

Allopurinol Induction on Histopathological Structure of the Liver in Male Mice (*Mus musculus*)

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Abstract. Allopurinol is used to reduce total uric acid levels in the body into oxypurinol which can inhibit xanthine oxidase. Allopurinol inhibits the precursors of uric acid formation, xanthine, and hypoxanthine. However, consumption of the drugs can cause side effects on the liver. The aim of the research was to determine the effect of allopurinol induction on the liver histopathology of male mice (*Mus musculus*) DDY strain. The method used in this research was an experimental design used post-test only that was divided into 4 groups of 4 mice per group. The control group (P0) was given 0.5% Na-CMC, and groups I, II, and III (P1, P2, and P3) were induced by allopurinol at 10 mg/kg BW, 20 mg/kg BW, and 30 mg/kg BW for 14 days. Allopurinol induction was performed by oral gavage. The results of the research showed that treatment with allopurinol caused changes in the mice's body weight, liver index, liver morphology, and histological structure of the liver tissue, including necrosis, steatosis, leukocyte infiltration, binuclear hepatocytes, hepatocyte swelling, congestion, sinusoid dilatation, and hemorrhage. The level of liver damage increased in line with the dose used. This research indicated that the higher the allopurinol level, the higher the level of alteration in the liver section structure. Long-term use of allopurinol can cause damage to the structure of mice liver (liver toxicity).

Keywords: allopurinol, histopathology, liver

Citation

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INTRODUCTION

Allopurinol is a drug that is widely used by the general population to reduce uric acid levels and prevent gout or hyperuricemia. In the treatment of hyperuricemia, there are two groups of drugs that are commonly used: uricosurics, drugs with the mechanism of action of increasing the body's uric acid excretion through urine, and uricostatics, drugs that work by reducing the formation of uric acid

from purines (Cendrianti et al., 2014). Allopurinol is a type of uricostatic drug that works by inhibiting the xanthine oxidase enzyme. In the body, the enzyme xanthine oxidase can be found in the liver and muscles. Xanthine oxidase activity is by converting the oxidation of hypoxanthine to xanthine, and xanthine to uric acid (Chengiz et al., 2012).

Action mechanism of this enzyme is through an oxidation reaction, in which oxygen atoms are transferred from molybdenum

to xanthine to form uric acid. Allopurinol has tens of times stronger affinity for the enzyme xanthine oxidase, so allopurinol will react more with this enzyme to form a product in the form of oxypurinol, which can inhibit the formation of uric acid in the body (Yulian, 2014). However, treatment of gout using allopurinol can also cause side effects such as allergies, fever, chills, leukopenia, kidney and liver failure, and digestive disorders (Liu et al., 2008).

Uric acid is the end product of nucleic acid and purine metabolism. Purines in the body, usually come from food consumption, conversion of nucleic acids in tissues into purine nucleotides, and de novo synthesis from purine bases. Purines are an important part of nucleic acids. Purines that are not used in the body will be converted into uric acid in large quantities. The production of uric acid in the body is synthesized mainly in the liver. The liver is the largest organ that plays an important function in metabolic processes and has an important role in detoxifying or degrading drugs, hormones, waste substances, and foreign compounds into inactive or water-soluble forms (Jayanegara, 2017). The liver is also the main place for synthesis, catabolism, absorption, and excretion of substances that enter the body, including drugs. Because of those roles, the liver is susceptible to damage due to the influence of certain chemicals or drugs. Liver damage is also noted in the Asia Pacific as one of the leading causes of death (Sarin et al., 2020).

Using the drug continuously and at inappropriate doses can cause liver damage. Liver damage can be influenced by several factors, such as the type of chemical substance, the dose used, and the length of exposure. Liver damage can cause some alterations such as necrosis, cholestasis, the gradual onset of hepatic dysfunction, or some changes in the

form of degeneration. Hammer & McPhee (2014) stated that the more severe the damage to the liver, the more degeneration that occurs becomes irreversible. The liver can also undergo necrosis caused by the direct effects of toxic agents.

Guzik et al., (2019) stated that allopurinol is a commonly used drug that rarely causes serious hepatotoxicity, but several case studies have shown that the use of allopurinol causes organ disorders. One case study by Iqbal et al., (2017) stated that the long-term use of allopurinol in an 83-year-old man suffering from a chronic disease showed the occurrence of granulomatous hepatitis. In another case study by Yu et al., (2013), there were 2 patients who had anti-tuberculosis treatment but had hyperuricemia and were given allopurinol 0.1 g orally 3 times a day, there was an effect after 2 weeks that the patient developed a rash and fever. According to Albir and Al-Kaisy (2019), histology is an important method that can be used to diagnose many liver diseases. The aim of the research was to determine allopurinol induction in the liver of male mice (*Mus musculus*) using several doses of allopurinol based on the histological study.

