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LIVER TISSUE EXAMINATION OF MICE USING 10% BNF FIXATION FOR 6 HOURS AND 16 HOURS

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ABSTRACT

The fixation process is the first stage in the manufacture of histopathological preparations which aims to preserve the tissue and harden the tissue, so that the tissue to be observed does not change in shape or size. BNF fixation solution (Neutral Buffer Formalin) 10% has been used as a routine fixative and It has been be the gold standard in histology laboratories for decades. The material used in this study was a 10% BNF solution because it is easier to use and it can be used to preserve tissue for a long period of time. This study aims to determine the differences in the microscopic results of the liver tissue of mice (Mus musculus) fixed with 10% BNF for 6 hours and 16 hours. This type of research is descriptive analytic. The research was carried out at the Barokah Laboratory/ dr.Mezfi Unita on 04-05 March 2022 with a total sample of 20 samples of mice liver tissue (Mus musculus). The results of the study on 10% BNF fixation for 6 hours and 16 hours showed that the average results were not good. The conclusion from the results of the study that there was no difference in the microscopic results of liver tissue that were fixed with 10% BNF for 6 hours and 16 hours using Hematorylin-Eosin staining. 10% BNF fixation with a time of 6 hours and 16 hours can be used as an alternative in histopathological examination in the short term and long term.

Keywords *: 10% BNF fixation, Hematoxylin Eosin, Liver*

INTRODUCTION

The world is currently facing public health problems due to the epidemiological transition, which is the transition of health problems from infectious diseases caused by viruses, bacteria, fungi and other microorganisms to non-communicable diseases. Now the country must address the problem of mobilizing existing resources to reduce non-communicable diseases that tend to increase such as cancer. Cancer is a non-communicable disease that affects health worldwide. The World Health Organization (WHO) calls liver cancer one of the leading causes of death in the world (Pangribowo, 2019).

Liver cancer or hepatocellular carcinoma (KHS) is a cancer that occurs due to abnormal growth of hepatocyte cells. The incidence of liver cancer in 2018 reached 841,080 cases so that it was ranked fifth in the incidence of cancer in the world and ranked fourth in cancer that causes death, namely 781,631 cases of death. Liver cancer in Indonesia is in the top 4 cancer cases with 18,468 new cases in 2018 and around 18,148 people died. So that laboratory tests must be carried out in order to diagnose cancer, one of which is histopathological examination (Puri et al., 2020).

Histopathological examination is very important in relation to the diagnosis of the disease because one of the considerations in the diagnosis is through the observation of the tissue that is suspected to be disturbed, in this experiment the organ examined histopathology is a mouse liver organ (*Mus musculus*) (Mulyono, 2013). The fixation stage in

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histopathological examination is also important to note because the fixation solution can provide an overview of the tissue in a living condition and the fixation process is the first stage in the preparation of histopathological preparations (Musyarifah, 2018).

Fixation is the chemical process of preserving biological tissue so as to prevent autolysis or the process of Decay (Rahmadani, 2018). The purpose of tissue fixation is to prevent autolysis and decay, maintain or preserve the state of cells and tissue elements to be identical to the living State, and coagulate the liquid tissue to facilitate the cutting of the preparation. Neutral Formalin Buffer (BNF) 10% is widely used as a routine fixative fluid and has been the Gold Standard in histology laboratories for decades (Suprianto, 2014). The advantage of using neutral buffer liquid Formalin 10% is to have a pH=7 (is a very good pH) its use is easier and can be used to preserve tissue for a long time. But kekuranganya power fixation is slower ie 12 to 24 hours (Miranti, 2010).

In general, tissue is colorless so it is difficult to examine tissue that is not colored under a microscope. Therefore, methods of tissue staining have been carried out by which it is possible to identify its components. Hematoxylin and eosin staining or commonly called H & E is the most common type of routine staining used in the process of making histopathological preparations (Irene, 2018).

The Hematoxylin-Eosin staining method is widely used in tissue staining. Hematoxylin is the main tissue dye that gives a bluish color to the cell nucleus and while eosin gives a red color to the cytoplasm (Aliviameita, 2022). All preparations show the cell nucleus, cell membrane, and extracellular matrix perfectly colored where the cell nucleus and cell membrane are blue, because colored by hematoxylin. Alkaline Hematoxylin will give color to the cell nucleus that is acidic so that it is colored blue-purple (Putri et al., 2022).

Based on previous studies by Rahmadani (2018) and Jahira (2018) showed the results, in Rahmadani's research (2018) the quality of tissue preparations fixed using 10% BNF for 6 and 24 hours resulted in observation of liver/kidney tissue with good microscopic picture assessment criteria marked with blue on the cell nucleus, red on the cytoplasm and connective tissue and color on uniform preparations both on the tissue, while for fixation for 7 days the microscopic picture obtained was not good (Rahmadani, 2018). Furthermore, the results of the study from Jahira (2018) fixation of rabbit kidney and liver organs with a 10% BNF solution and using Hematoxylin-Eosin staining with a fixation time of 8, 16, and 24 hours the average image results are good, namely bright blue in the cell nucleus, red (eosin) in the cytoplasm and color in uniform preparations.

