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Toxicity Test of Red Yeast Rice Extract as a Product of Solid Fermentation of *Monascus purpureus*

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Abstract
Red yeast rice (Known as Angkak in Indonesia) is a product of rice fermentation from Monascus purpureus, a specific type of mold that produces a secondary metabolite, namely monacolin K; found to be effective in reducing cholesterol levels. Subsequently, toxicity is the ability of a substance to induce functional, biochemical, or physiological disturbances leading to pain, and discomfort, as well as interfering with general body conditions. Therefore, this study aims to determine the potential toxic effects, mortality rates, and liver function damage, as indicated by SGPT and SGOT parameters, in rats. Solid fermentation method was used for rice fermentation. Test animals used were 25 white rats, exposed to graded doses: dose 1 (5mg/kg BW rats), dose 2 (50mg/kg BW rats), dose 3, (300mg/kg BW rats), and dose 4 (2000mg/kg BW mice). Observations were carried out for 14 days with a single dose. To determine liver damage, blood was taken through the lateral vein on day 14, the resulting data was processed using statistical analysis. The results showed that Angkak extract containing monacolin K did not exhibit a toxic effect and did not cause death. However, there was an impact on liver damage parameters, as evidenced by elevated SGPT and SGOT levels at doses of 300 mg/kg BW rats and 2000 mg/kg BW rats, exceeding normal levels.

Keywords: red yeast rice extract, monacolin K, toxicity, SGOT, SGPT

1. Introduction

The use of natural ingredients as the main components in medicine is a part of the culture of almost every nation in the world. Almost all the world's population depends on natural materials to maintain health. Subsequently, it is important to ensure th1e safety of using natural ingredients in traditional medicine to protect the community from adverse effects (Jafarizadeh-Malmiri et. al., 2019). One of the natural ingredients with potential in traditional medicine is Red yeast rice (Known as Angkak in Indonesia), which contains several active substances capable of treating various diseases such as hypertension, cholesterol, and thrombocytopenia (Younes et.al., 2018). Additionally, Angkak can also be used for the treatment of cases related to digestion, blood circulation, and lymph and stomach health (Merryweather and Hill, 2005).

Angkak is a product of rice fermentation by *Monascus purpureus*, frequently used for food coloring, preservation, as well as for medicine purposes (Pattanagul, 2007). *Monascus purpureus* produces pigments widely used in the food industry (Silveira et. al., 2013). There are three types of pigments derived from *Monascus* secondary metabolites, namely yellow pigments containing ankaflavin and monascin, orange pigments containing monascorubin and rubropunctanin, and red pigments containing monascorubramin and rubropuctamin

(Patakova, 20013). Fermentation process generates secondary metabolites that can be harnessed for medicinal purposes. One of the secondary metabolites produced during fermentation is Monacolin K, which can be used in the treatment of cholesterol (Demain, 2014). Monacolin K inhibits cholesterol synthesis by reducing the activity of HMG-CoA (3-hydroxymethyl-glutaryl Coenzyme A reductase), an enzyme that determines cholesterol biosynthesis (Bills, 2009). This enzyme is used as a drug in dietary programs. Studies have shown that lovastatin, a related compound, can reduce blood cholesterol levels by 11-32% and triglycerides by 12-19% (Heber, 1999). These results were further supported by Madrigal et.al. (2014) who stated that Angkak can suppress the increase in total blood cholesterol levels in rats by 49.28%.

Angkak extract, in addition to its cholesterol-lowering benefits, may also lead to side effects like myopathy and abnormal liver function test results. Therefore, it has the potential to cause significant issues if not properly monitored and treated (Klimek et.al., 2009). It is necessary to carry out a safety test of Angkak extract to determine a safe dose for the treatment of cholesterol. Based on the information provided, the researcher carried out Toxicity Test of the fermented Angkak extract from *Monascus purpureus*.

2. Methods

2.1. Substrate Preparation and Sterilization

The cleaned IR-64 rice was placed in an Erlenmeyer and sterilized using an autoclave at 121^0C for 15 minutes. All glass utensils were sterilized using an oven at 180^0C for 15 minutes.