MATERIALS AND METHODS

The study is a laboratory experiment with design post-test-only control group design. The research was carried out from May until November 2021 at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu.

0.5% Na-CMC Solution

0.5 grams of Na-CMC was weighed and dissolved in 10 ml of distilled water for about 15 minutes until it was clear like a gel, stirred until homogeneous, and diluted with distilled water up to a volume of 100 ml.

Allopurinol Solution

Allopurinol tablets were crushed, then weighed according to the mice's average weight based on the dose to be induced in mice. Allopurinol was dissolved in 10 ml of 0.5% Na-CMC solution and homogenized.

Treatment of Test Animals

This research was feasible to carry out and has received approval from the Animal Care and Use Committee of Universitas Bengkulu or Ethical Clearance with the number 48/KEH-LPPM/EC/2021. The mice used were male mice DDY strain aged 10-12 weeks with an average body weight of 25-30 gr. The mice were acclimatized for 7 days, and their body weight was weighed. Mice were divided into 4 treatments, each treatment consisting of 4 mice:

Group 1 (P0) as a control group without allopurinol induction (Na-CMC 0,5%)

Group 2 (P1) induced by allopurinol at a dose of 10 mg/kg BW

Group 3 (P2) induced by allopurinol at a dose of 20 mg/kg BW

Group 4 (P3) induced by allopurinol at a dose of 30 mg/kg BW

Mice were induced by allopurinol orally and given treatment for 14 days. At the end of the treatment, the mice's body weight was weighed. Furthermore, the mice were euthanized by cervical dislocation and operated on using a dissecting kit, the liver was taken and the liver was weighed. The liver morphology of the mice was observed before the fixation process was carried out. The liver index of mice was also measured by dividing the liver weight by body weight.

Liver Histology Preparation

Liver organs were isolated and washed

using 0.9% NaCl, then put into a bottle containing 10% NBF fixative. Fixation was carried out for 24-48 hours. The organs were washed using 70% alcohol and then dehydrated with 70%, 80%, 90%, and 100% alcohol for 60 minutes. It was cleared using toluene and infiltrated using 4 times liquid paraffin for 60 minutes. Embedding organs were implanted in liquid paraffin until harden and then sectioned by cutting the organ using a microtome. The coupes were attached to a glass object that has been smeared with Meyer albumin. The preparations were rehydrated into graded alcohol and then stained using hematoxylin-eosin, then dehydrated in graded alcohol and deparaffinated using xylene for 8-12 hours. The next stage was mounted tissue with a deck glass using canada balsam. The samples were then observed using optilab at 100x and 400x magnification to see the histology of the liver.

Histopathological Data

Histopathological data that have been obtained were analyzed based on tissue damage in the liver of mice after exposure to allopurinol induction. Observation of histology using 5 fields of view and was given a score of damage to each cell and then calculated the average of the score. The assessment of the degree of liver cell damage according to Baldatina (Table 1). For a semi-quantitative comparison of the structural changes, the abnormalities in the tissue sections were graded from 0 (normal structure), 1 (mild pathological changes), 2 (moderate pathological changes), and 3 (severe pathological changes) (Ibrahim et al., 2018).

Data Analysis

The data obtained were analyzed using the SPSS program with normality test data using Shapiro Wilk and followed by a One-

way ANOVA test. If the probability value (p) < 0.05 is significantly different, then it is continued with the Duncan Multiple Range Taste

(DMRT) test, p values less than 0.05 were considered statistically significant.

Table 1. Liver evaluation scoring parameters using Baldatina (2008)(ML)

Score	Percentage	Histological Change
0	< 25%	The liver undergoes hydropic degeneration, parenchymal degeneration, and apoptosis around the centrilobular (central vein)
1	25-50%	The liver undergoes hydropic degeneration, parenchymal degeneration, and apoptosis that extends to the middle area (midzone)
2	50-75%	The liver undergoes hydropic degeneration, parenchymal degeneration, and apoptosis that extends to the periportal
3	> 75%	The liver undergoes hydropic degeneration, parenchymal degeneration, and apoptosis that extends to the periportal (perilobular) zone

RESULTS AND DISCUSSION

The result of average body weight of mice in each treatment group can be seen in Table 2. It was shown that the control group of mice (P0) had a weight gain of about 0.23 gr. Meanwhile, the allopurinol induction treatment group had weight loss. In the P1 group treated with allopurinol 10 mg/kg BW had an average weight loss of 4.72 gr, while the P2 and P3 groups had an average weight loss up to 7.24 gr and 9.29 gr respectively. It shows that the induction of drugs using allopurinol can cause physiological stress in mice and will decrease appetite.

