P-ISSN : 2527-4627
Warmadewa Medical Journal
Available online http://ejournal.warmadewa.ac.id/index.php/warmadewa_medical_journal

WMJ (Warmadewa Medical Journal), Vol. 9, No.2, November 2024, Page. 64-69

Histopathological Study of Wistar Rat Liver Infected with *Schistosoma japonicum*

David Pakaya[1*], Varel Bramantio Gagola[1], Christin Rony Nayoan[2], Junjun Fitriani[2], Vera Diana Towidjojo[3]

[1]*Department of Histology, Faculty of Medicine, Universitas Tadulako, Palu, Indonesia*
[2] *Department of Pharmacology, Faculty of Medicine, Universitas Tadulako, Palu, Indonesia*
[3] *Department of Parasitology, Faculty of Medicine, Universitas Tadulako, Palu, Indonesia*
[*]*Correspondence: davidpakaya09@gmail.com*

Abstract

Schistosoma, including Schistosoma japonicum (S. japonicum), can live with an intermediate host, such as rats, and infect mammals, such as humans and rats. We can use a rat model to understand the pathophysiology of Schistosoma. The aim of this study is to describe the histopathological changes of Wistar rat liver infected with S. japonicum. This is a quasi-experimental study that employs a descriptive qualitative approach. The samples were 8-week-old male Wistar rats with an average weight of 250 to 350 g. The whole sample was made up of 16 rats that were given S. japonicum cercaria intraperitoneally. The rats were then split into 4 groups: the control group (C) ended on day 0, the T1 group ended on day 14, the T2 group ended on day 42, and the T3 group ended on day 60. We necropsied the liver, examined it histopathologically using hematoxylin eosin staining, and conducted a qualitative analysis. In the control group, we observed normal liver structure; in the T1 group, we observed hepatocyte degeneration, dilatation of liver sinusoids, and accumulation of inflammatory cells; in the T2 group, we observed similar conditions to the T1 group, including hepatocyte apoptosis; in the T3 group, we observed hepatocyte degeneration, hepatocyte necrosis, infiltration of inflammatory cells (PMNs), and thickening of connective tissue. In conclusion, there was gradual liver damage over the period of time in animal models, and the worst is in chronic conditions, which are dominated by fibrotic tissue, but no granulomas have been found.

Keywords: Schistosoma sp, tissue pathology, rat model.

INTRODUCTION

Trematodes from the genus Schistosoma cause schistosomiasis, a zoonotic disease. The World Health Organization's data from 2011 indicates that schistosomiasis infected over 240 million people in 78 countries, putting approximately 800 million people at risk of infection. There are four species of *Schistosoma* that can cause disease in humans, i.e., *S. haematobium*, *S. mansoni*, *S. japonicum*, and *S. mekongi*. *Schistosoma japonicum is considered the most dangerous species due to its large egg production and small size, which can complicate backwashing, make treatment difficult, and potentially lead to death.*(3) Schistosoma only occurs in Central Sulawesi (Napu highland, Bada highland) in Indonesia. (4)

In *S. japonicum* infection, the liver is the primary organ attacked by the eggs carried by the bloodstream. The tiny size of the sinusoidal-soidal capillary, which is easily trapped inside the liver, caused this. The response4+ cells induced by antigen from the eggs causes the formation of granuloma, which is formed from collagen fiber and a group of cells (macrophages, eosinophil,eosinophils cells) which surrouthathe egg. When *the Schistosoma* egg dies, granuloma will subside spontaneously, leaving a fibrotic lesion. Prolonged conditions can cause ascites and the formation of neovascularneovascularization, which is to rupture and bleeding.(5) The previouPrevious studies solely focused on histopathological characteristics in the chronic schistosomiasis condition, neglecting to examine periodic structural alterations. ication of liver histopathology in schistosomiasis conditions can explain the structural changes that occur in acute and chronic conditions, so it can be a reference time to carry out therapy. This is the novelty of the study: we can

evaluate the histological changes in each period of time.

Schistosoma sp. can live and infect not only humans but also other mammals, such as rats, pigs, dogs, cats, and others that act as intermediate hosts.(6) Rats can not only become intermediate hosts but also become infected with *S. japonicum*.(7) Therefore, studies investigating *S. japonicum* infection use rats as their animal model. The objective of this study was to describe histopathological changes in Wistar rat liver infected with *S. japonicum*.

