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Effect of Ethanol Extract of Moringa Leaf on Liver Histopathology of White Rat (*Rattus norvegicus*) Induced by Meloxicam

N.M.R. Suarni[1]*, N.G.A.M. Ermayanti[1], A.A.S.A. Sukmaningsih[1] and A.M. Deshmukh[2]

[1]*,[1]Animal Laboratory Biology Departement, Faculty of Mathematics and Natural Sciences Udayana University, Bali, 80361, Indonesia
[2]Microbiology Society, India

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ABSTRACT

The aim of this study was to find out the effects of ethanol extract of moringa leaf on liver histopathology white rats (*Rattus norvegicus*) induced by meloxicam. The experimental design used in this study was a Completely Randomized Design (CRD) with 5 treatments where K- was given only aquadest, K+ was given 8.4 mg/kg BW of meloxicam, P1 was given 8.4 mg/kgbw of meloxicam and 200 mg of moringa leaf extract, P2 was given 8.4mg/kg bw of meloxicam and 400 mg of moringa leaf extract and P3 was given 8.4 mg/kg BW of meloxicam and 600 mg of moringa leaf extract, and each treatment consisted of 5 replications. The treatment was carried out for 35 days. The parameters observed were hydropic degeneration, sinusoidal dilatation, venous congestion, fatty degeneration, necrosis, and inflammatory cell infiltration. The data obtained were analyzed by One Way ANOVA analysis and continued with Duncan's test. The results showed a significant effect ($P<0.05$) of the ethanol extract of moringa leaves on liver histopathology white rats induced by meloxicam. It can be concluded that the ethanol extract of moringa leaves in this study can prevent liver cell damage in white rat caused by meloxicam.

Key words : *Histopatology, Liver, Meloxicam, Moringa leaf, White rat*

Introduction

The inflammatory response is a tissue response to damaging physical or chemical stimuli. This stimulation releases inflammatory mediators histamine, serotonin, bradykinin, and prostaglandins, which cause an inflammatory reaction in the form of heat, pain, redness, and swelling, accompanied by impaired function (Katzung, 2004). Nonsteroidal anti-inflammatory drugs (NSAIDs) are nonsteroidal anti-inflammatory drugs. One of the drugs included in the NSAID class is meloxicam (Lanza *et al*., 2009). (Bindu *et al*., 2020) states that NSAIDs can cause two main clinical patterns of hepatotoxicity: acute hepatitis characterized by jaundice, nausea, and fever, increased serum levels of transaminase, and chronic active hepatitis characterized by serological and histopathological abnormalities. Meloxicam is safe if consumed in doses determined by a doctor, but meloxicam is also sold freely, so in the community, its use often needs to follow the rules. Using meloxicam that does not follow the rules, such as in the long term and high doses, will be toxic, causing disruption and damage to the liver and kidneys (Gretzer *et al.,* 2001) and (Burukoglu *et al.,* 2016).

Liver or hepar is the largest gland in the body with a wide variety of functions. The three essential functions of the liver are to form and secrete bile into

the intestinal tract; play a role in various metabolisms related to carbohydrates, lipids, and proteins; filter the blood, and get rid of bacteria and foreign bodies that enter the blood (Snell, 2012). Hepar synthesizes heparin, an anticoagulant substance with a critical detoxification function (Snell, 2012). As a detoxification organ, the hepar protects the body from various poisons and foreign bodies that enter the body by changing all foreign materials or toxins from outside the body. These foreign materials or toxins can be in the form of food, drugs, and other materials. The ability of this detoxification is limited, so not all incoming substances can be completely detoxified, but accumulate in the blood and can cause damage to hepatocytes. Hepar damage caused by toxic substances such as meloxicam can be prevented in various ways, including foods that contain high antioxidants.

One of the plants that contain high antioxidants is moringa. Phytochemical test results prove that moringa leaves contain high antioxidants (Kasolo *et al*., 2010) Moringa has 46 antioxidants and is a plant with the most natural sources of antioxidants. Antioxidants will work optimally with several antioxidants and other nutrients (Bey, 2010). Indahsari (2017) stated that ethanol extract at a dose of 1000 mg/200 BW could protect the livers of rats induced with toxic doses of paracetamol from inflammation, necrosis, and degeneration. Based on the above, a study on the effect of ethanol extract of moringa leaves on the liver histopathology of meloxicam-induced male white rats was conducted.