Weight gain in individuals was influenced by nutritional factors. Nutrients are

contained in a feed that enters the individual's body as feed consumption (Mardiati & Sitaswi, 2016). However, many factors cause a decrease in appetite in mice. Magfirah & Christin (2020) stated that a significant change in body weight is the easiest visible indicator and early indicator of a toxic effect from a given test. Body weight in toxicity studies showed that experimental animals who received high doses generally lost weight due to decreased appetite. Table 2 shows that the higher the dose given, the lower the body weight in male mice induced using allopurinol. The final weight of the treatment showed a significant difference. Allopurinol treatment at a dose of 30mg/kg BW caused the most weight loss compared to the other treatments.

Table 2. Body weight of male mice induced by allopurinol

Group	Body Weight Average		Increase Percentage (%)	Decrease Percentage (%)
	Pre-treatment (gr)	Post-treatment (gr)		
P0	27.95 ± 0.45	28.19 ± 3.23 ^a	8.58	-
P1	28.55 ± 0.23	23.83 ± 4.57 ^{ab}	-	16.5
P2	29.36 ± 0.27	22.12 ± 4.46 ^{ab}	-	24.66
P3	29.27 ± 0.51	20.08 ± 3.06 ^b	-	31.39


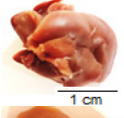

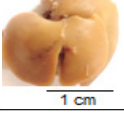
Data represent the mean ± standard deviation. Mean values with different letters over the same column are significantly different ($p < 0.05$) according to Duncan's Multiple Range Test. P0: mice control using 0,5% Na-CMC, P1: mice treated with allopurinol dose 10mg/kg BW, P2: mice treated with allopurinol dose 20mg/kg BW, and P3: mice treated with allopurinol dose 30mg/kg BW.

The results of the study showed that there were differences in the weight of the liver of mice between each treatment. In the control group, the mean liver weight was the highest compared to the other treatments of allopurinol, the higher the dose of allopurinol induced in mice, the lower the liver weight. In the P3 group, the average dose of allopurinol 30mg/kg BW was 1.16 gr, a difference of 0.18 gr from the control group (Table 3). The liver is an important organ in the detoxification process or defense against various foreign substances, bacterial invasion, and toxins, so the liver weight can change due to substance intake (Ceriana et al., 2018). Liver weight can decrease may be due to exposure to allopurinol, which can liver cells undergo necrosis. However, the liver weight of mice between treatments did not show a significant difference. Induction of allopurinol for 14 days did not significantly affect liver weight. Mean-

while, data regarding the liver index showed that the allopurinol induction treatment had a significant impact on the hepatosomatic index compared to the control group (or normal group).

Organ morphology is one way to determine the effect of a drug on an organ. Based on the morphology of the liver (Table 3), the control group (P0) did not change macroscopically, as well as P1 group that had a red liver color due to a lot of blood and healthy lobules. Significant changes in liver morphology are seen in P2 and P3 groups that looks paler. Color changes in the liver that become paler indicate a disturbance in blood flow to the liver (Lailatul et al., 2015). According to Westbrook (2016), the pale liver was caused by the rapid release of free fatty acids from enlarged visceral fat. Fortes (2017), also stated that the cause of pale liver is a toxic compound that causes fatty liver.

Table 3. Liver index and morphology of liver

Group	Liver weight (gr)	Liver Index	Liver Morphology
P0	1.34 ± 0.16	4.75 ± 0.21 ^a	
P1	1.32 ± 0.03	5.53 ± 0.34 ^b	
P2	1.21 ± 0.32	5.47 ± 0.16 ^b	
P3	1.16 ± 0.24	5.77 ± 0.16 ^c	

Data represent the mean ± standard deviation. Mean values with different letters over the same column are significantly different ($p < 0.05$) according to Duncan's Multiple Range Test. P0: mice control using 0,5% Na-CMC, P1: mice treated with allopurinol dose 10mg/kg BW, P2: mice treated with allopurinol dose 20mg/kg BW, and P3: mice treated with allopurinol dose 30mg/kg BW.

Differences in dosage also affect liver morphology due to several factors, such as prolonged exposure, duration of exposure, dose, and susceptible host cells. The intensity of exposure of a substance to an organ is increased, causing changes in morphology and function, these changes are generally reversible. Hepatotoxicity due to chemical compounds is a potential complication that almost always exists in any given chemical compound because the liver is the center of the metabolic disposition of all drugs and foreign substances that enter. The higher the concen-

tration, the more significant the toxic response in a tissue (Amalina, 2009).

Based on the scoring of the liver histology section (Table 4), it showed that the negative control and P1 group had normal tissue, P2 treated with allopurinol 20mg/kg BW showed mild damage, while P3 treated with allopurinol 30mg/kg BW showed moderate damage. The liver-scoring histopathology were significant differences in each treatment group with negative control. Group P3 showed severe damage compared to other treatments.

