

## Efek Sari Buah Cherry (*Prunus avium* (L.) L.) terhadap Efek Sedasi dan Waktu Tidur pada Mencit Putih Jantan

### The Effects of Cherry (*Prunus avium* (L.) L.) Juice on Sedative Effect and Sleep Duration in Male White Mice

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#### ABSTRACT

Sedation can be defined as the use of sedative medication to relieve intolerable and refractory distress by reducing patient consciousness. Sedation can cause mild depression in the central nervous system without loss of consciousness and calming effect and prevent seizures. Cherry is a plant with sedative potential effect, contain melatonin as a flavonoid compound. This study aims to determine sedative effect, effective dose, and sleep duration in white mice male after cherry juice administration. The rotarod test was conducted to determine the resistance time of tested mice on a rotating rod. The observed parameter was the length of the time the mice running on a rotating rod at a speed of 28 rpm. Cherry juice was prepared using a juicer and then filtered with muslin cloth and dried with vacuum dryer. Cherry juice was prepared in 3 doses, they were treatment dose 1, 2, and 3 with cherry juice of 8.3, 16.6, and 25 mg/20gBW, respectively. Aquadest was used as negative control (0.5 ml/ mice), while phenobarbital was the positive control at a dose of 0.8 mg/ 20gBW). The results showed that treatment dose 3 containing 25 mg/20gBW) cherry juice was the most effective dose with the fastest falling effect (13.41 second) on rotarod and the longest duration of sleep time (45.92 minute).

**Keywords:** cherry juice, duration of sleep, sedation effect.

#### ABSTRAK

*Sedasi dapat didefinisikan sebagai penggunaan obat sedatif untuk meredakan gangguan yang tidak dapat ditolerir dengan cara menurunkan kesadaran. Sedasi dapat menyebabkan depresi ringan pada sistem saraf pusat tanpa kehilangan kesadaran dan efek menenangkan serta mencegah kejang. Cherry merupakan tumbuhan dengan potensi efek sedatif, mengandung melatonin sebagai senyawa flavonoid. Penelitian ini*

*bertujuan untuk mengetahui efek sedatif, dosis efektif, dan lama tidur mencit putih jantan setelah pemberian jus kersen. Metode konduksi menggunakan uji rotarod dengan menentukan waktu resistansi mencit pada batang putar sebagai pekerjaan utama. Parameter yang diamati adalah lamanya tikus dapat berlari pada batang yang berputar dengan kecepatan 28 rpm. Proses pembuatan jus ceri dilakukan dengan menggunakan juicer kemudian disaring dengan kain batis untuk memastikan sari terpisah dari ampasnya lalu dikeringkan dengan vacuum dry. Jus ceri dibuat dalam 3 dosis perlakuan, yaitu perlakuan 1, 2, dan 3, masing-masing dengan jus ceri 8,3; 16,6; dan 25 mg/20gBB. Akuades digunakan sebagai kontrol negatif (0.5 ml) dan fenobarbital sebagai kontrol positif (0.8 mg/20gBB). Hasil penelitian menunjukkan bahwa perlakuan 3 dengan sari buah ceri sebanyak 25 mg/20gBB merupakan dosis yang paling efektif karena memiliki efek jatuh paling cepat (13.41 detik) pada rotarod dan durasi waktu tidur terlama (45.92 menit) setelah kontrol positif.*

**Kata kunci:** durasi tidur, efek sedasi, sari buah ceri.

## Introduction

Sleep is one thing that is very important and influences the function and psychology of the human body. The recommended sleep time for adults is 7 hours (Deacon, 2013). People in the world including Indonesia experience sleep disorders that are often faced and also show a significant increase known as insomnia. Insomnia is a sleep disorder that is mostly suffered, it is estimated that around 30% of adults experience insomnia and around 10% of them experience chronic insomnia (Ferrie et.al, 2013). The study mentioned the prevalence of insomnia in Indonesia reported 28 million people or 10 percent of the total population (Salbiah, 2018). The use of sleeping pills continues to increase in 2005-2010 due to the increasing number of sleep disorders (Conference et al., 2015).

