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Toxicity Effect by Binahong (*Anredera cordifolia*) Leaf Extract in Histopathology and Liver Weight of Guinea Pigs (*Cavia cobaya*)

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ABSTRACT

The aim of this research was to determine the toxic effect of *Anredera cordifolia* leaf extract on the *Cavia cobaya* liver which was evaluated by the histopathological examination of liver tissue. The materials used were 8 female guinea pigs 2.5 months old that were divided into 4 groups by simple random sampling, each treatment was given to 2 female *C. cobaya*. Treatments given were 0, 10, 50 and 90 mg of *A. cordifolia* leaf extract/head, designated as T0, T1, T2 and T3, respectively. Materials were given treatment daily as long as 10 days prepartum. All of the guinea pigs were slaughtered at day 11, and the liver were taken to examined their histopathological changes. Each of the liver tissues were processed by paraffin block-embedded and hematoxylin eosin (HE) staining method. The results of this study indicate the presence of albuminosa degeneration or mild degeneration (DH +) from group control and hydropic degeneration or moderate degeneration (DH ++) in all treatment groups and the weight of *C. cobaya* liver which was given an extract of *A. cordifolia* 50 mg/head was not significantly different from the control but was significantly different from 10 and 90 mg/head. The conclusion was Binahong's (*A. cordifolia*) leaves extract up to the dosage 90 mg/head had no significantly toxicity effect on the liver of guinea pigs (*C. cobaya*).

Keywords: *A. cordifolia*, Guinea pigs, Liver, Toxicity

Introduction

Chemical based drugs in their use have been reduced. Chemicals content in synthetic drugs if it was used in the long time can leave residues and result in toxicity. Liver was the central organ of the body's metabolism from the synthesis, modification, breakdown and excretion of substances needed for life. Liver can be damaged due to excessive dose drug induction (Beyene, 2016). Liver damage was characterized by changes in the microstructure of the anatomy. This has led to the increasing use of alternative medicines that are safe and easily available, namely herbal medicines derived from herbal plants. Herbal plants have been widely used for the treatment of various internal and external diseases.

A. cordifolia was one of the herbal plants that has benefits as an antibacterial, anticancer and antiinflammatory (Lestari *et al.,* 2015). The contents of *A. cordifolia* leaves were flavonoids, saponins, terpenoids, tannins, antioxidants and phytoestrogens (Miladiyah and Prabowo, 2015). According to Wijayanti *et al.* (2017), *C. cobaya* given 50 mg / head of *A. cordifolia* leaf extract can accelerate the onset of postpartum estrus in *C. cobaya* after giving 10 prepartum days to 10 days after postpartum. Flavonoid content in *A. cordifolia* extract can improve the profile of red blood in *C. cobaya* (Wijayanti *et al.*, 2016). Flavonoids have antioxidant activity that counteracted free radicals because they can damage liver tissue (Colle *et al.*, 2012).

The liver organ has a function in offering poisons. So it is necessary to protect against toxic effects on the liver. Binahong leaves have a toxic reduction effect on the liver. Mice induced by carbon tetrachloride, the effect of hepatotoxicity can be decreased by administering 289.8 ml / 200 g body weight of binahong leaf juice (Orbayinah and Kartyanto, 2008). According to Saba *et al.* (2010), antioxidant properties of *Cnidoscolus aconitifolius* extract could reduce the toxic effects on rat liver due to CCl4 administration. Ethanol extract of *A. cordifolia* leaves was used as rat hepatoprotector activity due to paracetamol induction (Olaleye *et al.*, 2006). Dollah *et al.* (2013) stated that supplementation of *Nigella sativa* up to the dose of 1 g/kg did not cause any

toxicity effect on the liver function and no changes in liver enzymes level. However, herbal plants usually were used at low doses so it was not known exactly how they affect liver tissue. Histology of liver tissue can describe the extent of tissue damage due to exposure to the drug. So it was necessary to conduct liver toxicity testing on *A. cordifolia* leaf extract against *C. cobaya*. so that herbal medicines given to livestock were safe to use. This study aimed to evaluate changes in liver tissue toxicity in *C. cobaya* after administration of *A. cordifolia* leaf extract.