Table 3. Scoring of liver histopathology

Group	Hepatocytes Damage Score	Min Score	Max Score	Level of Damage
	Average ± SD			
P0	0.18 ± 0.1 ^a	0.1	0.3	Normal
P1	0.83 ± 0.65 ^b	0.9	1.21	Normal
P2	1.57 ± 0.87 ^c	1.76	1.98	Mild
P3	2.08 ± 0.9 ^d	2.45	2.72	Moderate

Based on the microphotographs (Figure 1), the control group (P0) showed histology of the central vein without congestion, hepatocytes and sinusoids were normal, but in the P1, P2, and P3 groups treatment with allopurinol showed some tissue damage, there were leukocyte infiltration, congestion, and steatosis. Microphotographs using a magnification of 400x (Figures 2, 3, 4, and 5) showed histopathology with sinusoid dilatation, necrosis, hemorrhage, binucleation (binuclear), and hydrophilic degeneration.

Liver damage can be irreversible and reversible, with alterations in histopathology showing abnormalities of the tissue. Degeneration in liver tissue is a temporary change, but degeneration that occurs continuously can result in cell death. According to Maulida (2013), liver cell damage begins with a degeneration process or cell swelling. The swelling of cells is reversible, so it can return to normal. Figures 3 and 4 show a lot of cells with

steatosis. Fat degeneration or steatosis is an abnormal accumulation of triglycerides in parenchymal cells. One of the causes of steatosis is toxins (Mulyono, et al., 2013). Fat degeneration is caused by the accumulation of fat in the cytoplasm of cells and usually occurs in parenchymatous cells, such as liver cells. In hematoxylin-eosin (HE) staining, fat loss due to the dehydration process with alcohol will form vacuoles, so it is often called vacuole degeneration.

Leukocyte infiltration shown in Figures 3 and 4, is a living tissue reaction to all forms of injury. Injuries occur due to reactions between toxic substances and molecules in the body, both subcellular and cellular. Infiltration of inflammatory cells of leukocytes in the central vein is caused by damage to endothelial cells that are very sensitive to toxic substances, inflammation of the liver begins in the central vein as a reservoir for blood originating from the hepatic artery and portal

vein. The longer the toxic exposure occurs, the more inflammatory cell infiltration will

diffuse and spread from the portal area to the central area (Makiyah & Khumaisah, 2018).