Sedation drugs has mild depression effect in the central nervous system without loss of consciousness

and provide a calming effect, prevent seizures such as phenobarbital. Plants that have the potential as sedation are cherry fruit. Cherry fruit contains flavonoid compounds in the form of melatonin from various variations of flavonoids and procyanidins that contained in cherries, which can reduce the risk of Alzheimer's disease (Feng et.al, 2014). Melatonin is a hormone produced by the pineal gland that has an important role especially in the initiation of sleep. Melatonin has antioxidant activity that can help normalize heart rhythms and help healthy sleep patterns (Gandhi et.al, 2015). The effect of tart cherry (*Prunus ceracus*) gradual dose on phenobarbital induced mice extended sleep time with the best tart cherry dose was 0.156 ml and provide extended sleep time for 62.4 minutes induced phenobarbital (Wijaya, 2018).

The results of those study are the basis for conducting the sedative effect test and sleep duration of cherry

in white mice, because sweet cherry is one of the cherry variation species containing melatonin compounds and can improve healthy sleep patterns, it is thought to be able to be used as a more effective sedation compound. The objective of this study is to determine sedative effect and the most effective dose from sweet cherry juice.

## **Material and Methods**

### *Instruments*

Analytical balance (LabPRO®), juicers (Phillips®), oven (Menmert), furnace (Ney®), rotarod (rotarod apparatus®), vacuum drier (Ogawa®), 1 mL syringe, gastric sonde, and stopwatch are used in this study.

### *Materials*

The reagents used are phenobarbital, aquadest, sulfuric acid, hydrochloric acid 2N, FeCl<sub>3</sub>, HCl 2N, Mayer, Dragendorf, gelatin, 10% NaCl and Mg powder. Selected sweet cherries (obtained from the Melvin Fruit Supplier, Jakarta), were shiny blackish red in color and soft textured. The determination of sweet cherries were carried out at the Plant Conservation Research Center of the Indonesian Institute of Sciences (LIPI) Bogor. Experimental animals used in this study were 25 white male mice aged 2-3 months.

### *Experiments*

#### **1. Cherry juice processing**

Ripe sweet cherry fruit is prepared as much as 3.5 kg and then separated from the seeds and fruit stems. Then the cherries are washed with running water then processed

using a juicer, filtered and separated with the pulp, dried with vacuum dryer.

#### **2. Extract characterization test**

Determination of the yield of cherry fruit juice is carried out in the following steps. Weigh as much as 3500 g of cherries, then dry them. Calculation of excess juice by comparing the initial weight with the final weight obtained.

Extract characterization test includes determination of water content, determination of ash content and calculation of excess extract. Water content determination of sweet cherry juice is done by this following step, as much as 2 g of dry cherry juice which has been weighed carefully, put into a steamed cup that has been stored for 10 minutes in a 105°C oven, the juice is evaporated in a 105°C oven until its weight is constant (weight difference between weighing plates after dried i.e. 0.0025 g or 0.25%). The water content is calculated against the weight of the test material expressed in % w/w (Departemen Kesehatan RI, 2013).

A total of 2 g of dried sweet cherry juice is weighed carefully, put into a crucible that has been heated and weighed, slowly put at 600°C until the charcoal runs out, cooled. Then weighed until constant. Hot water can be added, stirred and filtered through an ash-free filter paper if the charcoal is not lost. The filter paper is heated along with the filtering results in the same crucible.

The filtrate is put into the crucible, evaporated and heated until constant weight. The total ash content is calculated against the weight of the test material expressed in% w/w (Departemen Kesehatan RI, 2013).

3. Phytochemical screening of cherry juice

Phytochemical testing is carried out to determine the class of compounds found in a plant. Phytochemical testing on dried cherry juice includes identification of alkaloids using Dragendorff reagent and Mayer reagent, flavonoids identification using Mg powder and strong HCl and tannins identification using 1% gelatin solution, 10% NaCl solution, and 3% FeCl solution.