METHODS

Preparation of Cercarial Suspension

The cercariae of *S. japonicum* were from the snail focus transmission area and were processed in the schistosomiasis laboratory in Napu, Central Sulawesi. The Oncomelania snails that were collected were cleaned with aquadest sterile water, and then the snail was crushed on a glass slide; 200 µl of aquadest sterile water was added. We evaluated the cercariae that were carried out under the microscope.

Animals and *S. japonicum* treatment

This is a quasi-experiment with a descriptive qualitative approach using 16 male Wistar rats, 8 weeks of age, with body weight (BW) of 250-350 g, divided into 4 termination groups and 4 replications. The schistosomiasis animal models were cared for inside individual cages and cleaned daily. Care was performed by placing the animal model in room temperature with 12 hours of light and 12 hours of dark lighting. The animals were given standard feed and water orally. All of the rats were injected with *S. japonicum* cercariae suspension in aquadest intraperitoneally (200 µl/rat) and were divided into 4 groups, ii.e., thecontrol group (C), which was terminated on day-0,day 0; theoups tergroup, which wasd on day-14, T2 groups teday 14; thed on daygroup, which wasd T3 groups terminated onday 42;0.1. Tthe T3 termgroup, which was time can present the norday 60. ute, and chronic condition of schistosomiasis. This study received ethical committee clearance from the Faculty of Medicine, Tadulako University No. 6367/UN 28.1.30/ KL/2019.

Histopathological analysis

he liver of the rats was necropsied and turned into paraffin blocks, cut using a Leica RM 2235 microtome© (Leica Biosystems, Nussloch, Germany) with a 4 µm width and stained with hematoxylin eosin. The results were observed qualitatively using an Olympus CX22 microscope (Olympus Europa SE & Co. KG, Hamburg, Germany) with the aid of OptiLab Advance software (Miconos, Indonesia) using 400x magnification and a numerical aperture (NA) of 1.25. The histological assessment was carried out qualitatively; the researcher was the investigator of the double-blinded histopathological assessment.

RESULTS

Table 1. Characteristics of rat models based on body weight

Groups	Body Weight (gr)
C	214.25±4.45
T1	275.67± 26.41
T2	212.67 ± 6.28
T3	198.33±19.48

Table 1 showed changes in the average weight of rats; we found the decrease of body weight in chronic conditions of the infection. General histological image of liver was composed of hepatocytes. The hepatocytes are piled up and forming cell layers with round-shaped nuclei, consisting of 1 or 2 nuclei and eosinophilic cytoplasm. This piled hepatocyte creates a structural unit called hexagonal liver lobules with a central vein in its center. Between the hepatocytes, there is a liver sinusoid that empties out to the central vein, in which Kupffer cells are found. Figure 1 generally depicts the liver structure.

DOI: https://doi.org/10.22225/wmj.9.2.10203.64-69

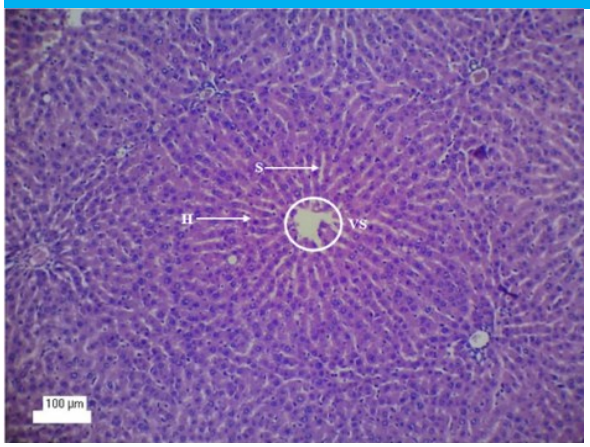

Figure 1. Histological structure of the liver. Consists of hepatocytes (H) arranged in rows with sinusoid (S) in between which empties out to the central vein (VS).

