

# Effect of Bran Red Rice Tea Product for Stamina of Male Mice (*Mus musculus* L.).

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## ABSTRACT

Today's society requires a stamina enhancer that is able to restore lost stamina in a short time. Red rice bran which is used to make red rice bran tea product brand 'X' is a natural stamina enhancer because it contains secondary metabolites, tocopherols, oryzanol, and carbohydrates. In addition, the red rice bran tea product brand 'X' also contains *Piper caninum* extract which contains alkaloids and flavonoids. This study aims to determine the effect of red rice bran tea product brand 'X' on the stamina of male mice. This study used a completely randomized design with 25 mice. Mice were divided into 5 treatment groups, namely K(-) given distilled water, K(+) given stamina enhancing tablets brand 'XX', P1 given 300 mg/kg BW dose of bran red rice tea product brand 'X', P2 given 600 mg/kg BW dose of rice bran tea product red brand 'X', P3 was given 900 mg/kg BW dose of red rice bran tea product brand 'X'. This research method is natural exhaustion. The results showed that bran red rice tea in the P1, P2, and P3 treatments was significantly able to increase the stamina of male mice with the mean difference in the T1 swimming endurance test being longer than T2. The best dose of red rice bran tea in this study was 900 mg/kg BW dose. The results of the phytochemical test showed that the red rice bran tea product brand 'X' contained flavonoids, alkaloids, phenolics, terpenoids, and saponins. It can be concluded that the bran red rice tea product brand 'X' in this study was able to increase the stamina of male mice.

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## 1. Introduction

People's lives in various countries are currently undergoing COVID-19 pandemic. The increase in the number of patients exposed to COVID-19 is still occurring because the virus continues to evolve. This pandemic period has greatly affected the community on how to fulfill good nutritional intake so that they can increase the body's immune system by consuming healthy food (Sibuea, 2021). Fulfilling adequate nutritional intake during pandemic is a problem for the community because of busy activities, high job demands, and high socio-economic status to meet daily needs. Stamina restoration is crucial because people need to recover lost energy in a very short time so that they can continue to carry out their daily activities with the help of synthetic stamina restorers (Praristiya, 2019). According to Ifada et al. (2021), excessive use of synthetic stamina recovery drugs and energy drinks can cause health problems because of the synthetic chemical compounds present in this kind of drugs. Currently, there are many natural ingredients that contain secondary metabolite compounds that function as tonic effects or restore stamina, one of which is red rice bran. Bran is a by-product of the process of milling rice plant into the rice which has various ingredients such as phenolic compounds, protein, fiber, vitamins, and minerals (Henderson et al., 2012).

According to Kusumastuti and Ayustaningwarno's 2013 research on red rice bran, the compounds contained in red rice bran are carbohydrates, protein, oryzanol, anthocyanin and tocopherols. One of the potential physiological activities of red rice bran is it can be made into a functional food such as tea. This bran red rice tea product brand 'X', apart from having a red rice bran composition, also contains *Piper caninum*. *P. caninum* is one of the plants in the betel order or commonly called forest betel which contains secondary metabolite compounds. According to Suriani's research (2019), *P. caninum* extract contains bioactive substances such as alkaloids, flavonoids, polyphenols, and steroids which have antifungal and antimicrobial properties. The activity of secondary metabolite content of red rice bran and *P. caninum* in bran red rice tea product brand 'X' is expected to affect natural stamina in mice.

## 2. Methodology

### 2.1 Time and place of study

The natatory exhaustion test of mice was carried out on 03 - 05 August 2022 and for the phytochemical test it was carried out on 13 November 2022. The research took place at the Animal Physiology Laboratory, Biology Study Program, Faculty of Science and Animal Nutrition and Feed Laboratory, Faculty of Animal Husbandry, Udayana University.