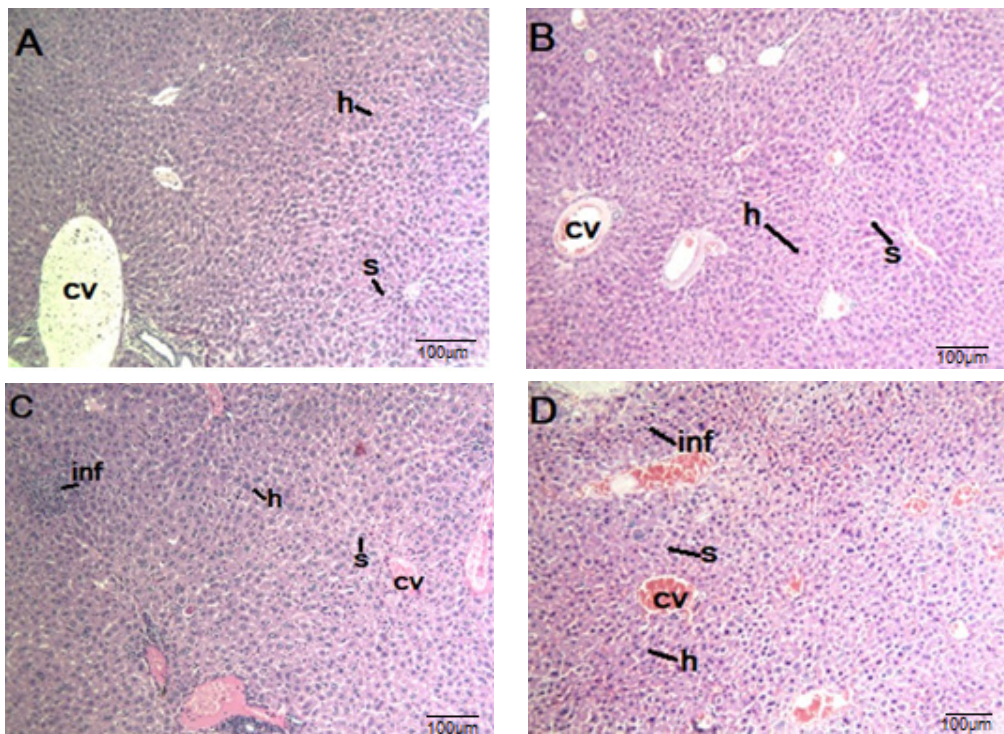


Figure 1. Microphotographs of the liver of control and treated male mice with 100x magnification. A. The control group (P0) showed normal histology, B. liver treated with an allopurinol dose of 10mg/kg BW (P1) showed central venous congestion and hydrophilic degeneration, C. liver treated with an allopurinol dose of 20mg/kg BW (P2) showed slight damage. in the form of hemorrhage, congestion, and leukocyte infiltration, D. liver treated with an allopurinol dose of 30 mg/kg BW (P3) which moderate damage in the form of congestion, necrosis, hydrophilic degeneration, fatty degeneration. Description:VC (central vein), S (sinusoid), H (hepatocytes), BD (bile duct), HPV (hepatic portal vein).

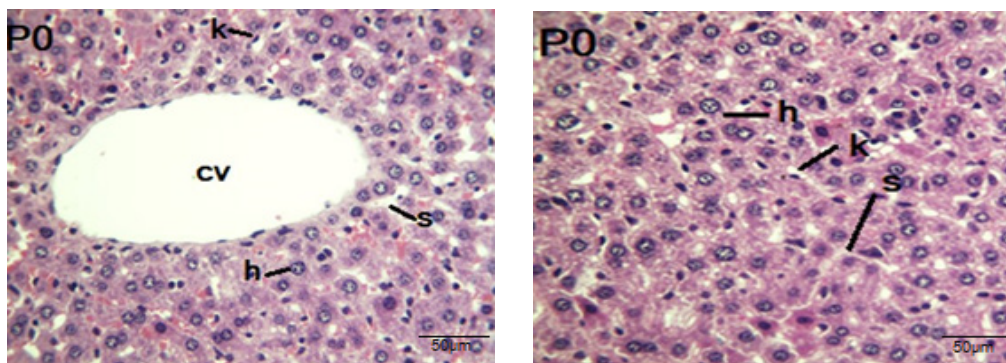


Figure 2. Liver histology of male mice in control group (P0) (400x magnification), showing normal central vein (cv), normal hepatocyte (h), Kupfer cells (k), and normal sinusoid (s).

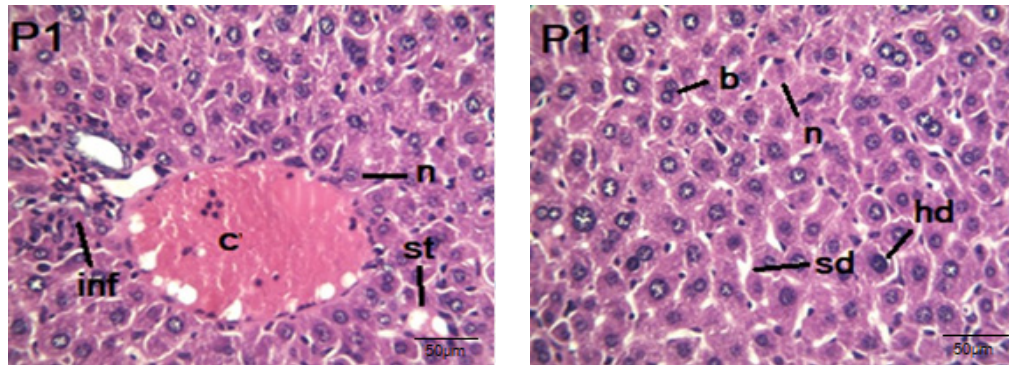


Figure 3. Liver histology of male mice induced by allopurinol at a dose of 10mg/kg BW (P1) (400x magnification) showing central vein congestion (c), leukocyte infiltration (inf), necrosis (n), steatosis (st), binucleation (b), sinusoid dilatation (sd) and hydropic degeneration (hd)

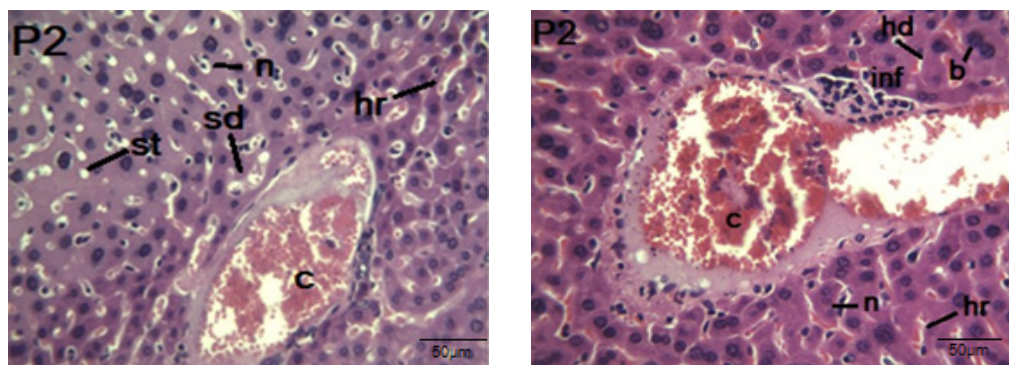


Figure 4. Liver histology of male mice induced by allopurinol at a dose of 20mg/kg BW (P2) (400x magnification) showing central vein congestion (c), necrosis (n), steatosis (st), sinusoid dilatation (sd), leukocyte infiltration (inf), binucleation (b), hydropic degeneration (hd) and hemorrhage (hr)

