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HEPATOTOXIC TEST OF BURDOCK ROOTS (*Arctium lappa L*) ON HISTOPATHOLOGY LIVER OF MALE WHITE RATS (*Rattus norvegicus*)

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Abstract

Burdock (*Arctium lappa L*) is a homologous medical plant that is considered having many advantages as a traditional medicine. Burdock root contains several substances such as inulin, essential oils, tannins, quercetin, iron, and arctigenin. This study was conducted to determine effect of burdock root extract on the liver histopathology of male white rats. This study used an experimental method with 20 rats and was divided into 4 groups. The positive control group was given gentamicin induction intramuscularly. The negative control group was given distilled water. The 150 mg/kgBW dose group and the 450 mg/kgBW dose group were given burdock root extract with treatment 14 days and surgery was performed in the 15th day to take hepatic organs to make histopathology preparations. The results of the study were interpreted by scoring and tabulated, the analysis was carried out descriptively with the results, in positive control group found mild damage, the negative group found no damage, in the extract dose group 150mg/kgBW found necrosis, bleeding and vacuolization, and in the extract dose group 450mg/kgBW found degeneration, necrosis, bleeding and vacuolization. On the other side, the parasitic worm *Taenia taeniaeformis* was found in the liver which led to the interpretation of the results being disturbed. In conclusion, the higher the dose of extract given is toxic to the liver.

Keywords: Burdock root extract (*Arctium lappa L*), liver, histopathology, toxic.

1. Introduction (Book Antiqua 10pt Bold)

Almost every country in the world has accepted the use of herbal medicine. The use of herbal medicine is quite popular, one of which is in Indonesia, where Indonesia is one of the countries that has abundant natural resources in the form of plants that can be utilized as herbal medicines where herbal medicine enthusiasts in Indonesia are still high. One of the plants that are widely spread in Indonesia and considered to have potential as herbal medicine is the burdock plant (*Arctium lappa L*). The plant with the scientific name *Arctium lappa L.* or commonly referred to as burdock, is a family of *Asteraceae* plants that that grow abundantly, especially Asia. Burdock plants are popular around the world because all parts of the burdock plant can be utilized. Burdock roots have several chemical compounds such as inulin, essential oils, tannins, resins, sugars, iron, calcium, quercetin, arctigenin, and vitamin C. Burdock root has traditionally has been used for treating infectious diseases much as sore throats, boils, rashes and various other skin disorders (Gurunanselage Don & Yap, 2019). The utilization of burdock roots (*Arctium lappa L.*) is focused on the roots which have various ingredients that can be antioxidants that function as free radicals and have potential as hepatoprotectors. Burdock roots are made into into extracts and then induced in the appropriate treatment group of rats to determine the activity against hepatotoxicity with histopathological examination as an indicator (Fauziah, 2021). According to

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previous research conducted by Ahmadgarpour et al., (2017) that burdock root has hypoglycemic properties and antioxidant effects, and has been used for the treatment of diabetes in traditional medicines. The study showed that *Arctium lappa* root extract, in a dose-specific manner, has anti-diabetic effects through hypolipidemic and insulinotropic properties. Therefore, this plant extract may be beneficial for the treatment of diabetes. Therefore, this plant extract may be useful in the treatment of diabetes. Burdock plants that are likely to be used as traditional medicine have the potential to be consumed in the short or long term, this will definitely cause side effects on the organs in the human body and one of these organs is the liver. Hepar in the human body functions to neutralize toxins. The causes of damage to hepatic cells include drugs, viruses, and several other compounds that are hepatotoxic (Fitriani et al., 2021).

The liver damage caused by toxic substances is influenced by a few factors, such as the dose given, the type of chemical substance, the length of exposure to the substance such as acute subchronic or chronic, and the higher the concentration/dose of the compound given, an even more toxic response. One such hepatotoxic agent or hepatic damaging agent caused by causing a reaction due to the accumulation of toxic substances in the hepatic system is gentamicin. Gentamicin is a prototype of the aminoglycoside class which is a bactericidal drug and gentamicin is an aminoglycoside that is often used and is known to be toxic to the kidneys (Muda et al., 2020). The study of burdock root is lacking, therefore this study was conducted to find concrete evidence to determine the potential of burdock root extract against hepatotoxicity with supporting examination of hepatic histopathology in male male white rat test animals.