### 2.2. Tools and materials

The tools and materials used in this study were adult male mice (*Mus musculus*), red rice bran tea product brand 'X' consisting of 99.5% red rice bran and 0.5% *P.caninum* leaf extract, distilled water, medicine brand 'XX' stamina enhancer, NaCMC, transparent container with a diameter of 30 cm, towel, 1 ml size syringe, ethanol, chloroform, magnesium powder, concentrated HCl, Liebermann Burchard reagent, FeCl<sub>3</sub>, ammonia chloroform, sulfuric acid, Mayer's reagent.

### 2.3. Methods

#### Research Design

This study used a completely randomized design (CRD) with random sampling method. The experimental animal is adult male mice. These mice were divided into 5 groups, so the total sample size was 25 mice. The test group consisted of group K(-) which was given distilled water, K(+) which was given the stamina enhancer drug brand 'XX', and the treatment group, namely P1 (bran red rice tea product at a dose of 300 mg/kg BW), P2 (bran red rice tea product dose of 600 mg/kg BW), and P3 (bran red rice tea product solution dose of 900 mg/kg BW).

#### Sample Preparation

Preparation of sample solution

The bran red rice tea product brand 'X' sample contains a composition consisting of 99.5% red rice bran and 0.5% leaf extract *P. caninum*.

#### Preparation of control solution

The negative control group was only given 0.3 ml of distilled water. The positive control group was given stamina enhancing drug brand 'XX', namely 24 mg stamina enhancing drug brand 'XX' dissolved in 2.5 ml 0.5% Na CMC.

#### Sample dose determination

Determination of the sample dose of bran red rice tea product brand 'X' was given to each treatment with doses based on Widarta and Arnata (2014). For a dose of 300 mg/kg BW, 90 mg of sample is dissolved in 5 ml of hot water. For a dose of 600 mg/kg BW, 180 mg of sample is dissolved in 5 ml of hot water. For a dose of 900 mg/kg BW, a sample of 279 mg/kg BW is dissolved in 5 ml of hot water (Handayani, 2020).

### Research Procedure

Experimental animals, namely male mice, were first acclimatized for 7 days by being given feed in the form of concentrate and clean water for drinking. After 7 days of acclimatization, the mice were given swimming exercises for 3 days. After swimming exercises, the mice were rested for 1 day and given treatment the next day.

Twenty-five mice were randomly divided into 5 treatment groups consisting of K(-), K(+), and the treatment of bran red rice tea brand 'X' with 3 different doses. Before treatment, the mice were put into five containers filled with water, each containing one mouse to do the initial swimming test method (natatory exhaustion test). An increase in the swimming duration of mice was observed from the start of swimming (during the struggling phase) until the mice experienced fatigue and did not move (during the floating phase). The mice were allowed to swim until they showed a signs of fatigue, i.e. there was no movement from all four legs, the body was bent, the tail was stretched, and the head was under water for 4-7 seconds.

After that, the fatigue time was recorded which was counted from two seconds after the mice were placed in a container filled with water until the mice showed signs of fatigue condition. This duration was recorded as the swimming endurance test time before treatment (T<sub>0</sub>). Mice were dried with a towel then returned to the cage and rested for 30 minutes. After that, the mice were given the test solution according to the treatment group.

The next step was giving the treatment orally using a 1 ml syringe and then leaving the mice for 15 minutes as an estimate of the absorption time of the test solution in the mice's body. After 15 minutes, the mice ran the swimming test again. The mice were swam until they showed signs of fatigue and then the fatigue time was recorded which was counted from two seconds after the mice were placed in a container filled with water until the mice showed signs of fatigue. This duration is recorded as the first period swimming endurance test time (T<sub>1</sub>). Mice were dried with a towel then returned to the cage and rested for 30 minutes. After that, the mice ran the swimming test again until fatigues reaction appeared as previously observed. This duration is recorded as the second period swimming endurance test time (T<sub>2</sub>). The data written is the average difference in swimming endurance tests, namely the average difference in swimming endurance tests in the first (T<sub>1</sub>) and second (T<sub>2</sub>) periods minus the average difference in swimming endurance tests before treatment (T<sub>0</sub>) (Lukman and Vivi, 2013) as follows: mice swimming endurance test = T<sub>1</sub> – T<sub>0</sub> and T<sub>2</sub> – T<sub>0</sub>.