Research Method

The experimental design used was Completely Randomized Design (CRD). Five treatments consisted of negative control (K-) given aquadest and CMC Na 0.5%, positive control (K+) given meloxicam 8.4mg/kgbb, and P1 given meloxicam 8.4mg/kgbb, P2 given meloxicam 8.4 mg /kgbb and P3 were given meloxicam. 8.4 mg/kgbb. The treatment was carried out for 35 days, where each treatment consisted of 5 replications, so 25 male white rats were used.The study variables included histopathological features of the liver parenchyma (normal, hydropic degeneration, fatty degeneration, dilated sinusoids, venous congestion, necrosis, inflammatory cell infiltration.

Histopathological Preparation of the Liver

Liver was fixed with formalin buffer followed by the paraffin method and Hematoxylin and Eosin (HE) staining. Observation and histopathological evaluation of the liver were carried out using a light microscope with 400x magnification and documented with an Optilab camera (Micronos).

Data analysis

Data were analyzed using costat with the One Way ANOVA test, and if the results showed significantly different results, then continued with Duncan's test.

Results and Discussion

The results showed several types of liver damage caused by meloxicam, such as hydropic degeneration, fatty degeneration, sinusoidal dilatation, venous congestion, necrosis, and inflammatory cell infiltration. All types of liver damage were present in all treatments, including the negative control, but in the positive control, where the animals were given meloxicam without moringa leaf extract, the amount of damage significantly increased (Figure 1,2,3,4 and Table 1).

Drug-induced liver damage is typical because the liver has a central role in the metabolism of many drugs. The liver is also responsible for metabolizing NSAID-class drugs, so almost all NSAID medicines are involved in liver damage. Many experimental studies have been conducted on various animal species to examine the toxic effects of NSAIDs on the liver. The results showed that using NSAIDs excessively caused mixed cell infiltration and bile duct proliferation in the portal area and hepatic parenchymal cell degeneration. Research using naproxen in Wistar rats showed increased ROS by increasing SOD and catalase. Increased ROS was supposed to be the underlying cause of significant genotoxic effects and a fall in liver function (Ahmad *et al*., 2018).

The average value of hydropic degeneration data in this study was: K-(0.2), K(7.6). P1(6,6), P2(2,9) and P3(1,0). Results Statistical analysis of the liver hydropic degeneration of white rats in this study showed a significant difference between K+ and K0, P1, P2 and P3. The higher concentration of the ethanol extract moringa leaves, better ability to prevent hydropic degeneration in the livers of white male rats. This can be seen at a concentration of 600mg/kg BW, which is not significantly different from the negative control (K-). Fat degeneration is fat accumulation in the cytoplasm caused by hepatocyte disorders due to food deficiencies and (toxic) poisoning

Table 1. Average data on the effect of ethanol extract of moringa leaves on the liver histopathology of meloxicam-induced male white rats

Treatment	Hydropic degeneration	Fat degeneration	Sinusoidal dilatation	Venous congestion	Necrosis	Inflammatory cell infiltration
K-	0,2d	0,6c	0,4e	0,2c	0,8c	0,6c
K+	7,6a	20,2a	25,6a	1,6a	10,0a	5,8a
P1	6,6b	10,6b	21,6b	1,4ab	6,0b	4,8b
P2	2,8c	1,2c	9,0c	0,8bc	1,4c	1,4c
P3	1,0d	0,75c	4,2d	0,25c	0,8c	1,0c

Note: different letters in the same column indicate significantly different (P<0.05)
K- = given aquadest and CMC Na 0.5%, (K+) = given meloxicam 8.4mg/kg BW, P1 = given meloxicam 8.4mg/kg BW and 200mg /kg BW *Moringa oleifera* ethanol extract, P2 = given meloxicam 8.4 mg/kg BW and 400 mg/kg BW *Moringa oleifera* ethanol extract and P3 = given meloxicam. 8.4mg/kg and 600mg/kg BW *Moringa oleifera* ethanol extract

(Berata *et al*., 2014). The average fat degeneration data are: K-(0.6), K+(20.2), P1(10.6), P2(1.2) and P(0.75). The statistical analysis results showed significant differences between K+ and K0, P1, P2 and P3. P2 and P3 were not significantly different from K- and P2 was also not significantly different from P3. The ethanol extract of moringa leaves 400 and 600 mg/kg has the same effect on preventing fat degeneration. This means that a dose of 400 mg/kg has prevented fat degeneration caused by meloxicam. Moringa leaves contain various antioxidants that can prevent damage caused by free radicals (Kurniasih, 2013).