Based on the above description, The aim of the study was to determine "liver tissue examination of mice using 10% BNF fixation for 6 hours and 16 hours".

RESEARCH METHODS

The type of research used is descriptive analytic. The study used a repeat sample of 20 samples of hepatic tissue mice (Mus musculus). The mouse sample that was examined was a male mouse with an age of 3 months with a body weight of 25 – 30 grams and healthy. Mice in the study amounted to 10 tail consisting of two groups of treatment fixation namely hepatic tissue mice in two pieces then in fixation for 6 hours and 16 hours using Hematoxylin eosin staining.

RESEARCH PLACE

The study was conducted in the laboratory of Barokah/ dr. Mezfi Unita Sp.PA Palembang.

RESEARCH TIME

The study was conducted in February – March 2022.

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POPULATION AND SAMPLE

The sample used mice (*Mus musculus*) taken male liver organs, age 3 months with an average weight of 25-30 gr. Post Test only Control Group Design. This study began with the process of adaptation of male mice (*Mus musculus*) for 7 days, Fed and watered (*Ad libitium*), the cage was replaced with a new one every 3 days (Alwi, 2016).

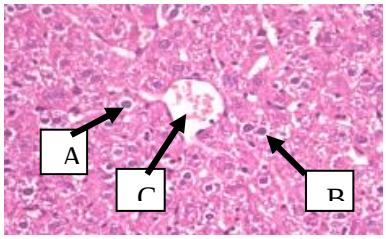
Hepatic organ fixation is carried out which aims to preserve the organ using a 10% solution (neutral buffer formalin) with a volume of 10x fixation solution or with a ratio of 1:9 tissue samples to be fixed, with a fixation time of 6 hours and 16 hours (Ellyawati, 2018). The process of making blocks of preparations so that organs can be cut with microtomes (Ravif, 2016).

RESULTS AND DISCUSSION

The study was conducted to determine the differences in the microscopic results of hepatic tissue in fixation with 10% BNF solution for 6 hours and 16 hours using Hematoxylin eosin staining. Animal tissue mice (Mus musculus) performed surgery and then taken fresh liver organs, tissue cut into 2 parts each inserted into the cassette that has been labeled then inserted into an airtight jar containing a solution of fixative BNF 10% then in fixation for 6 hours and 16 hours, then performed tissue management, staining HE and microscopic observation magnification 400x by a specialist in Anatomical Pathology.

The results of the observation of the microscopic picture of the tissue is read using three staining criteria score is score 1 (not good) if the blue color in the cell nucleus is not clear, the red color (eosin) in the cytoplasm and connective tissue is not clear and the color of the preparation is not uniform, score 2 (not good) if the blue color in the cell nucleus is less, red color (eosin) in the cytoplasm and connective tissue is less, and the uniformity of color in the preparation is less. But it can still be diagnosed, and score 3 (good) if the blue color in the cell nucleus, red color (eosin) in the cytoplasm and connective tissue and color in uniform preparations (Jahira, 2018).

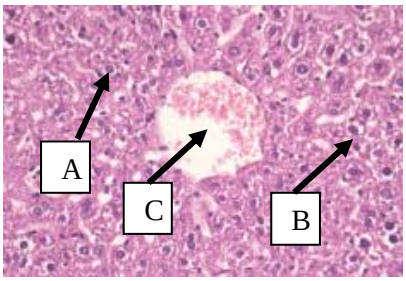
The results of microscopic observation of hepatic tissue with 10% BNF fixation for 6 hours using Hematoxylyn eosin (HE) Painting are attached in the form of images as follows:



Picture 1. Observation results fixation BNF 10% for 6 hours magnification 400x

The results of the observation of 10% BNF fixation for 6 hours using 400x magnification, Code (A) the cell nucleus is clearly visible in a dark purplish blue round shape and there is a child nucleus, code (B) the cytoplasm is clearly red and Code (C) the central vein there are erythrocytes.

The results of microscopic observations of hepatic tissue with 10% BNF fixation for 16 hours using Hematoxylyn eosin (HE) Painting are attached in the form of images as follows:



Picture 2. Observation results fixation BNF 10% for 16 hours magnification 400x

Observation of 10% BNF fixation for 6 hours using 400x magnification. Code (A) the cell nucleus is clearly visible dark purplish blue round shape and there are daughter nuclei, code (B) the cytoplasm is clearly red and Code

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(C) the central vein there are erythrocytes.

Difference of 10% BNF fixation result for 6 hours and 16 hours

Table 1. Differences in BNF fixation results 10% for 6 hours and 16 hours

Hasil Pemeriksaan			
Fiksasi	Tidak baik (skor 1)	Kurang baik (skor 2)	Baik (skor 3)
BNF 10% 6 jam	1 (10%)	8 (80%)	1 (10%)
BNF 10% 16 jam	0 (0%)	7 (70%)	3 (30%)
Total lapang pandang	20		

Results of hepatic tissue of mice (*Mus musculus)* in fixation BNF 10% for 6 hours and 16 hours on eosin haematoksilin staining there are 3 levels of quality is not good, not good and good. At 10% BNF fixation with 6 hours total 10 field of view got the result that is 10% tissue preparation with poor quality (score 1), 80% tissue preparation obtained with poor quality (score 2), and 10% good tissue preparation (score 3), while the fixation of BNF 10% with a time of 16 hours with a total of 10 field of view daptkan results are 70% tissue preparation obtained with poor quality preparation (score 2) and 30% good quality tissue preparation (score 3).