Materials and Methods

Experimental animals

A total materials used was 8 females of *C. cobaya* with body weight±425 g and 2.5 months old. The animals were maintained at 30±5^{o}C, fed ad libitum. They also had free access to water. The rearing and handling of experimental animals (guinea pigs) during the experiment were conducted according to the animal ethics mentioned in the Law of the Republic of Indonesia number 18, 2009.

Experimental design

A. cordifolia leaf extract was given based on the conversion of human weight to guinea pig (Pan *et al.*, 2016). Giving of *A. cordifolia* leaf extract of 0, 10, 50, and 90 mg /head with treatment design T0, T1, T2 and T3. Each treatment was given 2 female *C. cobaya* and *A. cordifolia* leaf extract were administered orally for 10 days prepartum (Wijayanti *et al.*, 2017; Wijayanti *et al.*, 2018).

Preparation of *A. cordifolia* leaf extract. *A. cordifolia* fresh leaves in the amount of 500 g was cleaned and milled. Subsequently it was macerated with 70% methanol 5.000 ml (1:10) in an Erlenmeyer tube (Miladiyah and Prabowo, 2015). The maceration process was carried out for 5 days at room temperature and stirred daily for 15 minutes. Filtration between the filtrate and residue was done after 5 days (Laksmitawati *et al*., 2017; Garmana *et al*., 2016), filtrate was obtained 2000 ml and then was evaporated to obtain a condensed extract further in the water bath with a temperature of 80^{o}C (Wijayanti *et al.*, 2018). Evaporation results were generated as much as 48.35 g.

Animal surgical procedures and sampling. *C. cobaya* has treatmented for 10 days prepartum, then postpartum first day was performed dislocation using overdose anesthesia (Thompson *et al.*, 2016) and was followed by rapid surgery to remove the liver organs. Liver organs was stored in 10% formalin solution to histopathology and toxicity testing (Thompson *et al.*, 2016; Samik and Safitri, 2017).

Observations of liver histology. Liver weight of each treatment was weighed to determine the weight after treatment. After that, the trimming process started from the tissues of the organs liver cut about 4 mm thick. The trimmed tissue was inserted in absolute alcohol for 1 hour then cleansed (clearing agent) using xylol for 1 hour. Then the tissue in the embedding cassette was transferred into the base mold and filled with liquid paraffin, it was attached wooden beam or to an *embedding cassette* (Zin *et al.*, 2013). The tissue sheet was taken and the slide placed clean diagonally into the water bath. Xylol (I) and xylol (II) for 5 min for staining respectively and followed by immersion in Absolute (I) and absolute (II) alcohols for 5 min (Samik and Safitri, 2017). The tissue sheets were immersed in eosin hematoxylin for 10 minutes and rinsed with running water (Cakir *et al.*, 2015). Then, 96% alcohol (I), alcohol 96% (II), absolute alcohol (III), absolute alcohol (Nithianantham *et al.,* 2011) was washed for 3 min followed by xylol (Thompson *et al.*, 2016; Cakir *et al.*, 2015; Pradana *et al.*, 2016).

Observation of histology and toxicity. Damage to hepatocyte structure was examined microscopically in accordance with the procedure (Hastuti, 2013). Examination was carried out from the center and triangle from 3 zones namely the centrilobular zone, midzonal zone and peripheral zone. The amount of hepatocyte damage was examined and calculated with conditions: damage I (albuminose degeneration), damage II (hydropic degeneration), damage III (fat degeneration) and IV damage (necrosis) of every 100 hepatocytes in each sub-field of view of the microscope. The calculation of hepatocytes was carried out with the aid of a micrometer filled (Zin *et al*., 2013). Damage to subcellular level of hepatocyte structure was observed by electron microscope type of transmission electron microscope (TEM).

Statistical analysis

Data was analyzed by univariate method (Duncan test) and descriptively for determining the effect of treatment on the observed parameters (hepar toxicity).