Figure 1 showed a liver lobule with a polygonal shape. Loose connective tissues form the sides of the lobule. The hepatic artery and portal vein are located within the connective tissue. We observed no histological changes or granuloma formation in any of the objects. Figure 2 reveals significant structural changes in hepatocytes, blood vessels, the distribution and accumulation of inflammatory cells, and fibrosis.

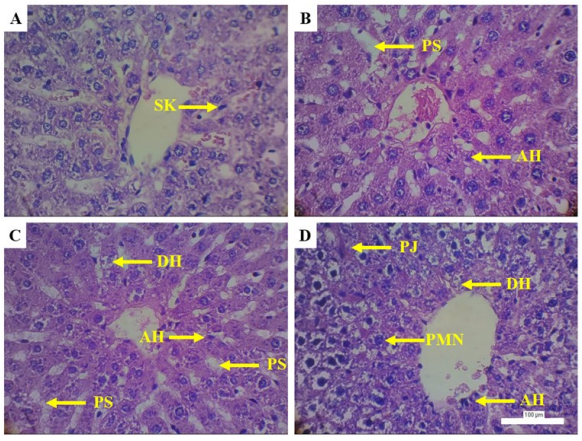

Figure 2. Histological structure of the liver of a Wistar rat infected with Schistosoma. (A) Histological image of the liver of group K with Kupffer cells (SK). (B) Histological image of the liver of group T1 with hepatocyte degeneration (DH) and sinusoid dilatation (PS). As shown in (C), the histological picture of group T2 showed hepatocyte degeneration (DH), hepatocyte apoptosis (AH), and sinusoid dilatation. As you can see in (D), the histological picture of group T3 shows hepatocyte degeneration (DH), hepatocyte necrosis (NH), inflammatory cells (PMNs) infiltration, and thickening of connective tissue (PJ). 400x magnification.

Figure 2 shows changes in hepatocyte structure during apoptosis in groups T1, T2, and T3. Apoptosis is characterized by small-sized apoptotic bodies with pyknotic and eosinophilic nuclei. Most cells also underwent necrosis with pyknotic nuclei. Furthermore, hepatocyte degeneration was found in groups T1, T2, and T3. This degeneration was characterized by edematous hepatocytes and irregular cytoplasm. Some of these hepatocytes experienced osmotic edema and rupture, a condition known as hydropic degeneration. In group T3, massive inflammatory cell infiltration was found, especially eosinophils. Meanwhile, the infiltration of inflammatory cells in groups T1 and T2 was not as massive as in group T3. In group T3, extensive thickening of connective tissue (fibrosis) from the edge of the central vein lumen to the sinusoid. A clearer fibrosis image from group T3 can be seen in Figure 3.

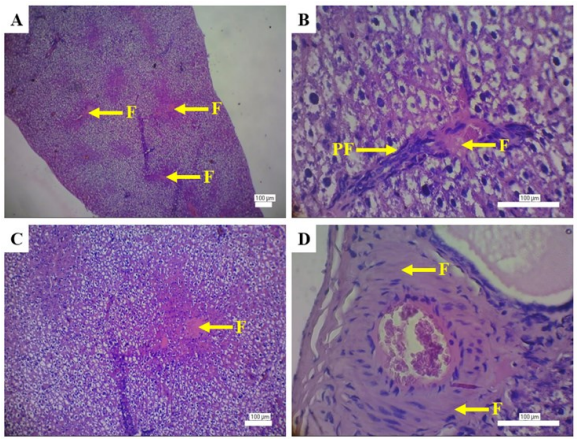

Figure 3. Histological structure of the liver of Wistar rats infected with *Schistosoma* treatment group 3. (A) fibrosis (F) is apparent in all parts of the liver tissue with extensive inflammatory cells accumulation. (B) Proliferation of fibroblasts (PF). (C) Fibrosis (F) image in the lumen of central vein. 100x magnification. (D) Fibrosis (F) image of blood vessel walls, accompanied by distribution of inflammatory cells. 400x Magnification.

Figure 3's observations on group T3 revealed extensive inflammation throughout the liver tissues. group T3 shown in Figure 3 found extensive inflammation throughout the liver tissues. This inflammation was

accompanied by extensive accumulation of connective tissue (fibrosis) from blood vessel lumen, including the central vein, thickening of blood vessel walls to the sinusoid. These fibrous strands partly connect the regions of the liver (bridging fibrosis). The formed fibrosis is derived from fibroblasts that underwent proliferation shown in Figure 3(B).