2. Method

Methods used in this research are experimental. Using 20 male white rats divided into 4 groups with each group totaling 5 rats, and given gentamicin antibiotics by intramuscular injection for the positive control group, given aquadest for the negative control group and given burdock root extract preparations orally for the 150 mg/ kgBW dose group and the 450 mg / kgBW dose group, and for 14 days then performed surgery, observed liver histopathology. Data analysis was obtained from tabulated examination data and analyzed descriptively qualitative. The study was conducted for 6 months from December 2023 to May 2024. The research was conducted in the laboratory of the faculty of pharmacy, science and technology, Al-Irsyad University, Ciacap, from making extracts to dissecting experimental animals, and making preparations at BLUD RSUD R. A Kartini Jepara. The research started with the preparation of burdock root extract by maceration method for 3 days. Then the determination of the dose of the drug is divided into 2 groups, namely the dose of 150mg / kgBW and 450mg / kgBW which will be given for 14 days in the form of a suspension solution using a 0.9% CMC-Na mixture orally. The next stage is the preparation of test animals, group division is carried out with Federer's calculation, divided into 4 groups with information: Group 1 (positive control): given gentamicin induction, Group 2 (negative control): given aquadest, Group 3 (low dose): was given a dose of 150 mg/kg BW extract Group 4 (high dose): given an extract dose of 450 mg / kg BW extract. Make sure the test animals weigh 200-350gr and are 2-3 months old. with acclimatization for 7 days before being given treatment. During the 14-day treatment, rats were fed 2 times a day adlibitum, and drank adlibitum. Rat cages were cleaned every 2 days. After 14 days, the rats were dissected on the 22nd day from the time of 7 days of acclimatization and 14 days of treatment. The surgery was accompanied by a veterinarian and the test animals were euthanized (killed) by cervical dislocation (neck dislocation), if it was felt that the test animals were lifeless, the test animals were immediately dissected and the hepatic organs were taken and the hepatic organs were were inserted into the urine cup containing 10% NBF solution to make histopathological preparations.

Histopathology preparations were examined under a microscope each at 8 microscopic field of view. Examination with a microscope was carried out with 100x magnification then continued with 400x magnification (Swarayana *et al.*, 2012). The changes observed were the presence of fatty degeneration, vacuolization, necrosis, and inflammatory cell infiltration. Histopathology

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preparations were observed and scored to obtain quantitative data.

The scoring system used is:
Score 0 = no histopathological damage
Score 1 = there is focal damage (mild)
Score 2 = multifocal damage (moderate)
Score 3 = there is diffuse damage (servere)
(Darmayanti et al., 2020).

3. Result and Discussion

Table 1. Characteristics of experimental animals before and after treatment

Sample characteristics	Before treatment			
	Positive Control	Negative Control	Dose 150mg/kg BW/day	Dose 450mg/kg BW/day
Rat species and strain	*Rattus Norve-Gicus*	*Rattus Norve-Gicus*	*Rattus Norve-gicus*	*Rattus Norve-Gicus*
Sex	Males	Males	Males	Males
Quantity of rats	5	5	5	5
Color	White	White	White	White
General condition	Move actively, healthy	Move actively, healthy	Move actively, healthy	Move actively, healthy

Sample character-istics	After treatment			
	Positive Control	Negative Control	Dose 150mg/kg BW/day	Dose 450mg/kg BW/day
Rat species and strain	*Rattus Norve-gicus*	*Rattus Norve-gicus*	*Rattus Norve-gicus*	*Rattus Norve-Gicus*
Sex	Males	Males	Males	Males
Quantity	2	3	4	3
Color	White brownish	White brownish	White brownish	White brownish
General condition	Move actively	Move actively	Move actively	Move actively

Active movement = Animals are always movinginquisitively

Healthy = The fur is clean, smooth and shiny, there are no fleas, the eyeballs look pink and clear, the mouth does not salivate continuously, the consistency of the feces is normal and solid, not liquid (Solihah & Haris, 2019).

