Description of Liver Histology of Mice (Mus musculus L.) after Giving Nano Herbal Haramonting (Rhodomyrtus tomentosa)

Putri Cahaya Situmorang1 and Syafruddin Ilyas1
1Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia, 20155

Keywords: Nanoherbal, Rhodomyrtus tomentosa, Liver.

Abstract: The objective of this study was to determine the description of liver histology of male mice (Mus musculus L.) after giving Nano Herbal Haramonting (Rhodomyrtus tomentosa). This research use the Completely Randomized Design (CRD), which consists of six groups of CMC 0.5% and Nano Herbal Haramonting (R. tomentosa) at dosage of (100; 141,42; 200; 282,82; 400)mg/20g body weight. The livers were made into preparations by using the paraffin method and Hematoxylin Erlich-Eosin staining (HE). The value of each cell with Manja Roenigk's histophatolgy model. Nano-R. tomentosa administration was not significantly different in body weight of mice (P>0,05), but It was significantly different to weigh of liver (P<0,05). The results of histological liver showed that there is a significant difference (P<0,05) between control group and treatment groups with damaged cells liver of male mice Mus musculus L.) after giving of Nano Herbal Haramonting (R. tomentosa).

1 INTRODUCTION

Nanoherbal is the herb that uses nanoscale technology (wave power) and use quality herbal raw materials. The use of herbal remedies has been practiced for thousands of years and a part of several countries such as Indonesia, China and India. Nanotechnology commonly refers to structures that are up to several 100 nm in size which can be increased up to 1000 nm. Nanotechnology and nano science studies have develops briskly during the past years in a large range of product results. It gives opportunities for the development of materials, including those for medical applications, where conventional techniques may reach their limits (Ratnam et al, 2006). Herbal medicines have less adverse effects as compared with modern medicines due to poor solubility, poor permeability, low bioavailability, instability in biological milieu and extensive first pass metabolism by developing new formulation as nano herbal medicines like nanoparticles, dendrimers, Nano crystals, Quantum dots, Nanosperes, Nanocapsules, herbal market get good feedback (Rinku et al, 2018) One study using nano herbal is Nano curcumin. In this study Nano-curcumin significantly inhibited the growth of MCF-7 breast cancer cell line and resulted in synergism cytotoxicity effects (Parisa et al, 2018) Haramonting (R. tomentosa) is an ornamental, evergreen shrub grows up to four meters. This plant species is native to southern and southeastern Asia (Awinita et al, 2005). The potential of R. tomentosa as a new source of health-promoting compounds such as dietary fibers, essential fatty acids, and phenolic compounds. A total of 19 phenolic compounds were tentatively characterized, including stilbenes and ellagitannins as major components, followed by anthocyanins, flavonols, and gallic acid. Piceatannol, a promising health-promoting stilbene component, was the major phenolic compound found in R. tomentosa fruits (Lai et al, 2013).

Hepatic sistem is the major organ system involved in the metabolism, detoxification and excretion of various endogenous and exogenous. Liver damage is always asssociated with necrosis, Strengthening of inbuilt protective mechanism or exogenous administration of antioxidant may be useful in protecting the liver (Pramodh et al, 2008). In vitro antioxidant activity of the different extracts of R. tomentosa has been reported by different methods (Geetha et al, 2010). Natural antioxidants are known to exert beneficial effects in hepatitis induced by antitubercular agents (Limsuwan et al, 2009).
Oxidative stress induced damage to hepatocytes has been found to have a key role in antitubercular drugs induced hepatitis (Shakun and Shmnan’ko, 1986). R. tomentosa extract demonstrated its free radical scavenging effects in concentration dependent manner and the results suggest that R. tomentosa extract can serve as a potent antioxidant (Sodhi, 1998). So in this study, we examined the effect of Nano- R.tomentosa to liver.

2 METHODS

2.1 Materials

The material used is male mice (Mus musculus L) strain DDW, Haramonting leaves (R.tomentosa) obtained from plantation residents in Tapanuli North Sumatera, Feed the mice no. PB 551, staining Hematoxylin and Eosin. This research use the Completely Randomized Design (CRD) using 30 male mice with an average weight of 20-25g age 12-18 weeks. Male mice are kept in Animal Cages Biological Laboratory, Faculty Mathematics and Natural Science, Universitas Sumatera Utara. Experimental animal handling is done ethically (Ethical Clearance).

2.2 Making of Nano- R.tomentosa

R.tomentosa leaves are washed, then dried in accordance with the requirements of water content using high energy milling (HEM), then Simplicia as the destructive medium is inserted into the jar container, Inserting balls with larger diameter size then continued by inserting small balls and the last sample. The total volume of the balls and the samples entered does not exceed 2/3 of the volume of the jar. The usual Ball to Powder Ratio (BPR) is 20:1, 10:1, 8:1. Example BPR 20:1 means 1 gram of sample then milled with 20 grams of ball weight. Jar that has been filled with the ball, samples are closed tightly then mounted on the jar inside the HEM tool, then HEM is turned on for 2 hours.

2.3 Treatment and Observation of Hystology

The treatment consisted of 6 groups consisting of 5 male mice ie CMC 0.5% and Nano Herbal Haramonting (R.tomentosa) at dosage of (100; 141,42; 200; 282,82; 400)mg/20g bw in 14 days, then killed by the method of disclasio cervicalis to take the liver. Preparation of histologist by paraffin method and Hematoxylin Erlich-Eosin staining (HE) to observe the damage to liver cells due to the administration of Nano-R.tomentosa. Mixture is observed with magnification 400x. In each field of view counted 20 cells at random and in the value of each cell with Manja Roenigk's histopatholgy model (1: Normal, 2: Parenchymatous Degeneration, 3: Hydropic Degeneration 4: Necrosis) then the data processed with SPSS 22 program with Kruskal Wallis test.