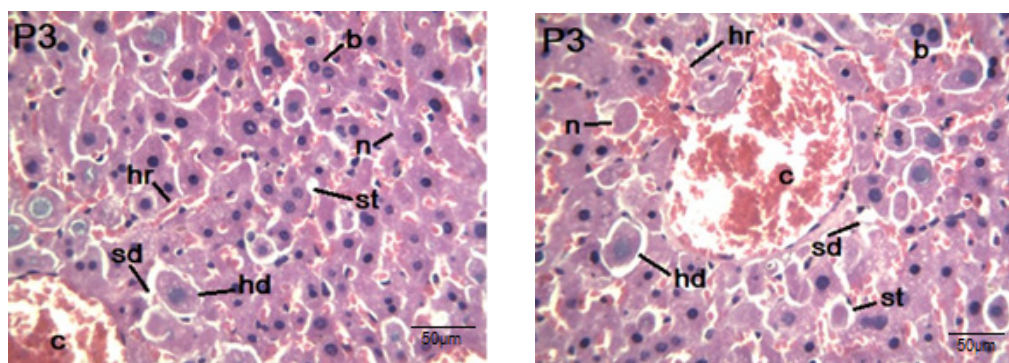


Figure 5. Liver histology of male mice induced by allopurinol at a dose of 30mg/kg BW (P3) (400x magnification) showing central vein congestion (c), necrosis (n), steatosis (st), sinusoid dilatation (sd), leukocyte infiltration (inf), binucleation (b), hydropic degeneration (hd) and hemorrhage (hr)

Photomicrographs in Figures 3, 4, and 5 showing dilatation of the hepatic sinusoids is a sign of liver sinusoid damage. Sinusoidal

dilatation in the liver of mice is thought to be caused by the presence of necrotic hepatocytes (Hayati & Sunaryo, 2014). Hepatocytes

undergoing necrosis have an irregular shape so the arrangement of hepatocytes in the lobules becomes irregular as well. As a result, the sinusoids bordering the hepatocytes become dilated. The results of this study are also similar to Ekinici et al. (2019) using a dose of 0.1 mg/kg of allopurinol in rats which showed that induction of allopurinol causes dilatation and accumulation of blood in sinusoids. Widening of the sinusoids can also be caused by high levels of toxicants in the blood that passes through the sinusoids to the central vein. Surasa et al., (2014) stated that sinusoids can easily come into contact with toxicants from hepatocytes (composed of endothelial cells). The sinusoids and hepatocytes lining by sub-endothelial clefts contain the microvilli of the hepatocytes. This facilitates the contact between the hepatocyte surface and the sinusoids, thus facilitating the exchange of compounds including toxicants.

Figures 3, 4, and 5 also show microscopic congestion is indicated by the presence of blood cells in the blood vessels, and their location fills the lumen in the blood vessels. Congestion is an increase in blood volume due to the dilatation of small blood vessels (capillaries) (Sijid et al., 2020). Hydrophilic degeneration is one of the lesions that occur due to congestion (shown in Figures 3, 4, and 5). Hydrophilic degeneration is the initial response of hepatocytes to toxic substances. Hydrophilic degeneration is a reversible change so that when the toxic exposure is stopped, the damaged cells will return to normal, but continued degeneration will lead to cell death. Liver cell death causes hepatocytes to be unable to return to their normal shape (irreversible).

The next stage of liver cell damage is necrosis, which is irreversible damage. Figures 3, 4, and 5 show the presence of hepatocytes necrosis. The results of the research by

Fitria (2017) also showed that the induction of allopurinol at a dose of 5 mg/kg BW caused necrosis which was characterized by pyknosis, karyorrhexis, and karyolysis. The final stage of liver damage is necrosis, in which cells die. Damage to hepatocytes is a manifestation of the effects of the metabolism of toxic substances in the liver. The increase in transaminase levels in serum is caused by transaminase-rich cells undergoing necrosis or destruction. Cell damage in the form of necrosis causes swelling of the nucleus and cytoplasm and then ruptures. The hallmark of necrosis is the appearance of cell fragments or liver cells without a nucleus or no visible cells accompanied by an inflammatory reaction, collapse, or enlargement of cells.

