed a rise in urea levels of 5.66 ± 4.58 mg/dl because this group was not given Curcuma extract and

was only given control drinks and feed, so the process of neutralizing ROS was only assisted by endogenous antioxidants which over time will run out due to increasing free radicals. The effect of maximum physical activity on kidney function, characterized by increased urea levels, has been widely studied. Chen et al.'s (2022) research conducted on mice that were swum to stress proved through morphological evaluation that there was kidney tissue injury and increased urea levels in the control group that was swum only compared to the resting group [21]. The average difference in urea levels can be seen in Table 1.

Table 1. Mean Urea Levels Before and After Intervention

Urea Level (mg/dl)	Groups				p^1
	K	P1	P2	P3	
	Mean±SD	Mean ± SD	Mean ± SD	Mean ± SD	
Before	72.16±7.90	65.00±5.72	74.33±9.24	79.50±8.04	0.033*
After	77.83±8.79	52.33±8.80	52.50±4.23	54.33±7.86	0.000*
Delta	5.66±4.58	-12.66±9.70	-21.83±7.60	-25.16±9.62	0.000*

Information:
1 The value of the One Way Anova test results on the same row
* There is a significant difference between the four treatments (ANOVA test, p<0.05)
K = control group (maximum physical activity)
P1 = Treatment 1 (maximum physical activity and 6.75 mg dose of temulawak extract)
P2 = Treatment 2 (maximum physical activity and temulawak extract dose 13.5 mg)
P3 = Treatment 3 (maximum physical activity and temulawak extract dose 20.25 mg)

The results of the One Way Anova test for the difference in urea levels, obtained the p-value at 0.000 (p <0.05), implying significant difference in urea levels across all groups. Further Post Hoc LSD test results for urea levels after administration of temulawak extract can be seen in Table 2.

The results of the Post Hoc LSD test showed a significant difference (p<0.05) between the control and P1 (p=0.000), the control and P2 (p=0.000), and the control group and P3 (p=0.000). Based on Table 1, all groups given temulawak extract experienced a decrease in urea levels. This findings indicate that administering temulawak extract at doses of 6.75 mg, 13.5 mg, and 20.25 mg to mice given maximum physical activity for 28 days can significantly reduce blood urea levels. This is in line with Chen et al. (2022) who reported that curcumin supplementation at a dose of 100 mg could significantly reduce urea levels by limiting reactive oxygen production in mice experiencing stress due to swimming [21].

Curcumin inhibits the production of reactive oxygen by increasing the activity of antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase through the activation of Nrf2 and Keap1 signaling pathways. Rosidi et al (2014) stated that temulawak extract with active antioxidants of 87.01 ppm has the potential as a good antioxidant [18].

Temulawak contains bioactive substances such as curcumin, demethoxycurcumin, and bisdemethoxycurcumin, which have antioxidant effects. Curcumin functions as an antioxidant that can repair kidney damage caused by ROS by increasing Nrf2 translocation, inhibiting oxidative stress, and increasing antioxidant enzymes. Nrf2 (nuclear factor-erythroid-2 related factor 2) is a type of transcription factor that regulates genes to encode antioxidants and antioxidant enzymes [22]. The role of curcumin as an antioxidant that wards off free radicals cannot be separated from the structure of the curcumin compound. Curcumin has a phenolic hydroxy group and a β diketone group. The phenolic hydroxyl group functions to capture free radicals in the first phase of the antioxidant process. The β diketone group functions to capture free radicals in the next phase. The structure of the curcumin compound significantly affects its antioxidant properties [19-20].

Table 2. Results of LSD Post Hoc Test of Urea Levels After Administration of Curcuma Extract for 28 Days

Treatment Group	*p value*			
	K	P1	P2	P3
K	-	0.000*	0.000*	0.000*
P1	-	-	0.970	0.656
P2	-	-	-	0.683
P3	-	-	-	-

Information:
* There is a significant difference between the four treatments (Post et al., $p<0.05$)
K = control group (maximal physical activity)
P1 = Treatment 1 (maximum physical activity and 6.75 mg dose of temulawak extract)
P2 = Treatment 2 (maximum physical activity and temulawak extract dose 13.5 mg)
P3 = Treatment 3 (maximum physical activity and temulawak extract dose 20.25 mg)

Combination of temulawak rhizome extract and starfruit fruit on the histological picture of the kidneys of diabetic rats induced by Streptozotocin. The findings of the study suggest that a single treatment of temulawak extract at a dose of 17.5 mg/kgBW caused improvements in the histological structure of the kidneys as evidenced by a decrease in glomerular diameter, narrowing of the Bowman space, and a decrease in proximal tubules that experienced necrosis (Alipin et al, 2017). Research on rats given curcumin supplements at doses (12.3 ml, 24.6 ml, and 61.5 ml) and a swimming exercise test with a load to exhaustion for 28 days showed a significant decrease in urea levels [29]. Other researchers stated that giving 200 mg of curcumin to mice that were swum to exhaustion for 8 weeks showed a decrease in blood urea levels ($p<0.05$), curcumin was able to regulate the elevated expression of Nrf2 and HO-1, effectively reducing oxidative stress due to excessive swimming, thereby increasing the expression of BCL-2 related proteins, reducing Bax expression, inhibiting kidney apoptosis and protecting the kidney structure to function properly [30].

4 CONCLUSION

Administration of temulawak extract (Curcuma xanthorrhiza Roxb) at various doses can reduce blood urea levels in male Wistar rats (Rattus norvegicus) with maximum physical activity.

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