The results of the study on microscopic examination of hepatic tissue mice (Mus musculus) at 10% BNF fixation with a total time of 6 hours 10 field of view got the results that is 10% with criteria not good (score 1) that is a preparation with a quantity of interpretation of the results of hepatic tissue preparations in the cell nucleus is not good blue and red cytoplasm is not good, then 80% of the preparation obtained with poor quality (score 2) that is a preparation with a quantity of interpretation of the results of hepatic tissue preparations in the cell nucleus is not good blue and cytoplasm is not good red color, then 80% of the preparation obtained with poor quality (score 2) that is a preparation with a quantity of interpretation of the results of hepatic tissue preparations in the cell nucleus is not good blue and cytoplasm is not good red color, and 10% good preparation (score 3) is the preparation with the quantity of interpretation of the results of hepatic tissue preparations in the nucleus of cells of good blue color and cytoplasm of good red color.

On fixation BNF 10% with time 16 hours with a total of 10 field of view got the result that is 70% preparation obtained with poor quality (score 2) is the preparation with the quantity of interpretation of the results of hepatic tissue preparations in the nucleus of the cell color blue is not good and cytoplasm red is not good and 30% good preparation (score 3) is the preparation with the quantity of interpretation of the results of hepatic tissue preparations in the nucleus of the cell color blue is good and cytoplasm red is good.

The next research Data was analyzed descriptively. Descriptive statistics is part of statistics regarding data collection, presentation, determination of statistical values, making diagrams or drawings about something, here the data is presented in a form that is easier to understand or read (Siswanto, 2013). The results were analyzed descriptively showed that there was no significant difference between the fixation of BNF 10% for 6 hours and 16 hours. In the research that has been carried out, the results of good quality are the highest level of the three categories, because the quality is able to meet the criteria for interpreting the results that have been set on histological examination (Fauzi, 2018).

The results of the study there is no difference examination of hepatic tissue mice (Mus musculus) in fixation BNF 10% for 6 hours and 16 hours using Hematoxylin eosin

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staining, according to researchers this happens because it has the same fixation results, namely by providing an overview of the shape, arrangement of cells, cell nuclei, and cytoplasm, as well as the arrangement of connective tissue fibers (Jahira, 2018). BNF 10% is widely used as a routine fixative fluid and has been the Gold Standard in histology laboratories for decades (Suprianto, 2014).

The standard time of fixation of 10% BNF is 12 – 24 hours using Hematoxylin eosin staining. An alternative method that can be used is 6 hours to accelerate fixation and to maintain the cell nucleus and cytoplasm. An alternative method of fixation was carried out for 6 hours using 10% BNF on Hematoxylin eosin staining to get good results, that is, the color in the cell nucleus and cytoplasm was good on average. BNF 10% is a fixation fluid used for tissue preservation in routine histological examinations. This fixation fluid was chosen because its use is younger and can be used to preserve tissues in a fairly short period of time (Rahmadani, 2018)

The results are in line with research conducted by Rahmadani, et al., (2018) regarding the fixation of 10% BNF for 6 hours and 24 hours resulted in observations of hepatic/renal tissue with a tissue size of 1x1 cm the assessment criteria showed a good microscopic picture of 10% BNF fixation for 6 which was marked with blue on the cell nucleus, red on the cytoplasm and connective tissue and color on uniform preparations, this is because the 10% BNF solution has a fairly good penetration ability in the tissue.

According to jahira, et al., (2018) fixation of rabbit kidney and liver organs with 10% BNF solution and using Hematoxylin eosin staining with fixation time of 8, 16, and 24 hours the results of the preparation images are bright blue in the cell nucleus, bright red in the cytoplasm and uniform preparations.

Examination of liver tissue microscopic results on Hematoxylin eosin staining is influenced by several factors, where the basic hematoxylin will color the structure in the acidic tissue is mainly part of the core. Whereas eosin dye is acidic which colors alkaline cell structures when the cytoplasmic color becomes paler and fainter, blurred boundaries between cells can be caused by too high PH, dehydration with alcohol for too long, too thin cutting, inadequate coloring time. It is according to the acid-base bond in the Hematoxylin staining of Eosin that in the results of fixation in these tissues there is no significant difference (Hayyusari, 2018).

CONCLUSSION

In the fixation of hepatic organs of mice (Mus musculus) with a 10% solution of BNF (neutral buffer formalin) using Hematoxylin eosin staining with a fixation time of 6 hours and 16 good average image results are bright blue in the cell nucleus, red color (eosin) in the cytoplasm and color on uniform preparations. So that the fixation solution of BNF (neutral buffer formalin 10% can be used in tissue fixation with a shorter time.

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