### Phytochemical Screening

#### Flavonoid test

Bran red rice tea powder is added to 25 ml of ethanol and then filtered. The solution was added with chloroform and stirred until two layers were obtained, namely the chloroform layer and the solution layer. The solution layer was taken and placed in a test tube, then magnesium powder was added. After that it was dripped with concentrated hydrochloric acid (HCl), waited for the splash to disappear and the color change was observed (Riwanti dan Izazih, 2019).

#### Steroid and triterpenoid test

Bran red rice tea powder was added to 25 ml of ethanol and filtered. The solution was then dripped with chloroform and stirred until two layers were obtained, namely the chloroform layer and the solution layer. The chloroform layer was taken with a dropper and dripped onto the plate. The plate was then added with a few drops of anhydrous acetic acid and concentrated sulfuric acid (Liebermann Burchard reagent), then the color change was observed. If it turns red or purple it contains terpenoids, if it turns blue or green it contains steroids (Susanti et al., 2014).

#### Tannin test

Red rice bran tea powder is added to 25 ml of ethanol and then filtered. The solution was then added with chloroform and stirred until two layers were obtained, namely the chloroform layer and the solution layer. The solution layer is taken with a dropper pipette and dripped onto the plate. Then drops of gelatin on the plate and added drops of FeCl<sub>3</sub>. The presence of tannins is indicated by a blue change in color (Makalalag et al., 2015).

#### Saponin test

Bran Red rice tea powder was taken and added to 25 ml of ethanol then filtered. The solution is then added hot distilled water and shaken for 5 minutes. Foam that does not disappear after shaking for 5 minutes indicates the presence of saponins (Putri dan Lubis, 2020).

#### Phenolic test

Bran red rice tea powder is added to 25 ml of ethanol, then filtered. The solution was then added with chloroform and stirred

until two layers were obtained, namely the chloroform layer and the solution layer. The solution layer was taken with a pipette and transferred to a new test tube. The solution was added with FeCl<sub>3</sub> (iron (III) chloride). Formation of a green color at the lip of the solution indicates the presence of phenolic compounds (Reiza et al., 2019).

#### Alkaloid test

Bran Red rice tea powder was placed in a test tube and added with 10 ml of chloroform. The solution was added with 0.05 M chloroform ammonia, stirred and then filtered. The filter results were transferred to a new test tube. The solution in the new test tube was added with 0.5 ml of 2 N sulfuric acid, then stirred until two layers were obtained between the sulfuric acid and chloroform. A layer of 2 N sulfuric acid was taken and put into a test tube then added with 1 drop of Mayer's reagent and let it sit to form a precipitate. (Aliwu et al., 2020).

#### 2.4. Data analysis

The data obtained from the study were analyzed using the SPSS for Windows version 17 program with One Way ANOVA test. This test is used if the data distribution is normal and homogeneous, and is continued with Duncan's test if it is significantly different ( $p < 0.05$ ). If the data distribution is not normal, then the Kruskal-Wallis test is used (Yulianita dan Effendi, 2015).

### 3. Results and Discussion

#### Swimming endurance test results of the first day and the second day

The results of statistical tests on the first day of natatory exhaustion test showed that in the first period of the test (T1) the K(-) treatment group with a mean difference of -185 seconds was significantly different ( $p < 0.05$ ) from the other treatment groups. K(+) treatment group with an average difference of 335 seconds was not significantly different ( $p > 0.05$ ) from P3 treatment (red rice bran tea solution dose of 900 mg/kg BW) which had an average difference of 259 seconds. P1 treatment group (red rice bran tea solution dose of 300 mg/kg BW) with an average difference of 145.60 seconds was not significantly different from P2 treatment group (red rice bran tea solution dose of 600 mg/kg BW) which had an average difference of 134 seconds. The results of the second period (T2) of the test showed that P1 with a mean difference of -146 seconds was significantly different from all treatment groups. Treatment of P2 with an average difference of -257.80 seconds was not significantly different from K(-) which had an average difference of -286.40 seconds. The results of the average difference in P3 of 83 seconds are not significantly different from K(+) which has an average difference of 128.20 seconds.