4. Animal studies

a. Ethical review

This research was conducted after an ethical review that was reviewed by the Animal Ethics Commission of Pakuan University. All procedures for the maintenance and treatment of experimental animals have passed the ethical review by the Ethics Committee for the Use of Experimental Animals, Faculty of Mathematics and Natural Sciences, Pakuan University, with the ethics committee's decision letter Number. 74 / KEPHP-UNPAK / 10-2019.

b. Preparation of tested solutions

Preparation of the test solution was done by dissolving 1000 mg of dried cherry juice in 20 ml of aquadest (dose 3 solution),

for the second dose solution was made by dissolving 0.83 ml of the dose 3 solution then adding up to 5 ml aquadest, the first dose solution was made by dissolving 0.41 ml 3 dosage solution in aquadest add up to 5ml. Each mouse was to accept 0.5 ml solution/ 20 g BW.

c. Acclimatization

All 25 experimental animals used in this study were weighed, their physiological conditions were observed, divided randomly into 5 groups and each group consisted of 5 animals as replications. The animals were adapted for 7 days in a cage, fed and drank standard (ad libitum) and then weighed each mice after 7 days.

d. Experimental animal treatments

Treatment carried out on experimental animals by administering cherry fruit juice at doses of 8.3, 16.6, and 25mg/ 20gBW, as well as positive control (phenobarbital at 0.8 mg/20gBW) and negative control (distilled water at 0.5 ml/mice). The negative control function in the study used as a comparison and a solvent for the making of positive control solutions and the preparation of test solutions (Manawan F, Defny SW, dan Frenly W, 2014).

To determine the effect of sedation on tested animals, the Rotarod method was used where the experimental animals were placed on a rotating rod with a diameter of 3 cm. Abnormal test

animals will need a short time to run and also need a fast time to fall. This shows that the test animals are under the effect of sedation.

Rotarod speed was set at 28 rpm, the time required for mice to maintain position on the rotarod was then recorded. The length of time mice can survive to run on rotating wheels or rotarod is a parameter of motor activity, balance and physical balance. Normal mice will maintain position on the rotarod for a long time. The existence of a minimum neurological disturbance such as sedation is shown by the inability of the mice to hold their position and fall faster.

#### 5. Data analysis

The data obtained were processed by the ANOVA test followed by Duncan's post hoc test. Anova test was conducted to determine the effect concentration to sedation effect and sleep onset on male white mice. Duncan's post hoc test was used to determine the differences of sleep time onset and the effect of sedation between test groups.

### Results and Discussion

The cherry fruit used is 3500 g. The drying results obtained were 187.4 g of dried cherry juice so that the yield of cherry fruit juice was 5.35%. Extract characterization testing is carried out on water content and ash content, water content testing is conduct with gravimetric method, this is the simplest

method with the principle of water evaporation and the amount of weight loss is calculated by constant weighing at the time after and before heating at 105°C (AOAC, 2019). High moisture content results in a short shelf life and facilitates the growth process of microorganisms. The water content of the cherry juice in this study is 4.28%. This amount meet the requirements in general <10% (DepKes RI, 2000).

The result of cherry juice ash content is 2.46%. Measurement of ash content aims to determine the mineral contained in food. The measured ash content is inorganic material that does not burn in the process while organic material burns. The results of the ash content test from cherry fruit juice meet the requirements because the ash content requirements in general are <10% (DepKes RI, 2000). Based on this study, dried cherry juice met the requirements and there are not many impurities in the dried juice.

The organoleptic test results showed that the dried cherry juice is thick and sticky, has yellowish brown color, has a strong characteristic aromatic odor and has a bitter sour taste as shown in Table 1.