3 RESULT AND DISCUSSION

The result will be discussed in 3 subsection i.e average weight of body, weight of liver and damage of liver cells.

3.1 Weight of Body

![Figure 1. Average of weight of male mice after giving Nano-R.tomentosa. (X ± SD).](image)

The results of average body weight of mice CMC 0.5% group has the highest average weight while the lowest weight is in T1 (Figure 1). Nano-R.tomentosa administration was not significantly different in body weight of mice (P>0.05). Nano-R.tomentosa does not affect the weight of male mice, Allegedly due to outside variables that can not be controlled such as the psychological condition of the mice that is affected by the surrounding environment, repeated treatment, different appetite and fights between mice.

3.2 Weight of Liver

The result of statistic test P<0.05 showed that Nano-R.tomentosa administration at each treatment was significantly different in weight of liver except in Control group with T1, T3, T4 and T5, T1 group with T4 and T5, T2 group with T3, T3 group with T4
(P>0.05). T5 group has the highest average liver weight while the lowest liver weight is in T2 (figure 2). T1 higher than T2, and also T1 higher than control is assumed because the outside variable is difficult to control such as weight of body, different appetite and activity of mice. The liver is the largest organ in the body that is involved in the body's metabolism, neutralize toxins and toxic substances through the detoxification process by cell kupfer in the body. So that the weight of the liver can be increased or decreased due to the entry of excessive toxic substances in the body.

### 3.3 Damage of Liver Cells

Table 1. Average Hepatocyte Normal and degree of damage (Parenchymatous Degeneration, Hydrophic Degeneration and necrosis) after giving Nano-R. tomentosa (\( \bar{X} \pm SD \)).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Normal</th>
<th>Parenchymatous Degeneration</th>
<th>Hydrophic Degeneration</th>
<th>Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMC 0.5%</td>
<td>14.40 ± 1.66</td>
<td>4.56 ± 1.47</td>
<td>4.20 ± 3.00</td>
<td>7.52 ± 4.05</td>
</tr>
<tr>
<td>T1</td>
<td>13.60 ± 1.50</td>
<td>4.88 ± 1.42</td>
<td>5.16 ± 2.81</td>
<td>9.12 ± 4.09</td>
</tr>
<tr>
<td>T2</td>
<td>12.30 ± 1.14</td>
<td>5.52 ± 1.94</td>
<td>12.5 ± 2.48</td>
<td>10.24 ± 4.01</td>
</tr>
<tr>
<td>T3</td>
<td>6.76 ± 1.51</td>
<td>6.80 ± 2.00</td>
<td>12.5 ± 4.30</td>
<td>22.72 ± 8.54</td>
</tr>
<tr>
<td>T4</td>
<td>4.84 ± 1.52</td>
<td>7.76 ± 2.60</td>
<td>11.5 ± 3.32</td>
<td>29.76 ± 8.01</td>
</tr>
<tr>
<td>T5</td>
<td>3.40 ± 1.35</td>
<td>6.80 ± 2.71</td>
<td>9.48 ± 3.84</td>
<td>40.16 ± 8.06</td>
</tr>
</tbody>
</table>

The results P<0.05 showed cell damage due to nano-R. tomentosa administration is significantly different from each treatment except Control group with T1 (P>0.05). Description of liver damage is different for each treatment (figure 3). Damage with Parenchymatous degeneration occurs lowest in CMC.
0.5% (Control) and highest in T4 (table 1) due to the mice are also given aquades so that the cells can regenerate. In liver organ with parecmatous degeneration may improve, cells undergoing necrosis over time will be replaced with new liver cells due to cell regeneration process in liver organ (Geetha et al., 2012). Parenchymatous degeneration is the mildest degenerate level. In parenchymatous degeneration cells, granules are found in the cytoplasm due to the precipitate that causes the cytoplasm to become turbid and swelling of the cells. Hydrophic Degeneration is low in control, and increases in T2 and T3 (table 1). This degeneration is more severe damage, there are vacuoles containing water and cytoplasm that do not contain fat and glycogen. This change is generally a result of metabolic disorders such as hypoxia or chemical poisoning. This degeneration is also reversible although it may be irreversible if the cause of the injury persists. The process of Necrosis increases from Controls to P5 and Normal cells decreases from control to treatment level (table 1) (figure 3). At each dose level, the toxic ingredients in the liver are getting out of process, causing parenchymatous degeneration, hydrophic degeneration and necrosis in liver. The target of a toxic substance in the body is the molecular structure of transport of bile acids, membranes, intracellular fats, proteins and nucleic acids. As a result the target molecule becomes a non-functioning unit and may activate secondary pathways such as apoptosis, necrosis, autophagy and mitochondrial disorders and other immunological reactions (Kandena et al., 2011).

4 CONCLUSIONS

Cell damage due to Nano-R. tomentosa administration is significantly different from each treatment (P<0.05) and Giving Nano herbal Haramonting (Rhodomyrtus tomentosa) with excessive doses can cause a decrease in hepatic weight and damage to liver cells, So the liver loses its function especially the detoxification function of toxic.

ACKNOWLEDGEMENTS

We are grateful to Directorate of research and community service, Directorate general of research and development, Ministry of research, Technology, and Higher Education in accordance with research and community service funding agreement for budgeting year 2018 (Fund of the research postgraduate team 1) to funding our research.

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