The results of statistical analysis on the second day of the test showed that in the first period swimming (T1), K(-) with an average difference of -159 seconds and K(+) with an average difference of 368.40 seconds was significantly different from the other treatment groups. The P1 treatment group with an average difference of 146.60 seconds, P2 with an average difference of 113.80 seconds, and P3 with an average difference of 221.80 seconds had no significant difference. The results of the second period (T2) statistical analysis, namely K(-) with a mean difference of -252.20 seconds, were significantly different from the other treatment groups. P1 treatment group with an average difference of -106.80 seconds was not significantly different from P2 which had an average difference of -71.40 seconds. P3 treatment group with an average difference of 106.60 seconds was not significantly different from K(+) which had an average difference of 135 seconds. The results on the first day and the second day can be seen in table 1.

Table 1. Results of statistical analysis of the mean difference between the first and second days of natatory exhaustion

Treatment	Difference in swimming duration of mice (seconds)			
	First Day		Second day	
	(T1-T0)	(T2-T0)	(T1-T0)	(T2-T0)
K(-)	-185 ± 43,15 <sup>a</sup>	-286,40 ± 44,09 <sup>a</sup>	-159 ± 86,72 <sup>a</sup>	-252,20 ± 88,50 <sup>a</sup>
K(+)	335 ± 124,37 <sup>c</sup>	128,20 ± 43,49 <sup>c</sup>	368,40 ± 122,22 <sup>c</sup>	135 ± 118,28 <sup>c</sup>
P1	145,60 ± 108,68 <sup>b</sup>	-146 ± 67,64 <sup>b</sup>	146,60 ± 79,99 <sup>b</sup>	-106,80 ± 99,01 <sup>b</sup>
P2	134 ± 19,07 <sup>b</sup>	-257,80 ± 77,54 <sup>a</sup>	113,80 ± 101,74 <sup>b</sup>	-71,40 ± 21,79 <sup>b</sup>
P3	259 ± 43,18 <sup>c</sup>	83 ± 13,77 <sup>c</sup>	221,80 ± 105,49 <sup>b</sup>	106,60 ± 85,55 <sup>c</sup>

Note:

1. Different superscript letters behind numbers in the same column indicate significant differences between treatments ( $p < 0.05$ )
2. The number after the (±) sign indicates the deviation standard
3. Negative results in the table indicate that the average swimming time of mice at T0 is longer than T1 or T2

Based on data from the results of the natatory exhaustion test on the first day, it showed that the average difference in the first period (T1) the test with the K(-), K(+), P1, P2, and P3 treatments as a whole has a longer mean difference in swimming duration compared to the swimming endurance test in the second period (T2). Comparison of the average difference between the first period (T1) and second period (T2) of the natatory exhaustion test can be seen in Figure 1.

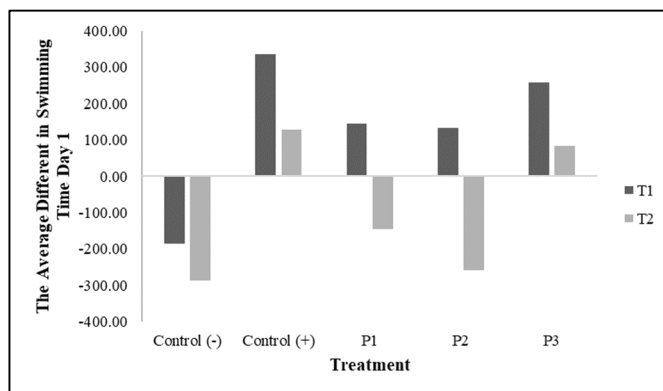


Figure 1. Graph of the average difference in swimming time day 1

Based on data from the results of the natatory exhaustion test on the first day, it showed that the average difference in the first period (T1) the test with the K(-), K(+), P1, P2, and P3 treatments as a whole has a longer mean difference in swimming duration compared to the swimming endurance test in the second period (T2). Comparison of the average difference between the first period (T1) and second period (T2) of the natatory exhaustion test can be seen in Figure 2.