2.2. Rice Fermentation as Solid Media

Rice was inoculated with several mL of *Monascus purpureus* suspension and nutrient broth. The mixture was stirred until homogeneous, and fermentation was carried out at a temperature of 27-32^0C for about 14 days.

2.3. Extraction

Angkak produced by fermentation process was ground until it became smooth, and the powder was extracted using ethyl acetate solvent using a Waterbath shaker. The resulting supernatant was filtered using filter paper.

2.4. Toxicity Test

2.4.1. Selection of Test Animals

Test animals used were healthy rats aged about 6-8 weeks, with a minimum weight of 120 grams. Mice were acclimatized for 1 week.

2.4.2. Preparation and Treatment of Test Animals

A total of 25 test animals were prepared and divided into 5 groups, with 4 groups receiving treatment and 1 group serving as the control. Each group consisted of 5 test animals. Subsequently, the animals used for the testing profess were not given food before treatment. Mice were not given any food for 14-18 hours, however, they were given drinking water. After the process, the animals were weighed and administered test preparation orally.

2.4.3. Dosage Determination

For other acute toxicity doses, the dose from the evaluation results based on the hazard criteria listed in the Regulation of the Head of the Food and Drug Supervisory Agency number 7 of 2014 concerning Guidelines for In Vivo Nonclinical Toxicity Test is 5 mg/kg rats, 50 mg/kg rats, 300 mg/kg BW rats, 2000 mg/kg BW rats.

2.4.4. Observation

Observations were carried out on the activity of rats for 14 days, including tremors, seizures, salivation, diarrhea, lethargy, weakness, sleep, and coma. Additionally, death and liver damage were observed using SGPT and SGOT parameters.

2.4.5. Determination of SGPT Level

On the 14^{th} day, blood samples were collected through the lateral vein, placed into an ephendroph tube, and then centrifuged at 2500 rpm for 30 minutes. The samples were measured at a wavelength of 340 nm and, incubation temperature 37^0C. Serum was then added with SGPT Kit reagent.

2.4.6. Data Analysis

The data was processed using SPSS.

3. Results and Discussion

3.1. Substrate Preparation and Sterilization

The sterilization process serves the purpose of eliminating living microorganisms present in equipment and materials. This is achieved by denaturing proteins within enzymes and the cell walls of these microorganisms (Bharti et.al., 2022)

3.2. *Monascus purpureus* Breeding

The breeding of *Monascus purpureus* was carried out using PDA inclined media. PDA provides the necessary nutrients for the growth of the fungus like *Monascus purpureus*. Additionally, a selective medium was added to prevent the growth of unwanted microorganisms without inhibiting the growth of target microorganisms (Bonnet, et. al., 2020).

Table 1. Multiplication Morphology of *Monascus purpureus* in PDA (Potato Dextrose Agar) Culture

Breeding Age	***Monascus purpureus* culture development**
1	Grow white dot
2	White hyphae grow
3	Orange thin mycelium
4	Orange mycelium thickened
5	Orange mycelium turned red
6-7	The red color becomes a lot and evenly
8	Medium shriveled and red
9	Medium shriveled and dark red
10-14	The red color was getting more and more evenly distributed

3.3. Rice Fermentation as Solid Media

In fermentation process including *Monascus purpureus*, three distinct phases can be identified. The first stage is the adaptation phase, also known as the lag phase, during which microorganisms acclimate to their new environment. The second stage is the exponential phase, where microorganisms grow in the presence of a carbon source, and nitrogen is derived from primary metabolites, energy, and water for growth and development. The third stage is the stationary phase, in which *Monascus purpureus* cells engage in fermentation by secreting secondary metabolites in the form of dyes and other compounds (Singgih, 2015).

3.4. SGPT Measurement Results

Table 2 shows an increase in ALT levels from each dose when compared to the control group. In rats administered a single dose of Angkak extract at doses 3 and 4, SGPT levels indicated liver damage beyond the normal range. Figure 1 shows that the mean value of SGPT levels has increased, where the higher the dose, the higher the mean SGPT levels.