The sedation effect was tested using a rotarod device. This is used as a pharmacological screening to determine the effect of drugs that act on motor coordination and rodents balance, because sedation effect lead to motor activity decrease. The working principle of rotarod is to determine the endurance time of the experimental animal on

rotating the rotating rod at a certain speed. If the animal started to fall, it can be stated that the animal has motor balance disorders (Depkes RI, 2000)

**Table 1.** Results of organoleptic test and quality of cherry juice

Parameter	Result
<b>Organoleptic</b>	
Odor	Strong aromatic
Form	Thick juice
Colour	Yellowish-brown
Taste	Bitter
<b>Physicochemical</b>	
Water content	4.28 %
Ash content	2.47 %

The sedation effect was tested using a rotarod device. This is used as a pharmacological screening to determine the effect of drugs that work on motor coordination, the balance of rodents because sedation can reduce motor activity (Rosenfeld and Loose, 2007). The working principle of the rotarod is to determine the endurance time of the experimental animal on rotating the rotating rod at a certain speed. If the animal tries to fall, it can be stated that the animal has motor balance disorders (Deacon, 2013). After being given the treatment, the experimental animals were rushed to the rotarod device and the length of time the mice remained on the rotating rods with a stopwatch were calculated. The parameters observed were the length of time the experimental animal could survive running on a rotating rod at a predetermined speed. Observation data

on the sedation effect of each group can be seen in Table 2.

Table 2 shows different ability of mice to survive on rotarod before and after treatment. There was a decrease in the survival time of the mice after being given treatment. Anova test results showed that the results of the dose factor had a significant effect on sedation in male white mice ( $p < 0.05$ ). Duncan's continued test was carried out to determine the differences between the test groups. The results of Duncan's test showed that the negative control was significantly different from all treatment factors for the dose of cherry juice and the positive control, which means aquadest as a solvent had no sedation effect in male white mice. So that appearance of the sedation effect is resulting from cherry fruit juice and phenobarbital as well.

From Table 2 it can be seen that the survival time of mice on rotarod in the phenobarbital group as a positive control was significantly different from the negative group using aquadest. This shows that the experimental procedure carried out is valid. Duncan's test results showed that phenobarbital was in the same column as cherry juice 25mg/ 20 g BW (marked with the same superscript in Table 2). The resulting sedation effect from 3<sup>rd</sup> dose was comparable to that of the positive control.

**Table 2.** Average mice survival time on rotarod

Treatment	Weight (g)	Mice survival time (second)	
		Before treatment	After treatment
Phenobarbital	31.6±1.52	126.63±5.85	9.44±1.03 <sup>a</sup>
Aquadest	29.4±1.82	135.16±3.44	120.51±12.57 <sup>c</sup>
Dose 1	30.2±2.05	134.91±3.59	41.56±2.24 <sup>b</sup>
Dose 2	31.4±1.64	130.81±8.13	37.28±1.75 <sup>b</sup>
Dose 3	32.2±1.34	123.89±7.74	13.41±1.19 <sup>a</sup>

Note: Numbers followed by different superscript letters (a, b, c) in the same column show a significantly different effect ( $p < 0.05$ )

After the sedation effect was tested using a rotarod device, then an observation was made of the duration of sleep in mice to see the effect of falling asleep from the beginning of sleep to returning to normal or returning to activity. Prior to the duration of sleep duration, the mice were initially assessed by the onset of sleep time by seeing the loss of mice unconsciousness and no reaction to the environment and calculated using a stopwatch. The sleep time onset of mice can be seen in Table 3. After the sleep time onset of walking mice, the duration of sleep was tested by observing from the beginning of the mice the loss of reflex until the mice were able to return to their four legs to the base of the experiment and counted using a stopwatch. The sleep duration of mice can be seen in Table 4.

Based on observations of sleep time onset and effects of sedation all treatment doses had a sedating effect on white male mice. All of those treatment doses have have a faster sleep time onset and ability to survive on the rotarod faster than negative control. On Table 2 and Table 3, positive control group and all treatment dose groups

were significantly different from the negative control.