Result and Discussion

Giving of *A. cordifolia* leaves extracts at different doses than others. Histology of liver without administration of *A. cordifolia* leaf extracts suffered liver change from *C. cobaya* in the form of albuminosa degeneration (Figure 1A). This indicates that *C. cobaya* before the treatment had suffered from an infection or other disorder that caused degeneration. Mild degeneration in 0 mg / head of *A. cordifolia* leaf extract was affected by the physiological conditions of the experimental animals before being used for research. Tamayanti *et al.* (2014) and Olaleye *et al*. (2006) states that damage to liver tissue can be caused by two factors, namely internal factors such as endurance and external factors such as the influence of substances or other diseases, feeding and drinking that were not in accordance with standards, conditions of cages that are less ideal and stress factors. Based on Figure 1A. change in the form of albuminosa degeneration in the

cytoplasm which was characterized by turbidity, clots and cytoplasm which mixed.

The change to liver structure in groups of 10 and 90 mg / head in the form of bile ducts that appear dilated, the border of the sinusoid which begins to thin, hydropic degeneration and inflammation. This was due to the influence of the content of compounds present in *A. cordifolia* leaf extract. According to Wijayanti *et al*. (2018), the content of flavonoids, saponins, alkaloids and vitamin C in *A. cordifolia* leaves has a positive effect on kidney *C. cobaya* with a certain dose. Flavonoids as antioxidants can act as an antidote to free radicals (Wijayanti *et al.,* 2017; Mushollaeni *et al*., 2014). Flavonoids function to protect cell membranes from oxidative stress. Flavonoids play a role in breaking the chain of free radical reactions and preventing apoptosis or necrosis of hepatocytes. Flavonoid scavenging activity begins with the administration of hydrogen groups or electrons in free radicals. Giving hydrogen groups to free radicals will produce flavonoid radical molecules and stable molecules (RH). Flavonoid radicals have lower activity than free radicals. The flavonoid radicals will bind to other radicals into non-reactive compounds (Miladiyah and Prabowo, 2015). The nucleus of picnotics was more visibly dense and dark with inflammation/inflammation cells beginning to spread.

Giving of 90 mg/head *A. cordifolia* leaf extract the inflammatory change had widened and thickened (Figure 1D) compared with 10 mg / head (Figure 1B). The difference in the level of inflammation was caused by a dose of administration. Change to liver tissue was caused by exposure to substances that can cause free radicals so that organ cells were changed. Destructive effects in the body will soon be responded to by tissue in the form of inflammation (inflammation) as a form of the body's defense mechanism (Colle *et al*., 2012). Flavonoids can protect the cytoplasmic membrane in the liver tissue from turbidity caused by free radicals (Ajiboye *et al*., 2010). The treatment with 50 mg / head *A. cordifolia* leaf extract (Figure 1C) showed