Table 2. Interpretasi Kerusakan Organ Hepar pada Hasil Pewarnaan Hematoxilin Eosin (HE)

Group	observed parameter score					
	Degeneration (Nicrosteosis)	Necrosis	Blockage ofblood vessels	Hemorrhage	Vacuolization	Desc.
Kontrol Positif	0	1,5	0	0	1,5	Worms found in hepar
Kontrol negatif	0	0	0	0	0	Worms found in hepar
Dose 150mg/kgBW/day	0	1,80	0	2,4	3,00	Worms found In hepar
Dose 450mg/kgBW/day	3,00	3,00	0	2,25	2,25	Worms found in hepar

Score description:
0= no damage
1= mild
2= moderate
3= severe

In Table 2. in positive control group found signs of necrosis and vacuolization with a damage score of 1.5 from the damage score obtained it can be said that the positive control group suffered minor damage because the score score of 1.5 entered into score 1. mild damage was found in other damage parameters such as degeneration, blockage of blood vessels or bleeding no change or damage occurred.

While in the negative control no changes or damage to hepatic cells were found as seen from the score obtained in all parameters of damage is 0. Changes in hepatic cells occurred at a dose of 150mg / kgBW / day day did not occur cell changes such as degeneration and blockage of blood vessels, but but in dose group there were changes in hepatic cells in the form of necrosis with a score of 1.80 where the score is still included in the score 1 which means the damage is mild. As for the bleeding damage that occurs in the 150mg/kgBW/day dose group with the damage score is 2.4, the value included in moderate damage and the severest damage in this group is that the hepatic cells experience vacuolization with a damage score of 3.00 which means the level of damage is severe. After being observed in Table 2, the higher the dose given, the greater the damage score obtained, this can be seen in the

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450mg/kgBW/day dose group where the damage parameters occur degeneration and necrosis with a score of 3 which means that the damage is severe, and the bleeding and vacuolization damage obtained a score of 2.25 which means that the damage is included in the the moderate group. However, in the four groups all get results in the form of the discovery of worms in the hepatic organ and it affects the results obtained.

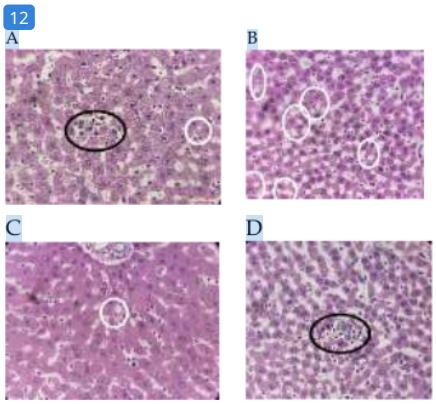

Figure 1. Results of Microscopic Examination of HE Painting Group 1 Positive Control (Gentamicin), magnification 400x

In the results of Figure 1. microscopic examination of hepatic tissue with Hematoxilin Eosin (HE) painting of the positive control group induced by gentamicin, it was found that there was focal necrosis marked with black circles in figures A and D. There was also degeneration marked in white circles in figures B, and C.

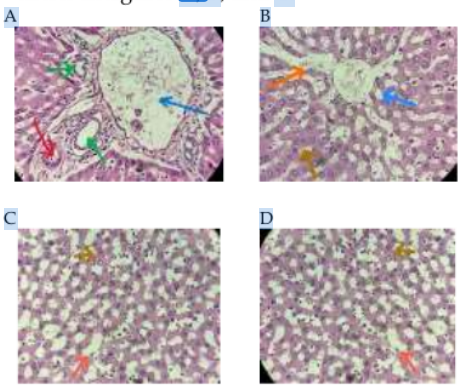

Figure 2. Results of Microscopic Examination of HE Painting Group 2 Negative Control, magnification 400x

In the results of microscopic examination of hepatic tissue with Hematoxilin-Eosin (HE) painting of the negative control group which was only given aquadest alone found Segita portal consisting of portal vein (blue arrow),mductus biliaris (green arrow) and artery hepatica red arrow in Figure A. Vena centralis (blue arrow), sinusoid (orange arrow) which separates the hepatic cell plate (brown arrow) in Figure B. Sinusoid (orange arro hepatic cells (brown arrow) in Figure C and D.