**Table 3.** Average effect of cherry fruit dosen on sleep time onset

Treatment	Sleep time onset (min)
Phenobarbital	61.32±4.69 <sup>a</sup>
Aquadest	307.41±34.55 <sup>c</sup>
Dose 1	119.59±36.18 <sup>b</sup>
Dose 2	99.46±16.35 <sup>b</sup>
Dose 3	66.89±4.58 <sup>a</sup>

Note: numbers followed by different superscript letters (a, b, c) in the same column show a significantly different effect ( $p < 0.05$ ).

Positive control has the most effective sedation effect with a rotarod endurance time of 9.44 seconds, while the treatment dose 3 cherry juice is the most effective dose as sedative with a resistance time of 13.41 seconds. Similar with the results of the Duncan analysis of the effect of treatment on the sedation effect on rotarod (Table 2), Duncan analysis of treatment effect on sleep time onset showed that the results of the negative control were significantly different from the positive controls (indicated by different superscripts in Table 3). So that the method used is valid. Positive control and all doses of



cherry juice were significantly different from negative control, which means that the treatment given had an effect on the experimental animals. Meanwhile, the best dose of cherry juice in providing sleep time onset was 25 mg/20g BW, this dose had a comparable effect with phenobarbital administration as a positive control.

**Table 4.** Average effect of dose of cherry fruit on sleep time duration

Treatment	Duration of sleep (min)
Phenobarbital	51.58±2.14 <sup>d</sup>
Aquadest	6.98±0.73 <sup>a</sup>
Dose 1	15.60±0.98 <sup>b</sup>
Dose 2	18.61±0.66 <sup>b</sup>
Dose 3	45.92±4.67 <sup>c</sup>

Note: numbers followed by different superscript letters (a, b, c) in the same column show a significantly different effect ( $p < 0.05$ ).

The results of Duncan's analysis of the effect of treatment on sleep duration were similar to the two previous test results regarding onset and survival on rotarod. From Table 4, it can be seen that the sleep duration of the negative control was significantly different from all treatment groups so that it can be stated that this method is quite valid and that treatment with phenobarbital and cherry juice has a sedative effect on mice. Meanwhile, the dose of cherry juice which has an effect comparable to that of phenobarbital is a dose of 25mg/20g BW.

Cherry fruit contains melatonin which is also a hormone produced and secreted by the pineal gland, retina and

intestines. Melatonin synthesis is controlled both endogenously via circadian rhythms and by external light stimulation (Zhu and Zee, 2013, Liu (2004). Melatonin has many benefits for the body, apart from being insomnia, melatonin can also relieve jet lag problems, reduce free radicals in the body, boost the immune system, are anti-inflammatory and anti cancer effects (Peuhkuri dkk., 2012).

The mechanism of action of melatonin could occur through binding to its receptors. One of the most abundant receptors in the hypothalamus, namely MT1, is mainly located on the SCN (Suprachiasmatic Nucleus) which releases neurotransmitters, which functions to maintain awareness and alertness. The bond between melatonin and MT1 on the SCN will reduce the excitation of neurotransmitters on the SCN so that melatonin plays a role in the sleep process.

Based on observations using rotarod, the onset and duration of sleep, it can be concluded that cherries have a sedative effect with the most effective dose of 25mg/ 20gBW. This result suiTable with another study that explained administration of cherry concentrate can improve quality and prolong sleep time for insomniacs. Cherry fruit can act as a GABA receptor agonist, where cherries with their flavonoid and alkaloid content can bind or interact with GABA receptors so that they can increase the activity of hypnotic



or sedative-hypnotic effects (Pigeon et al, 2010).

### Conclusion

Cherry fruit juice has a sedative effect based on the ability to survive on the rotarod, sleep time onset and duration. The most effective dose of cherry juice extract is 25 mg/20gBW based on analytical statistic of survival time on rotarod and sleep time onset, these dose is not significantly different from phenobarbital as positive control. In line with these results, the longest duration of sleep in treatment group present at the dose 25 mg/20gBB with a time of 45.92 minute. This value is the closest to the sleep duration of the positive control group.

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