The sinusoidal dilation average is K- (0.20), k+ (25.6), P1(21.6), P2(9.0), and P3 (4.2). The statistical analysis results showed significant differences between K- and K+, P1, P2, and P3. Even though K- was significantly different from all the treatments given to moringa leaves, it can be said that the greater the concentration of moringa leaf extract given, the higher the effect in preventing dilatation of the liver sinusoids of white male rats in this study. Data on venous congestion of the liver cells of male white rats in this study were K-(0.2), K+(1.6), P1(1.4), P2(0.8), and P3(0.25) . The average liver cell necrosis data were K-(0.8), K+(10.0), P1(6.0), P2(1.4) and P3(0.8). Inflammatory cell infiltration was K-(0.6), K+(5.8), P1(4.8), P2(1.4) and P3(1.0). The results showed the same effect of the ethanol extract of moringa leaves on venous congestion, necrosis, and inflammatory cell infiltration. This is shown from the statistical analysis results that there is a significant difference between K+ and K-, P1, P2, and P3 and no significant difference between K-, P2, and P3.

Meloxicam causes necrosis or tissue damage without mononuclear cell infiltration; in this research is thought to be due to apoptosis. Moreover, COX-2 inhibitor drugs might cause liver damage

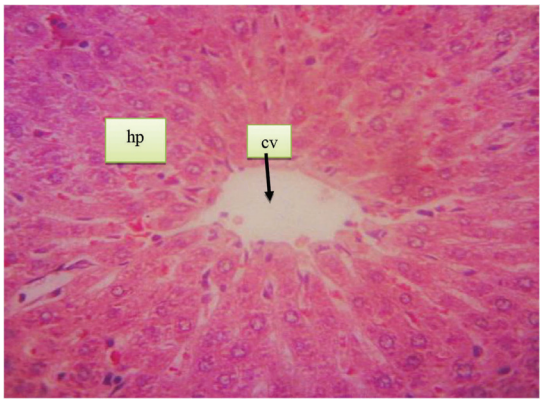

Fig. 1. Light micrographs of white rat liver of negative control (K-). cv = central vein, hp = normal hepatosit. Mic Mag x 400 H&E

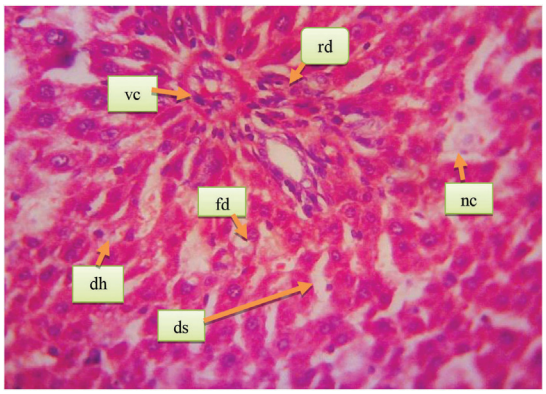

Fig. 2. Light micrographs of white rat liver of positive control (K+). vc = venous congestion, rd= inflammatory cell infiltration, dh= hydropic degeneration, fd= fat degeneration, ds= sinusoidal dilation, nc= necrosis without mononuclear cell infiltration. Mic Mag x 400 H&E

through prostaglandin pathways (Fernando, 2010), so the absence of prostaglandin would induce mitochondrial apoptosis (Najat *et al*., 2019).

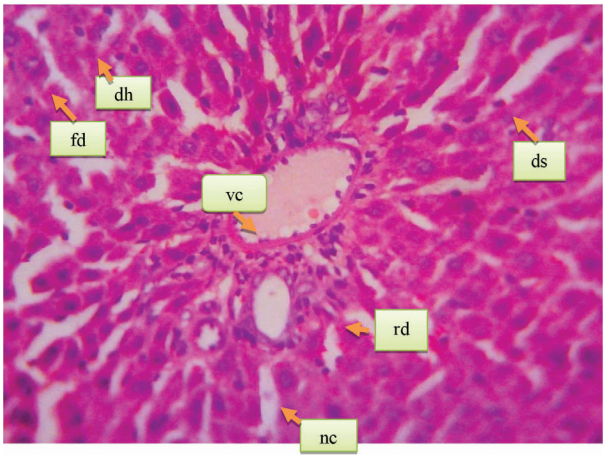

Fig. 3. Light micrographs of white rat liver of P1 (given meloxicam 8.4mg/kg BW and 200mg /kg BWethanol extract of *Moringa oleifera* leaf). vc = venous congestion, rd= inflammatory cell infiltration, dh= hydropic degeneration, fd= fat degeneration, ds= sinusoidal dilation, nc= necrosis without mononuclear cell infiltration. Mic Mag x 400 H&E