Induction treatment using allopurinol in various doses of 10 mg/kg BW, 20 mg/kg BW, and 30 mg/kg BW showed hepatotoxicity in the alteration structure of liver tissue. The higher the dose of allopurinol induction, the greater the damage to liver tissue. Allopurinol consumption should not be used in the long term to prevent liver tissue damage that can interfere with the work and function of the liver.

CONCLUSION

Allopurinol induction can cause effect on the liver tissue. The microphotographs of the liver tissue section show alterations in a liver structure such as leukocyte infiltration, necrosis, hemorrhage, steatosis, sinusoidal dilatation, binuclear, congestion, and hydrophilic degeneration. A higher dose of allopurinol was given in male mice for 14 days, in line with the amount of liver tissue damage.

AUTHOR CONTRIBUTION

D.F.L. designed the research and wrote

the manuscript, F.Z analyzed the data, A.P.S and S.S. carried out the experiment.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

- Albir A. A. & Al-Kaisy A.Z. (2019). Histological Investigation of the Hepatic Tissue in Mice Induced by Soft Laser with Different Doses. *Biomedical & Pharmacology Journal*, 12(2), 989-992. DOI: 10.13005/bpj/1726.
- Cendrianti F., Muslichah, S. & Umayah E. (2014). Uji Aktivitas Antihiperurisemia Ekstrak n-Heksana, Etil Asetat, dan Etanol 70% Daun Tempuyung (*Sonchus arvensis* L.) pada Mencit Jantan Hiperurisemia. *Jurnal Pustaka Kesehatan*, 2(2), 205-209.
- Cengiz S., Cavaz, L., Yurdakoc, C. & Aksu, S. (2012). Inhibition of Xanthine Oxidase by Caulerpenyne from *Caulerpa prolifera*. *Turkish Journal of Biochemistry*, 37(4), 445-451. DOI: 10.5505/TJB.2012.98698.
- Ceriana, R., Fitri, S. & Sari, W. (2018). Perubahan Berat Organ Hati, Ginjal, Limfa, Otak, Lambung, Testis, Jantung dan Paru-paru Mencit yang Diberi Ekstrak Batang Sipatah-patah (*Cissus quadrangularis* Salisb.). *Journal of Healthcare Technology and Medicine*, 4(1), 127-134. DOI: 10.33143/jhtm.v4i1.201.
- Ekinci, O., Eren, T., Gapbarov, A., Gacemer, M., Ozkinet, T. & Celik, F. (2019). Effects of Octreotide and Allopurinol on Liver Histology and Functions in Surgical Jaundice: An Experimental Study. *Albanian Journal of Trauma and Emergency Surgery*, 3 (1), 1-10. DOI: 10.32391/ajtes.v3i1.34.
- Fitri, R. (2017). Pengaruh Terapi Ekstrak Teh Hijau (*Camellia Sinensis*) Terhadap Kadar Asam Urat, Xantin Oksidase (XOD), Malondialdehid (MDA) Dan Gambaran Histopatologi Hepar pada Tikus (*Rattus Novergicus*) Hiperurisemia. *Thesis*. Malang: Universitas Brawijaya. Retrieved from: <http://repository.ub.ac.id/id/eprint/9289/>.
- Fortes, R.C. (2017). Nutritional Implications in Chronic Liver Diseases. *Journal of Liver Research, Disorders & Therapy*, 3(5), 131-135. DOI: 10.15406/jlrdt.2017.03.00071.
- Guzik P., Kim, C., Kumbum, K. (2013). Severe Allopurinol-Induced Liver Injury. *The American Journal of Gastroenterology*, 119, 1311-1312. DOI: 10.14309/01.ajg.0000598956.00340.
- Hammer, S. J. & McPhee, S. J. (2014). *Pathophysiology of Disease: An Introduction to Clinical Medicine*. USA: McGraw-Hill.
- Hayati, S. & Sunaryo. (2014). Efek Hepatoprotektor Fraksi Asetat Daun Sangitan (*Sambucus Canadensis* L.) pada Tikus Sprague Dawley. *Media Farm*, 11(1), 55–61. DOI: 10.12928/mf.v11i1.1397.
- Ibrahim, K. E., Al-Mutary, M. G., Bakhiet, A. O. & Khan, H. A. (2018). Histopathology of the Liver, Kidney, and Spleen of Mice Exposed to Gold Nanoparticles. *Molecules*, 23(8), 1848. DOI: 10.3390/molecules23081848.