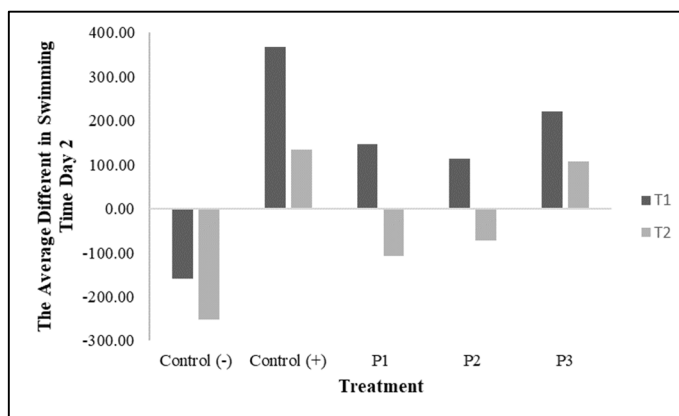


Figure 2. Graph of the average difference in swimming time day 2

### 1. Phytochemical test results

Test for phytochemical compounds, namely flavonoids, alkaloids, steroids/terpenoids, tannins, saponins, and phenolics. The results of the phytochemical tests in Table 3 show that the red rice bran tea product contains flavonoids which are indicated by a reddish color change. bran red rice tea products also contain alkaloids as evidenced by the formation of a white precipitate. Terpenoid compounds are also found in red rice bran tea products with a reddish color change. Other compounds that are also found in red rice bran tea products are saponins with the formation of foam and phenolic compounds which are indicated by a change in color to green.

Table 3. Phytochemical test results for red rice bran tea products

Compounds group	Result	Note
Flavonoids	+++	Reddish color
Alkaloids	+++	Form a white precipitate
Steroids	-	No green color formed
Terpenoids	++	Reddish color
Tannins	-	No blue color formed
Saponins	++	Formed foam
Phenolics	+++	Greenish color

Note:

- +++ : form a precipitate / multiple colors / striking color
- ++ : form a precipitate / moderate colored
- + : form a precipitate / light colored
- : did not form a precipitate / no discoloration

#### 4.1. Discussion

Five different treatments were given in two separate swimming periods, namely the first swimming period (T1) and the second swimming period (T2). This swimming period shows the absorption time of the treatment solution which aims to determine the most effective absorption time and the effect on the stamina of the mice. The K(-) treatment had a smaller average difference compared to the other treatments and did not show an increase in swimming duration. This was because the distilled water used did not have any effect on the mice's stamina so that the mice's stamina did not increase and they became tired quickly. The results of the average difference in the K(+) treatment showed a significant difference with the treatment group. This is because the stamina enhancing drug brand 'XX' contains several ingredients such as buffered vitamin C, contains vitamin A, vitamin E, and zinc which act as antioxidants, as well as ginseng extract, guaranine, vitamin B complex, L-Glutamic Acid, and minerals according to the Hemaviton Wellnes Center (2022).

The first period of the natatory exhaustion test (T1) experienced an increase in the mean difference in swimming duration when compared to the second period of the test (T2). This shows that mice treated with red rice bran tea can slow down the occurrence of fatigue. The most optimal absorption time of the red rice bran tea product to affect the stamina of mice is 15 minutes. According to Clifford et al. (2013), the presence of secondary metabolite compounds in the product solution will help speed up the absorption process in the digestive system with an absorption time between 15 minutes to 60 minutes. The dose that has the longest mean difference in swimming duration is 900 mg/kg BW. P3 showed a difference when compared to treatments P1 and P2, where P3 had the closest results to treatment K(+). This shows that the 900 mg/kg BW dose of red rice bran tea containing phytochemical compounds is more able to have a greater impact on increasing the stamina of mice. This is supported by Yang et al. (2016) which stated that higher doses would have the effect of increasing the stamina of mice because higher doses contain higher primary and secondary metabolites that affect the stamina of mice.

