JKR (JURNAL KEDOKTERAN RAFLESIA)
Vol. 7, No. 1, 2021
ISSN (print): 2477-3778; ISSN (online): 2622-8344
https://ejournal.unib.ac.id/index.php/jukeraflesia

TOXICITY TEST OF WHITE TURMERIC (CURCUMA ZEDOARIA) ON LIVER ORGANS IN WHITE MALE RATS

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Abstract
White turmeric is often used by the people of Indonesia as an herbal plant that has many benefits for the body, one of which is for the liver. But there are still many people who do not know the right and correct dosage in using this white turmeric. The purpose of this study was to determine the toxic dose of white turmeric extract to estimate the degree of damage caused by the extract to the liver. This research design uses experimental research methods by means of Post Test Only Control Group Design. The sample used in this study consisted of 5 rats in each group with a number of groups of rats consisting of 6 groups with a total sample of 30 male white rats of the Wistar strain and in each group different treatments were carried out. Sampling was done using the Simple Random Sampling technique. Rats were divided into 2 control groups, namely equates negative control and NaCMC positive control, and also 4 treatment groups with a dose of 250 mg/kg BW, 500 mg/kg BW, 750 mg/kg BW, and 2000 mg/kg BW. From the results, it was found that the histopathological picture of the control group did not show significant changes, but in the treatment group, the doses of 250 mg/kg BW and 500 mg/kg BW were very clear where there was a lot of severe damage and hydrophilic degeneration began to occur where cell swelling, vacuoles were also found. fat. However, this happens because the liver sample already has a history of disease so that it gives a picture of degeneration.

Keywords: acute toxicity test, extract of white turmeric *(Curcuma zedoaria),* liver histopathology, male white rat Wistar strain

Abstrak
Kunyit putih sering sekali digunakan oleh masyarakat Indonesia sebagai tanaman herbal yang memiliki banyak manfaat bagi tubuh, salah satunya bagi organ hati. Tetapi masih banyak masyarakat yang belum mengetahui dosis yang tepat dan benar dalam menggunakan kunyit putih ini. Tujuan dilakukannya penelitian untuk mengetahui dosis toksik dari ektrak kunyit putih untuk memperkirakan derajat kerusakan yang diakibatkan oleh ektrak tersebut terhadap organ hati. Rancangan penelitian ini menggunakan metode penelitian eksperimental dengan cara Post Test Only Control Group Design. Sampel yang digunakan dalam penelitian ini terdiri dari 5 ekor tikus setiap kelompok dengan jumlah kelompok tikus terdiri dari 6 kelompok dengan jumlah total sampel 30 tikus putih jantan jenis galur wistar dan di setiap kelompok dilakukan perlakuan yang berbeda- beda. Pengumpulan sampel dilakukan menggunakan teknik Simple Random Sampling. Tikus dibagi menjadi 2 kelompok kontrol yaitu kontrol negatif aquades dan kontrol positif NaCMC, dan juga 4 kelompok perlakuan dengan dosis 250 mg/KgBB, 500 mg/KgBB, 750 mg/KgBB, dan 2000 mg/KgBB. Dari hasil di dapatkan bahwa gambaran histopatologi kelompok kontrol tidak menunjukkan perubahan yang signifikan, tetapi pada kelompok perlakuan dosis 250 mg/KgBB dan 500 mg/KgBB terlihat sangat jelas dimana banyak kerusakan yang berat dan mulai terjadi degenarasi hidrofilik dimana pembengkakan sel, adanya di jumpai juga vakuola lemak. Namun hal ini terjadi kemungkinan sampel hati sudah memiliki riwayat penyakit sehingga memberikan gambaran degenerasi. Tetapi pada dosis 750 mg/KgBB dan 2000 mg/KgBB dijumpai sel yang mulai membaik dengan menunjukkan adanya perbaikan dimana berkurangnya degenerasi parenkimatosa dan degenerasi hidrofik tetapi masih dijumpai kelainan hati yang abnormal yaitu masih adanya pembengkakan pada sel-sel. Dengan kesimpulan semakin tinggi diberikan dosis dalam perlakuan maka memberikan efek yang positif atau baik terhadap perbaikan kerusakan hati.