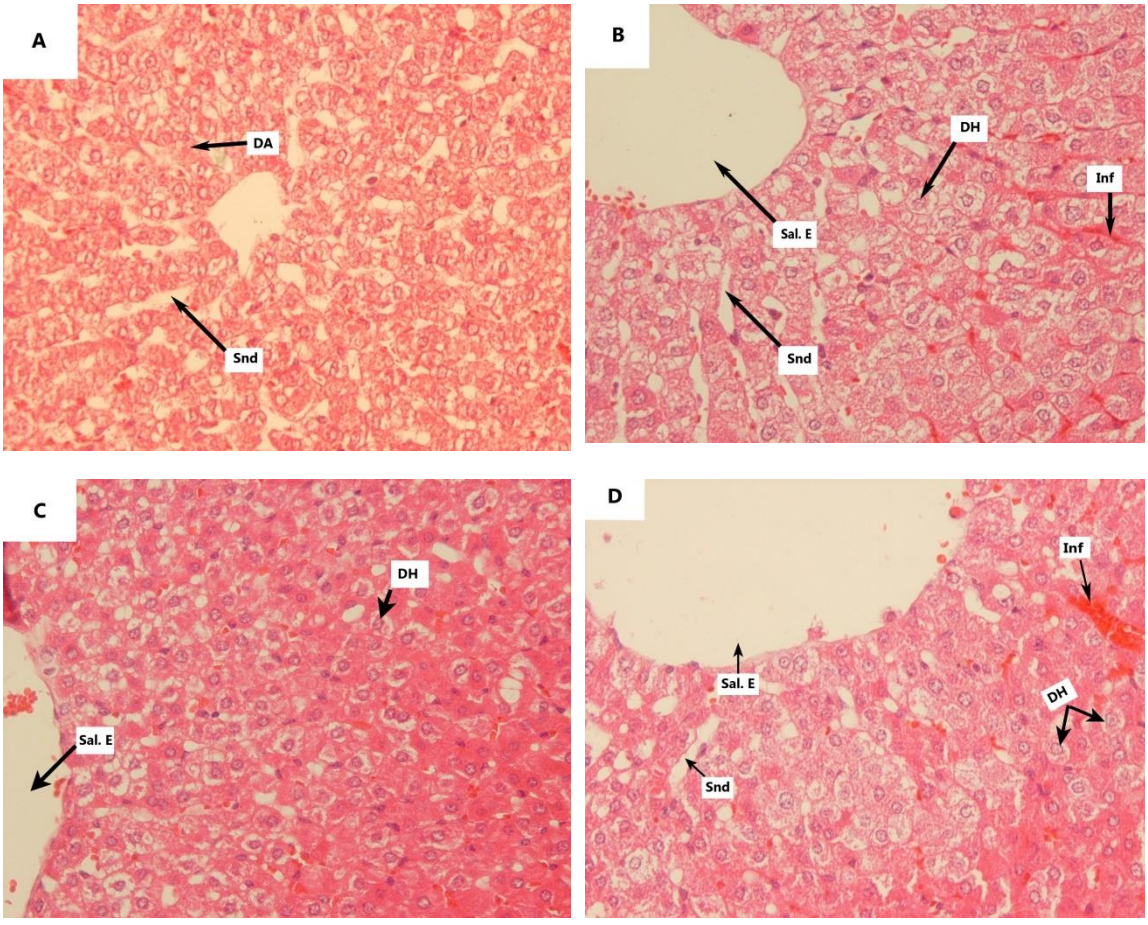

Figure 1. Hematoxylin-eosin stained liver sections (x400) of *C. cobaya*. Snd (sinusoid), sal E (bile duct), VP (portal vein), Inf (periportal inflamation), DA (albuminosa degeneration), DH (hydropic degeneration), Ifn (inflamation), A (0 mg/head): DA and Snd appear with filling granules which are proteins that damage the membrane, B (10 mg/head) : E duct begins to widen and DH appears, namely changes in cell membrane permeability, enlarged cells and vacuoles seen in the cytoplasm, periportal membrane becomes inflamed with tissue freezing, C (50 mg/head) : DH has decreased inflammation in the tissue, D (90 mg/head) : E duct begins to widen, and DH experiences accumulation of inflammation in membrane tissue, and acute inflammation (ifn) with dilation of blood vessels.

that the liver change structure was seen in the bile duct which was dilated and experienced hydropic degeneration. The core of the picnotic begins to thicken, the cytoplasm undergoes vacuolization. There was fluid that settles in the cytoplasm.

The lining of the liver tissue undergoes half-field degeneration. Hydropic degeneration and moderate degeneration in the administration of 10, 50 and 90 mg / head of *A. cordifolia* leaf extract was the effect of the increase in the dose given. According to Hastuti (2013), Hydropic degeneration was in the cytoplasmic condition of the cell containing water and there was cleared spaces in the cytoplasm. Cytoplasm works to regulate the entry of ions. Interruption of the sodium potassium pump when regulating the influx of ions can cause hydropic degeneration (Huang *et al*., 2010). Metabolic disorders in the liver *C. cobaya* also causes hydropic degeneration. Hydropic degeneration was reversible, when exposure to toxic substances was stopped the cells that were changed will return to normal (Tamayanti *et al*., 2014; Yousef *et al*., 2010).