DISCUSSION

The clinical condition of schistosomiasis disease, characterized by malaise lasting more than 6 weeks after infection, led to weight loss in group T3. Malaise can cause decreased appetite, which leads to inadequate nutrition, thus causing weight loss. (5)

Pathological condition caused by schistosomiasis was due to the accumulation of eggs trapped between The accumulation of eggs trapped between sinusoids caused schistosomiasis, a pathological condition. These eggs became antigens on the liver tissue surrounded by inflammatory cells and fibrotic tissue. This study found no eggs in the liver preparation. This may be due to a very small number of eggs, thus could not be found in the tissue section used in the preparation.(8) Furthermore, the treatment was only conducted during acute conditions. Granuloma gives the best image in chronic conditions, which can be seen in the study,(1) which used a 120-day period.

Histological changes found in this study include hepatocyte degeneration. Degenerated hepatocytes appeared edematous with irregular cytoplasm, and some of them underwent hydropic degeneration. This hepatocyte degeneration occurred because of an immunological reaction to Schistosoma metabolites, especially eggs (antigen). This antigen induces the secretion of type 1 helper T cells (Th1) as an inflammatory response involving TNFα, ILThis antigen triggers the release of type 1 helper T cells (Th1), leading to an inflammatory response that involves the expression of TNF, IL-1, IL-2, and IL-6. L-2, and IL-6 expression. This process continues to the destruction of hepatocytes. This destruction is caused by cytotoxic substances in the form of granzyme, which triggers apoptosis in hepatocytes. (9, 10, 11, 11)

Group T1, T2, and T3 showed the infiltration of inflammatory cells, especially eosinophils. This inflammatory cell was mostly found in the area surrounding the blood vessel and the portal vein. Inflammatory cells appear because of antigen exposure or schistosoma metabolites, which triggers an immune reaction. The body will mobilize various inflammatory components to damage the location where antigens were found.(9) This process will trigger a non-specific immune response; thus, the body will increase the production of eosinophils. Besides, Kupffer cells in the liver will also phagocytize those antigens. This is a response to maintain the structure and integrity of tissues.(12) Hepatocytes are classified as cells that are very quick to perform repair processes in the event of an injury. When the cells damage occurs due to this inflammation, the hepatocytes are quickly replaced with the good ones. In group T3, the infiltration of inflammatory cells seems massive because of the chronic process. (13) Sinusoid dilatation was found in the observation. This condition occurred because of two things, i.e., decreased hepatocytes and fibrosis. Decreased hepatocyte is already explained above, while fibrosis occurred due to the iFibroblastsprocess tthatriggers the proliferation of fibroblasts. (9,14) Fibroblast will differentiate into myofibroblasts, forming the accumulation of collagen which create new connective tissue (fibrosis). This fibrosis extends from blood vessel lumen, central vein and portal vein and accumulate in the sinusoid.(15, 16) The accumulation of connective tissue in the blood vessel will cause permanent damage to blood flow changes in the liver and hepatocyte perfusion. The liver fibrosis can trigger from many different cellular mechanisms that are involved. It's stars from the hepatic stellate cells (HSCs) as the predominant cell type.(17) The activation of HSCs is facilitated by the formation of inflammatory egg granulomas and liver fibrosis that are from the immune cells and

from enhancing NK- and T-cell activation and the regulating of T helper cell balance. (18)

CONCLUSION

In conclusion, there was gradual liver damage over the period of time in animal models in acute conditions. The histological structure changes that occur are reversible, and the worst is in chronic conditions, which are dominated by fibrotic tissue, but no granulomas have been found in this study.

RECOMMENDATION

We recommend evaluating the molecular mechanism related to granuloma and fibrosis *S. japonicum* infection and extending the observation period of the animal model to 120 days to evaluate the granuloma more clearly.

ACKNOWLEDGMENT

We would like to thank Schistosoma Laboratory in Napu for the support and supervision in this study.