***Keywords**: uji toksisitas akut, ekstrak kunyit putih (Curcuma zedoaria), histopatologi hati, tikus putih jantan galur wistar*

INTRODUCTION

Of the many varieties of plants in Indonesia and which can be developed as herbal medicine, one of them is white turmeric (Curcuma zedoaria).[1] Although it is rarely heard, it turns out that white turmeric has also been used quite often by Indonesian people who use white turmeric only as an additional ingredient/seasoning in cooking.[2]

With the development of the times, so that most people have recognized the benefits of this white turmeric so that people use and utilize this white turmeric not only as a cooking ingredient but also as herbal medicine.[3] Because white turmeric contains an active compound in the form of curcumin. Curcumin is useful as an anti-inflammatory, anti-inflammatory, and antioxidant that can prevent gene destruction, besides that it also contains other compounds, namely essential oils, which are useful as antineoplastic (anticancer) and have been shown to inactivate the growth of breast cancer cells.[4] However, many people only know how to consume without knowing the safe dose of white turmeric, because there is still a lack of research on this matter, so people only know that herbal medicines do not have side effects if used more than the proper dose.[5]

Seeing the use of white turmeric in society as herbal medicine which is quite large and often uncontrolled, further research is needed on the safety of using turmeric.[1] One way that can be used to find a safe dose of use in medicinal ingredients is by using a toxicity test. Testing the safety of drugs/materials before being used by humans can be done through a series of toxicity experiments using experimental animals.[2]

The purpose of this study was to determine the toxic dose of white turmeric extract to estimate the degree of damage caused by white turmeric extract to the liver of white rats.

So because there is still a lack of research on white turmeric, I am hereby interested in knowing and researching more about the use of toxic doses of white turmeric, especially the liver, so that in the future people will know that consuming herbal medicines is not arbitrary but in accordance with safe doses.

METHODS

This research is experimental research with The Post Only Control Group Design method. The sample of this study used 30 male white rats of the Wistar strain. The sample was collected using the

Simple Random Sampling technique and met the criteria, namely healthy male white rats, aged 6-8 weeks and bodyweight 160-200 mg. The sample used in this study consisted of 5 rats in each group, with the number of groups of rats consisting of 6 groups and each group being treated differently. Rats were divided into 2 control groups, namely equates negative control and NaCMC positive control, and also 4 treatment groups with a dose of 250 mg/kg BW, 500 mg/kg BW, 750 mg/kg BW, and 2000 mg/kg BW. This research was conducted at the Pharmacology Laboratory and Histology Laboratory, Faculty of Medicine, University of North Sumatra, from March 25, 2021 to May 5, 2021.

This research began with the manufacture of white turmeric extract by maceration method using 96% ethanol which had previously been distilled 10 times the weight of white turmeric powder. The white turmeric powder was then soaked with 96% ethanol and then filtered again repeatedly and concentrated with a rotary evaporator so that it became an almost concentrated extract which was then evaporated in a water bath until a concentrated extract was obtained.

Test animals must be fasted for 14-18 hours before being given treatment. After fasting, the animals were weighed and given ethanol extract of white turmeric rhizome using a probe. Observations were made every day for 14 days since the mice were given treatment. Furthermore, on day 22 the rats were sacrificed (euthanized) using ether. Then surgery was performed to remove the liver. The liver tissue that has been taken is then put into a container containing 10% formalin. After that, the preparation of gastric histopathological preparations using Hematoxylin Eosin (HE) staining was carried out at the Histology Department, University of North Sumatra.

After liver histopathology preparations, 3 samples from each group were taken to observe the microscopic structure with a magnification of 10x40. Then calculated the weight score of the level of hepatocyte damage with the criteria of Manja Roenigk as shown in the table below:

No	Damage rate	Score
1	Normal	1
2	Parenchymatous degeneration	2
3	Hydropic degeneration	3
4	Necrosis	4

Table 1 Scoring of the level of hepatocyte damage according to the criteria of Manja Roenigk.[6]

Procedure and Data Analysis

The results of the study, both from the control group and the treatment of the histopathological features of the liver, were carried out using the normality test using Shapiro Wilk. If the data were normally distributed (P>0.05), then continued with the analysis of variance test (ANOVA) to determine the toxicity level of white turmeric extract at various doses. However, if the variance is significantly different (P<0.05), the Kruskal Wallis test is used with the help of the SPSS (Statistical Package for the Social Sciences) for the Windows program.