Table 2. SGPT level

No	Group	SGPT Level			Mean
		1	2	3	
1.	Control	31,1	45,8	53,6	43,50±11,425
2.	Dose 5 mg/kg BW rats	104,3	132,4	141,5	126,07±19,392
3.	Dose 50 mg/kg BW rats	122,4	151,5	164,3	146,07±21,472
4.	Dose 300 mg/kg BW rats	143,8	167,4	175,7	162,30±16,55
5.	Dose 2000 mg/kg BW rats	179,2	173,0	180,1	177,43±3,866

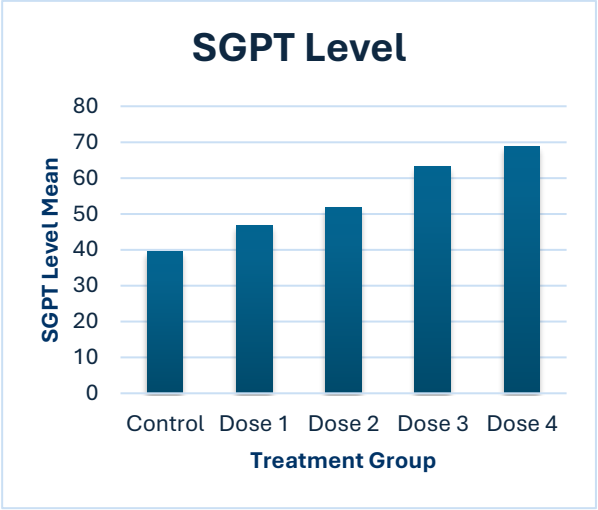

Figure 1. SGPT Level Mean

Based on the Shapiro-Wilk statistical test, the data obtained by all groups were normally distributed with a significant value ($p>0.05$). Homogeneity analysis confirmed that the data was homogeneous, with a significant value ($p>0.05$). The results of the ANOVA test had a significant difference among the groups with a significant value ($p<0.05$). Based on the LSD test in the control group at a dose of 5 mg/kg rats and 50 mg/kg rats, there was no significant difference in the increase in SGPT levels with significant values ($p>0.05$).

Additionally, while at a dose of 300 mg/kg rats and 2000 mg/kg BW rats, there was a significant difference in the increase in SGPT levels with a significant value ($p<0.05$).

3.5. SGOT Measurement Results

SGOT enzymes are more abundant in the heart, kidneys, and brain compared to the liver. Elevated serum enzyme levels suggest cell leakage and compromised liver cell membrane integrity due to toxicity. The high level of SGOT in comparison to the level of SGPT proves that not only the cytosol was damaged, but mitochondria were also affected by the increased SGOT levels.

Table 3. SGOT level

No	Group	SGOT Level			Mean
		1	2	3	
1.	Control	42,9	40,5	35,3	39,57±3,885
2.	Dose 5 mg/kg BW rats	54,9	48,2	37,6	46,90±8,723
3.	Dose 50 mg/kg BW rats	59,7	53,1	42,2	51,67±8,838
4.	Dose 300 mg/kg BW rats	71,1	63,9	54,8	63,27±8,168
5.	Dose 2000 mg/kg BW rats	85,2	63,0	58,3	68,83±14,36

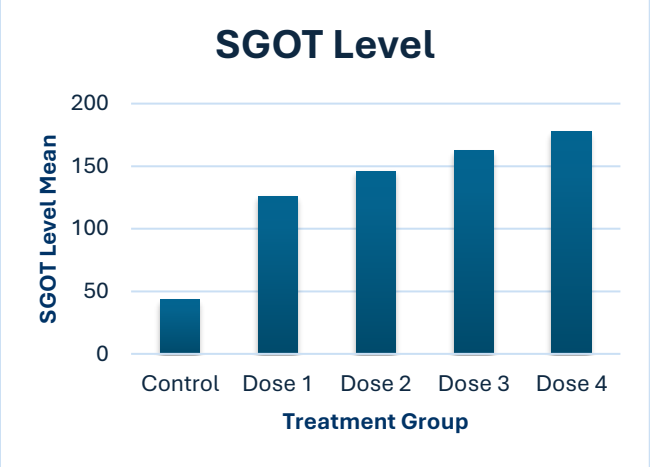

Figure 2. SGOT Level Mean

Table 3 shows that there is an increase in SGOT levels at each dose when compared to the control group. Figure 2 shows that the average value of SGOT levels increased along with the increase in the number of doses.