REFERENCES

1. Amaral, K., B., Silva, T., P., Dias, F., F., Malta, K., K., Rosa, M., M., Costa -Neto, S., F., 2017. Histological assessment of granolomas in natural and experimental *S. mansoni* infections using whole slide imaging. *Journal plos one.* 13;12(9):e0184696.
2. World Health Organizatation. 2017. Schistosomiasis. Available from: URL:http//www.who.int/mediacentre/factsheet.fs115/en/.
3. Nurjana., M., A., Samarang. 2013. Infeksi schistosoma japonicum pada hospes reservoir tikus di Dataran Tinggi Nau Kabupaten Poso Sulawesi Tengah Tahun 2012. *Media Litbangkes* 23(3):137-142.
4. Nurwidayati. A., 2015. Variasi genus keong di daerah fokus keong perantara schistosomiasis di dataran tinggi lindu, Sulawesi Tengah, *Balaba* 11 (2);59-66.
5. Rusjdi, R., S. 2013. Hubungan respon imun dan perubahan patologi Schistosomiasis. *Majalah kedokteran andalas*. 35(2):81-90.
6. Sriwahyuni, Ratianingsih R., Hajar, 2016. Kendali optimal model siklus hidup cacing schistosoma japonicum dengan prinsip minuman pontryagin. *Jurnal vektor penyakit*. 10 (2); 51-58.
7. Angeles, J.M.M., Leonardo, L.R., Goto, Y., Kirinoki, M., Villacorte E.A., Hakimi, H. et al. 2015. Water buffalo assentinel animals for schistosomiasis survailance. Bull World Health Organ. 1;93(7):511-2.
8. Hams, E., Gabriella Aviello, G., Fallon, P.G. 2013. The Schistosoma granuloma: friend or foe? *Front Immunol*. 15;4:89.
9. Kumar, V. Cotran, R.S., Robbins, S.L. Alih Bahasa: Pendit, B.U., 2007. Buku ajar patologi Robbins Ed. 7. *EGC*: Jakarta.
10. Zhang, M., Tian, F., Gao, Y., Ji., M., Wu, G. 2010. Ultraviolet attenuated cercariae of Schistosoma japonicum fail to effectively induce a Th1 response in spine of up-regulating expression of cytotoxicity-related genes in C57BL/6 mice. *J Biomed Resc*. 24 (4):277-284.
11. Liu, Z., Zhang, L., Liang, Y. and Lu, L. 2022. Pathology and molecular mechanisms of Schistosoma japonicum-associated liver fibrosis. *Front Cell Infect Microbiol.* 12:1035765.
12. Olveda, D., U., Li, Y., Remigio, M., O., Alfred, K., L., Vinluan, M., L., Harn, D., A., *et al*., 2013. Bilharzia: Pathology, Diagnosis, management and control. *Trop Med Surg.* 1 (4):135.
13. Llanwarne, F., & Helmby, H. 2020. Granuloma formation and tissue pathology in *schistosoma japonicum* versus *schistosoma manso*ni infection. *Parasite Immunol.,* (2020):e12778.
14. Shimada, M., Kirinoki, M., Shimizu, K., Kato-Hayashi, N., Hayashi, A., Chigusa, Y. 2010. Characteristics of granuloma formation and liver fibrosis in murine schistosomiasis mekongi: a morphological comparison be-

tween Schistosoma mekongi and S. japonicum infection. *Parasitology*. 137(12):1781–1789.
15. Andrade, Z.A. 2009. Schistosoma and liver fibrosis. *Parasite Immunol.* 31(11):656-663.
16. Elbaz, T. dan Esmat, G. 2013. Hepatic and Intestinal Schistosomiasis: Review. *J Adv Res*. 4(5):445–452.
17. Huang, P., Ma, H., Cao, Y., Zhan, T., Zhang, T., Wang, X. et al. 2022. Activation of primary hepatic stellate cells and liver fibrosis induced by targeting TGF-β1/ Smad signaling in schistosomiasis in mice. *Parasites & Vectors*. 15:456.
18. Yang, L., Sun, L., Cao, Y., Wang, Q., Song, A., Zhu, R. et al. 2022. MUL-T1-Encoding DNA Alleviates Schistosomiasis-Associated Hepatic Fibrosis via Modulating Cellular Immune Response. *J Inflamm Res*. 16:15:4027 -4045.