Result

Sub Result

Liver Histopathology Results After Giving White Turmeric Extract

The results of each treatment group with data collection using liver histopathological images using tissue preparations using Hematoxylin Eosin (HE) staining. The slides were viewed under a light microscope at 10x and 40x magnification with five large fields of view.

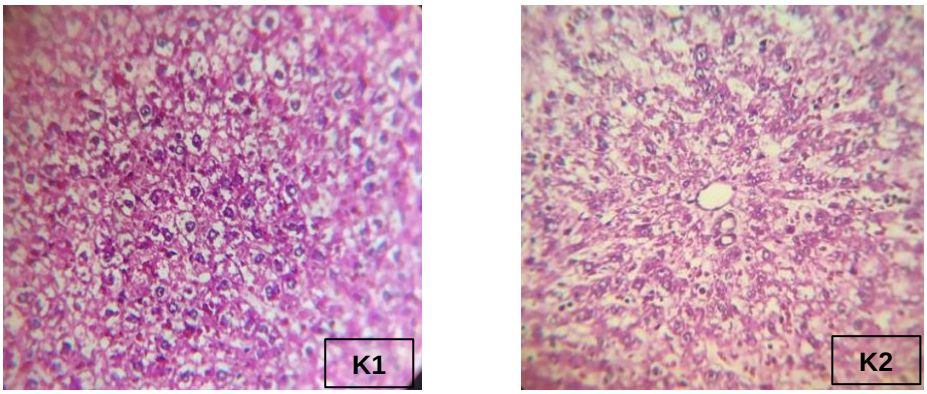


Figure 1 Histopathological description of K1 & K2.

Description: In general, the histopathological picture of the liver looks normal, there is no damage, has a round nucleus located in the middle, but it turns out that there are a few enlarged cells.

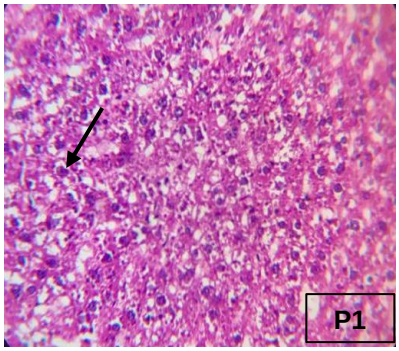


Figure 2 Histopathological picture of P1 liver (250 mg/Kg BW).

Description: In the arrow shown, there is an abnormality, namely parenchymatous degeneration (fatty) so that where there are cells, they appear enlarged.

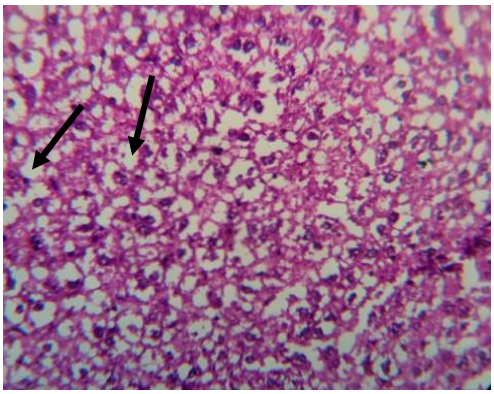
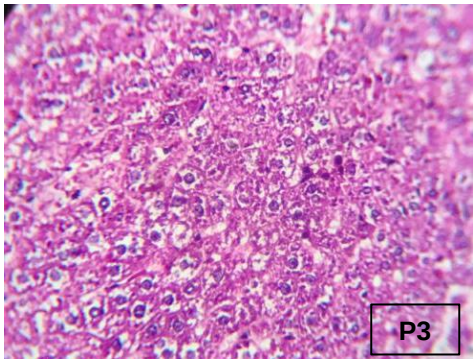


Figure 3 Histopathological description of the liver P2 (500 mg/Kg BW).

Description: In the arrow shown, the abnormality is parenchymatous degeneration (fatty) and hydrophilic degeneration so that where there are cells that look enlarged, there are also granules in the cytoplasm, fat vacuoles but no necrosis has occurred.