- Iqbal, U., Siddiqui, H.U., Anwar, H., Chaudhary, A., & Quadri, A.A. (2017). Allopurinol-Induced Granulomatous Hepatitis: A Case Report and Review of Literature. *Journal of Investigative Medicine High Impact Case Reports*, 1-3. DOI: 10.1177/2324709617728302.
- Jayanegara, D. (2017). Gambaran Histopatologi Hati Mencit (*Mus musculus*) yang Diberi Paparan Ibuprofen Dosis Bertingkat. *Skripsi*. Makassar: Fakultas Kedokteran Universitas Hasanuddin.
- Lailatul, N., Lyrawati, D. & Handaru, M., (2015). Efek Pemberian Asam Alfa Lipoat terhadap Kadar MDA dan Gambaran Histologi pada Hati Tikus Wistar Jantan dengan Diabetes Melitus Tipe 1. *Jurnal Kedokteran Brawijaya*, 28(3), 170-176. DOI: 10.21776/ub.jkb.2015.028.03.2.
- Liu, X., Chen, R., Shang, Y., Jiao, B. & Huang, C. (2008). Lithospermic Acid as a Novel Xanthine Oxidase Inhibitor has Anti-inflammatory and Hypouricemic Effects in Rats. *Chem-Biol Interact*, 176(3), 137-142. DOI: 10.1016/j.cbi.2008.07.003.
- Liu Y., Jiang Y., Dong X.D., et al. (2013). Liver Injury due to Allopurinol: two case reports. *Adverse Drug Reaction Journal*, 15(4), 231-233. DOI: 10.3760/cma.j.issn.1008-5734.2013.04.020.
- Magfirah & Christin, V. (2020). Analisis Profil Bobot Badan Tikus dan Gejala Toksik Pada Pemberian Ekstrak Etanol Daun Parang Romang (*Boehmeria virgata*) terhadap Tikus Putih (*Rattus norvegicus*). *Galenika Journal of Pharmacy*, 6(1), 1-6. DOI: 10.22487/j24428744.2020.v6.i1.13928.
- Makiyah, A. & Khumaisah, L. L. (2018). Studi Gambaran Histopatologi Hepar Tikus Putih Strain Wistar yang Diinduksi Aspirin Pasca Pemberian Ekstrak Etanol Umbi Iles-iles (*Amorphophallus variabilis* Bl) Selama 7 Hari. *Majalah Kedokteran*, 50(2), 93-101. DOI: 10.15395/mkb.v50n2.1323.
- Mardiati, S. M. & Sitasiwi, A. J. (2016). Pertambahan Berat Badan Mencit (*Mus musculus* L.) Setelah Perlakuan Ekstrak Air Biji Pepaya (*Carica papaya* Linn.) Secara Oral Selama 21 Hari. *Buletin Anatomi dan Fisiologi*, 1(1), 75-80. DOI: 10.14710/baf.1.1.2016.75-80.
- Maulida, A. (2013). Pengaruh Pemberian Vitamin C dan E Terhadap Gambaran Histologi Hepar Mencit (*Mus musculus* L) yang Dipajankan Monosodium Glutamat (MSG). *Skripsi*. Medan: Fakultas Matematika dan Ilmu Pengetahuan Alam Universitas Sumatera Utara.
- Mulyono, A., Farida, D. H. & Soesanti, N. (2013). Histopatologi Hepar Tikus Rumah (*Rattus tanezumi*) Infektif Patogenik *Leptospira* spp. *Jurnal Vektora*, 5(1), 7-11.
- Sarin, S. K., Kumar, M., Eslam, M., George, J., Mahtab, M. et. al. (2020). Liver diseases in the Asia-Pacific region: a Lancet Gastroenterology & Hepatology Commission, *Lancet Gastroenterol Hepatology*, 5(2), 167-228. DOI: 10.1016/S2468-1253(19)30342-5.
- Sijid, S. A., Muthiadin, C., Zulkarnain, Hidayat, A. S. & Amelia, R. R. (2020). Pengaruh Pemberian Tuak Terhadap Gambaran Histopatologi Hati Mencit (*Mus musculus*) ICR jantan. *Jurnal Pendidikan Matematika dan IPA*, 11(2), 193-205. DOI: 10.26418/jpmipa.v11i2.36623.
- Surasa, N. J., Utami, N. R. & Isnaeni. (2014). Struktur Mikroanatomi Hati dan Kadar Kolesterol Total Plasma Darah Tikus Putih Strain Wistar Pasca Suplementasi Minyak Lemuru dan Minyak Sawit. *Biosaintifika*, 6(2), 141– 151. DOI: 290

- 10.15294/biosaintifika.v6i2.3778.
Westbrook R. H., Dusheiko G. & Williamson.
(2016). Pregnancy and Liver Disease.
Journal of Hepatology, 64, 933-945.
DOI: 10.1016/j.jhep.2015.11.030.
- Yulian, M. (2014). Potensi Biodiversitas
Indonesia Sebagai Inhibitor Xantina
Oksidase dan Antigout. *Lantanida
Journal*, 1(1), 1-15. DOI: 10.22373/
lj.v2i1.666.