The level of *C. cobaya* change (Table 1) without the administration of *A. cordifolia* leaf extract (0 mg / head) was found that liver cell change was still mild degeneration (DH +). Changes only in certain parts do not meet part of the field of view. *C. cobaya* treatment group which was given of *A. cordifolia* leaves extracts as much as 10, 50 and 90 mg / head experienced moderate degeneration changes (DH ++). Changes in the structure of liver cell histology from mild degeneration to moderate degeneration because there was an increased in the dose of *A. cordifolia* leaf extract given. The histological picture of the administration of 50 mg / head *A. cordifolia* leaf extract showed the lightest hydropic degeneration between administration of 10 and 90 mg / head. Flavonoids were antioxidants and protect cell damage due to free radicals (Miladiyah and Prabowo, 2015). The higher the concentration, the greater the response (therapeutic response and toxic response). Of all the treatments there were no deaths in *C. cobaya*. According to Adedapo *et al.* (2009), acute toxicity test on *M. oleifera* extract did not cause death in animals even at a dose of 2.000 mg / kg.

Flavonoids as antioxidants can counteract free radicals from the compounds of *A. cordifolia* leaf extract. The number of doses given up to 90 mg / head in *C. cobaya* with the emergence of inflammation as a form of defense of the body against attacks of biochemical radiation. Polyphenol compounds such as flavonoids can inhibit oxidation reactions through the mechanism of radical scavenging by donating one electron to unpaired electrons in free radicals so that the amount of free radicals decreases (Olaleye *et al.,* 2006). Flavonoids was thought to have an effect on inhibiting liver damage by binding to free radicals so that the impact on the liver decreases. Free radicals will cause disruption of hepatocyte membrane integrity, which causes the release of various enzymes from hepatocytes so that this was an indicator of liver damage (Saba *et al*., 2010).

Based on Table 2 The weight of *C. cobaya* who was treated with 50 mg/head was not significantly different from the animals that were not treated. However, it was significantly different from the groups giving 10 and 90 mg/head. The weight of the liver is lighter because the liver does not store much toxins. Flavonoids help maintain liver function to keep warding off toxins caused by free radicals and drugs. Heavier organ weights indicate the occurrence of steatosis, which is fatty in liver cells which is seen as a symptom of toxic effects directly (Al-Ashban *et al*., 2010). Changes in heart weight are not always fatal (critical), because the liver is an organ that has tremendous growth capacity (Korani *et al*., 2011).

Vitamin C in *A. cordifolia* leaf extract as an antioxidant can counteract free radicals arising from exposure to excess secondary substances. If this is allowed, malondialdehyde (MDA) will occur in cells due to the free radicals reacting more quickly with cells in the body. According to Shinde *et al*. (2012), the mechanism of action of cellular antioxidants is to interact directly with free radicals, to prevent the formation of reactive oxygen types, to change the type of reactive oxygen to be less toxic and to repair cell damage that arises.

Table 1. Histophatology of liver tissue *C. cobaya* after giving *Anredera cordifolia* leaf extract

Treatment (mg/head)	Liver
0	DH+
10	DH++
50	DH++
90	DH++

DH+ = Liver degeneration I (albuminosa degeneration/mild degeneration); DH++ = Liver degeneration II (hydropic degeneration/moderate degeneration).

Table 2. The average value of the weight of *C. cobaya* liver organs

Treatment (mg/head)	Weight of liver (g)
0	12.75±6.04[a]
10	15.67±5.12[b]
50	12.54±6.23[a]
90	9.33±7.21[c]

The mean ± SD in one column followed by the same superscript letter did not difference significantly at the 95% confidence interval (Duncan Test, p<0.05).

The liver has the ability to regenerate, loss of liver tissue due to the work of toxic substances will spur a mechanism by which liver cells begin to divide. The regeneration process was controlled by substances called khalones which inhibit mitotic division of certain cells (Dollah *et al*., 2013). The amount of khalones produced decreases when the tissue was damaged.

Conclusions

Binahong's (*A. cordifolia*) leaves extract up to the dose of 90 mg/head had no significance toxicity effect on the liver of guinea pigs (*C. cobaya*).

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