Based on the Shapiro-Wilk statistical test, the data obtained by all groups were normally distributed with a significant value ($p>0.05$). Based on the homogeneity analysis, the data was homogeneous with a significant value ($p>0.05$). The results of the ANOVA test had a significant difference with a significant value ($p<0.05$). Based on the LSD test in the control group at a dose of 5 mg/kg BW rats, dose of 50 mg/kg BW rats, doses of 300 mg/kg BW rats, and 2000 mg/kg BW rats, there was a significant difference in the increase in SGPT levels with significant values ($p< 0.05$).

Conclusions

Based on this study, it can be concluded that the Angkak extract containing Monacolin K demonstrated a lack of toxicity, as evidenced by the absence of toxic effects and no observed deaths in the test animals. However, the Angkak extract containing Monacolin K did have an impact on liver damage parameters, particularly at doses of 300 mg/kg BW and 2000 mg/kg BW in rats, where elevated levels of liver enzymes indicated potential liver damage.

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Conflicts of Interest

The authors declare no conflict of interest.

References

Bharti, B., Li, H., Ren, Z., Zhu, R., & Zhu, Z. (2022). Recent advances in sterilization and disinfection technology: A review. *Chemosphere*, *308*, 136404.

Bills, G., Overy, D., Genilloud, O., & Peláez, F. (2009). Contributions of pharmaceutical antibiotic and secondary metabolite discovery to the understanding of microbial defense and antagonism. In *Defensive mutualism in microbial symbiosis* (pp. 275-316). CRC Press.

Bonnet, M., Lagier, J. C., Raoult, D., & Khelaifia, S. (2020). Bacterial culture through selective and non-selective conditions: the evolution of culture media in clinical microbiology. *New microbes and new infections*, *34*, 100622.

Demain, A. L. (2014). Valuable secondary metabolites from fungi. Biosynthesis and molecular genetics of fungal secondary metabolites, 1-15.

Heber, D., Yip, I., Ashley, J. M., Elashoff, D. A., Elashoff, R. M., & Go, V. L. W. (1999). Cholesterol-lowering effects of a proprietary Chinese red-yeast-rice dietary supplement. *The American journal of clinical nutrition*, *69*(2), 231-236.

Jafarizadeh-Malmiri, H., Sayyar, Z., Anarjan, N., & Berenjian, A. (2019). *Nanobiotechnology in food: Concepts, applications and perspectives* (pp. 81-94). Berlin, Germany: Springer.

Klimek, M., Wang, S., & Ogunkanmi, A. (2009). Safety and efficacy of red yeast rice (Monascus purpureus) as an alternative therapy for hyperlipidemia. *Pharmacy and Therapeutics*, *34*(6), 313.

Madrigal-Santillán, E., Madrigal-Bujaidar, E., Álvarez-González, I., Sumaya-Martínez, M. T., Gutiérrez-Salinas, J., Bautista, M., ... & Morales-González, J. A. (2014). Review of natural products with hepatoprotective effects. *World journal of gastroenterology: WJG*, *20*(40), 14787.

Merryweather, L. M., & Hill, S. H. A. (2005). Dictionary of food science and technology.

Patakova, P. (2013). Monascus secondary metabolites: production and biological activity. *Journal of Industrial Microbiology and Biotechnology*, *40*(2), 169-181.

Pattanagul, P., Pinthong, R., Phianmongkhol, A., & Leksawasdi, N. (2007). Review of angkak production (Monascus purpureus). *Chiang Mai J Sci*, *34*(3), 319-328.

Silveira, S. T., Daroit, D. J., Sant'Anna, V., & Brandelli, A. (2013). Stability modeling of red pigments produced by Monascus purpureus in submerged cultivations with sugarcane bagasse. *Food and Bioprocess Technology*, *6*, 1007-1014.

Singgih, M., & Julianti, E. (2015). Food colorant from microorganisms. *Beneficial microorganisms in food and nutraceuticals*, 265-284.

Younes, M., Aggett, P., Aguilar, F., Crebelli, R., Dusemund, B., ... & Wright, M. (2018). Scientific opinion on the safety of monacolins in red yeast rice. *EFSA Journal*, *16*(8), e05368.