Moringa is often called a miracle plant with very high nutrients and vitamins. Bey (2010) stated that Moringa contains 46 antioxidants, one of which is flavonoids. Flavonoids in moringa leaves are a real hepatoprotection that can prevent liver cell damage. According to (Wiryawan, 2008), several extracts containing flavonoid compounds be used as hepatoprotective, including honey, carrots, and black cumin. In this study, rats treated with moringa leaf extract at doses of 200, 400, and 600 mg/kg and meloxicam 8.4 mg/kg showed that there was a significant difference in the amount of damage to liver cells compared to the positive control, namely mice given meloxicam without moringa leaf extract. In the treatment given moringa leaf extract, liver cells' damage decreased with the higher concentration. Of all the parameters observed, administration of moringa leaf extract at a dose of 400 mg/kg BW prevented rat liver damage caused by meloxicam with the same results as negative control (K-).

Bey (2010) stated that A vitamin substance in dried moringa leaf ten times was more than in carrot. The highest vitamin substance is the most potent antioxidant, so it can prevent free radicals, which come from the environment or feed. The synergy effect between E vitamin and iso-flavones is considered to strengthen both as phenolic antioxidants and, therefore can stop the chain reaction of lipid peroxidation of unsaturated fatty acids in the cell membrane phospholipids, the free radical accumulation. In terms of this, it is supported by Zn's role in maintaining cell membrane integrity (Corah, 1996). Flavonoids can be antioxidants and prevent damage

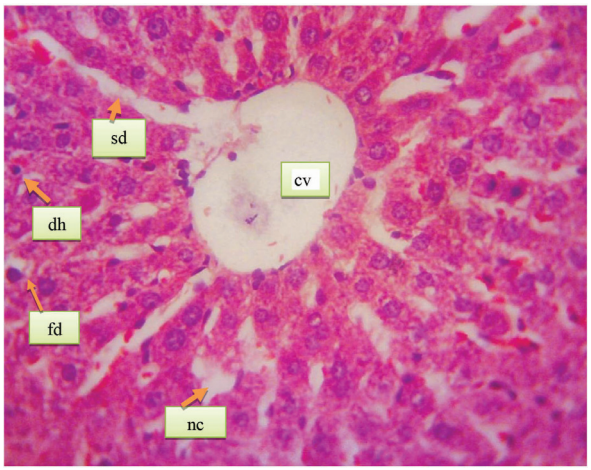

Fig. 4. Light micrographs of white rat liver of P2 (given meloxicam 8.4mg/kg BW and 400mg /kg BW ethanol extract of *Moringa oleifera* leaf cv = central venous, dh= hydropic degeneration, fd= fat degeneration, ds= sinusoidal dilation, nc= necrosis without mononuclear cell infiltration. Mic Mag x 400 H&E

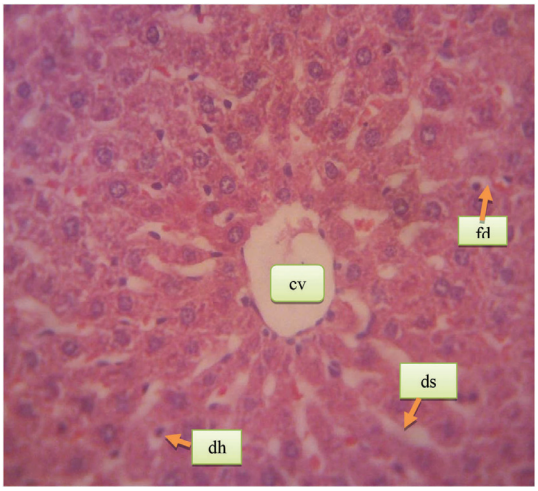

Fig. 5. Light micrographs of white rat liver of P3 (given meloxicam 8.4 mg/kg BW and 600 mg /kgBW ethanol extract of *Moringa oleifera* leaf cv = central venous, dh= hydropic degeneration, fd= fat degeneration, ds= sinusoidal dilation. Mic Mag x 400 H & E.

caused by free radicals, whereby Flavonoids act as scavengers about direct free radicals (Nijveldt *et al*., 2001).

Conclusion

Moringa leaf ethanol extract can prevent liver damage caused by meloxicam, such as hydropic degeneration, fatty degeneration, sinusoidal dilatation, venous congestion, necrosis, and inflammatory cell infiltration. The optimal dose of Moringa leaf ethanol extract in this studiis 400 mg/kgBW.

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