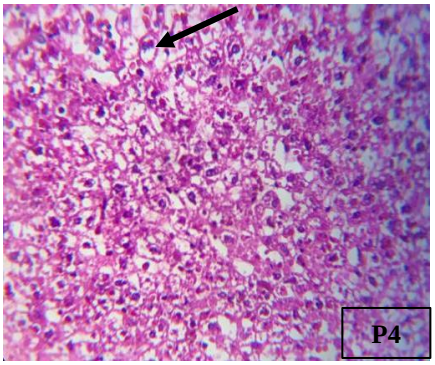


Figure 4 Histopathological features of the liver P3 & P4 (750 mg/Kg BW & 2000 mg/Kg BW).

Description: In the arrow shown, it shows an improvement where the parenchymatous degeneration is reduced but there are still abnormal liver abnormalities, marked by cells that still look enlarged.

Based on the microscopic observations that have been made as in the picture above, there was very little damage to the control group. The damage began to occur in the treatment group were in the 250 mg/kg BW group where parenchymatous degeneration (fatty) began to occur, but at a dose of 500 mg/kg BW there was very specific damage which was a visible enlargement of the cells and the presence of granules in the cytoplasm of fat vacuoles, this is due to the possibility that samples from the liver already have a history of disease so that they provide a more specific picture of degeneration, but at doses of 750 and 2000 mg/kg BW it shows a better histopathological picture.

Liver SPSS data results after administration of white turmeric extract

In the data that has been analyzed using SPSS, it was found in the ANOVA test and Post Hoc test that each control group and treatment group showed $P<0.05$, which means that there is a significant difference in liver damage. With the conclusion that the higher the dose given in the treatment, the better or positive effect on the repair of liver damage.

DISCUSSION

An oral acute toxicity test is a test carried out using test animals to detect toxic effects that are noticed within a certain period of time after administration of a substance given in single doses or in repeated doses.[7] Toxicity effects that can arise after carrying out this test are marked by stressed rats, sunken eyes, inactive movements, while non-stressed rats can be seen from active movements, normal appetite.[8] And from the results of observations for 14 days after being given white turmeric extract from each group showed that the rats did not show symptoms of toxicity (stress).

From the results of observations on the degree of damage on histopathological tests observed at a dose of 250 mg/kg BW, it was seen that there was parenchymatous degeneration, this degeneration was the mildest where there was swelling of the cells and turbidity of the cytoplasm due to the appearance of granules in the cytoplasm due to protein deposits. Parenchymatous degeneration occurs due to failure of oxidation which causes accumulation of water in the cell, due to impaired transport of proteins that have been produced by ribosomes.[9]

At a dose of 500 mg/kg BW, it was very clear where there was a lot of severe damage and hydrophilic degeneration began to occur where cell swelling, fat vacuoles were also found. These changes are generally the result of metabolic disorders such as hypoxia,[5] and although there is severe degeneration, no cells have died (necrosis). Hydropic degeneration is basically the same as parenchymatous degeneration, the degeneration is also reversible. However, the degree of hydropic degeneration is more severe than the degree of damage to parenchymatous degeneration.[10] Hydropic degeneration shows the presence of water-filled vacuoles in the cytoplasm that do not contain fat or glycogen.[11] Doses of 250 mg/kg BW and 500 mg/kg BW showed that this degeneration occurred possibly because the samples from the liver already had a history of disease affecting the liver of rats, so at this dose, there was considerable damage.

However, at doses of 750 and 2000 mg/kg BW, it showed a better histopathological picture where there was reduced parenchymatous degeneration and hydropic degeneration, but abnormal liver abnormalities were still found, namely swelling of the cells. So with this white turmeric with high doses still has a good effect on the liver. Although this white turmeric extract causes changes in the histological structure, these changes are reversible.

CONCLUSION AND SUGGESTION

Based on the results of research that has been done, it can be concluded that the administration of white turmeric rhizome extract did not cause stress levels and even death to mice. Doses of 250 mg/kg BW and 500 mg/kg BW show a picture of parenchymatous and hydrophilic degeneration, this is due to the possibility that samples from the liver already have a history of disease so that it gives the impression of fairly severe degeneration. However, the doses of 750 mg/kg BW and 2000 mg/kg BW showed a better histopathological picture, this indicates that the higher the extract dose, the better the histopathological picture of the liver. And from this study, it was concluded that in a dose of 2000 mg/KgBB it was still safe for consumption by humans.

The research carried out still has many shortcomings, so with this, it is necessary to do further research on white turmeric extract for chronic toxicity tests so that higher doses can damage the liver.

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