In addition to natatory exhaustion tests, phytochemical tests were also carried out. This red rice bran tea solution contains several phytochemical compounds such as flavonoids, alkaloids, terpenoids, saponins, and phenolics. According to Pratiwi and Simaremare (2020), several plants that contain phytochemical compounds have a tonic effect which can increase stamina in experimental animals. The tonic effect will spur and strengthen all organ systems and stimulate repair of muscle tone cells carried out on the central nervous system. Red rice bran tea product with a dose of 900 mg/kg BW can increase the stamina of mice, one of which is because it contains flavonoid compounds. Flavonoids can increase the stamina of mice by inhibiting the phosphodiesterase enzyme which has the function of converting cyclic cAMP to AMP. This AMP compound will stimulate the phosphorylase enzyme from inactive to active which will then convert glycogen in the body into glucose 1 phosphate. Glucose 1 phosphate will then be converted into glucose 6 phosphate by the enzyme gluco phosphomutase. The formation of glucose 6 phosphate will be an additional source of energy in carrying out activities. Flavonoids can also increase stamina by increasing hepatic glycogen content and reducing lactic acid in the muscles (Li and Zhang, 2013).

Flavonoids in the metabolism of mice will stimulate the central nervous system to trigger the locomotion system in moving the muscles so that the mice can swim longer because the energy produced by the muscles becomes more (Putriastuti dkk., 2007). In addition to flavonoids, red rice bran tea products are also proven to contain alkaloid compounds. The alkaloid compound comes from the presence of *P. caninum* in red rice bran tea products. Alkaloids can affect the increase in the stamina of mice through the sensory system which has a role in delivering receptors to the central nervous system by acting as an adenosine antagonist. Adenosine is a neurodepressant that binds to A1, A2a, A2b and A3 receptors in the brain which will cause several effects such as inhibition of acetylcholine, adrenaline, dopamine, and serotonin which can activate sleep promoting neurons thereby reducing muscle movement, reducing blood pumping by the heart which causes the supply reduced blood flow to the brain, causing drowsiness. Alkaloids will bind to the receptors of the adenosine, namely A1, A2a, A2b, and A3 receptors, then cause the reverse effect of adenosine, which increases muscle movement and facilitates blood flow to the brain, thereby making the body more active (Januarti et al., 2020).

Terpenoid compounds and saponins are also compounds that have been proven to be found in red rice bran tea. The sensory system in mice will receive signals from terpenoids that enter the mice's body. Terpenoids will give a signal to the muscles to help dilate blood vessels so as to make blood circulation smooth, provide a calming effect, and increase stamina. Saponins contain lead saponins and triterpene acids in the form of esters from sugar which have antioxidant properties so they can counteract free radicals. (Sunarti et al., 2017). According to Estiasih et al. (2021), several components such as phenolic compounds, vitamin E (tocopherols and tocotrienols), B vitamins, and oryzanol present in red rice bran tea products have high antioxidant activity. Antioxidants can increase cellular level metabolism by producing a lot of energy so that ATP production increases. Vitamin B

has a function as a coenzyme that helps metabolize energy in the body. By activating the nervous system, vitamin B will result in an acceleration of the heart rate to pump blood and oxygen, as well as stimulate an increase in blood sugar levels. (Putriastuti et al., 2007). Red rice bran tea products also contain other compounds such as carbohydrates and proteins. Carbohydrate metabolism in the body will use glucose as the main fuel for cell activity which will be transported throughout the body via blood. Protein is also one of the main sources of energy besides carbohydrates which function for growth, development and muscle formation (Hidayah dan Sugiarto, 2013).

#### 4. Conclusion

The combination of red rice bran and *P. caninum* as a tea product of bran red rice brand 'X' with a dose of 300, 600 and 900 mg/kg BW can affect the stamina of mice, namely by increasing the stamina of mice, especially after the absorption time of 15 minutes in the first period the natatory exhaustion test. The most optimal dose to increase the stamina of mice from a combination of red rice bran and *P. caninum* as a red rice bran tea product brand 'X' is a dose of 900 mg/kg BW.

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