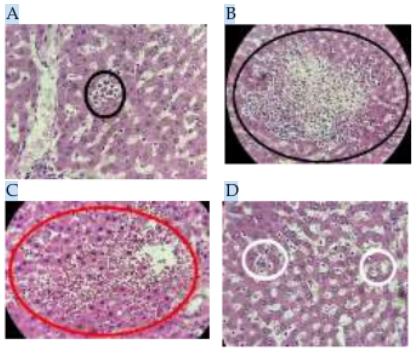

Figure 3. Results of Microscopic Examination of HE Painting Group 3 Dose 150mg/kgBW/day, magnification 400x

In the results of microscopic examination of hepatic tissue with Hematoxilin-Eosin (HE) painting of the 150mg / kgBW dose group found focal necrosis marked with black circles in Figure A and B, found degeneration marked with white circles in Figure D and the presence of bleeding marked with red circles in Figure C.

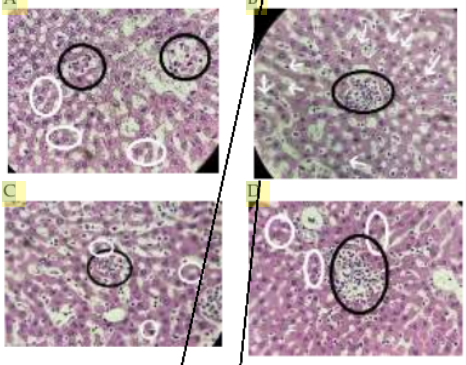

Figure 4. Results of Microscopic Examination of HE Painting Group 4 Dose 450mg/kgBW/day magnification 400x

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In the results of microscopic examination of hepatic tissue with Hematoxilin-Eosin (HE) painting of the 450mg/kgBW/day extract dose group found focal necrosis marked with black circles in pictures A, B, C and D. There was also degeneration marked in white circles in figures A, B, C and D and there was extensive degeneration in figure B marked with white arrows.

The results of microscopic observation of hepatic histopathology of male white rats from the positive control group, negative control, 150mg / kgBW dose, and 450mg / kgBW dose showed some hepatic tissue damage. This is not only related to toxic substances and important for human survival, but also where toxic substances are metabolized and concentrated. The damage seen with the difference in mean interpretation can be seen in Table 2.

Basically every chemical substance must have toxic properties and every poisoning is determined by the dose and method of administration if given continuously or given in excessive doses, the chemical substance will be toxic. Many factors can be used to determine whether the substance is toxic, but the dose is the most important factor, to determine the substance is toxic. The toxic effect is due to the biochemical interaction between the toxicant in the extract and the cells in the organ (Nofrian & Wijayahadi, 2017). Cell damage that occurs is also likely to be caused by the environmental conditions of rats both from cages, food given to test animals that experience stress levels, and other internal factors that are thought to be a factor in the occurrence of hepatic cell damage in experimental animals (Melisa et al., 2022).

From these microscopic observations we know the histopathological picture and changes that occur in the hepatic organs of experimental animals using 400x magnification which can be seen in figures 1, 2, 3, and 4. And in the scoring results in table 2 and the microscopic picture it can be concluded that the greater the dose given, the greater the hepatic damage that occurs in experimental animals. From these microscopic observations we know the histopathological picture and changes that occur in the hepatic organs of experimental animals using 400x magnification which can be seen in figures 1, 2, 3, and 4. And in the scoring results in table 2 and the microscopic picture it can be concluded that the greater the dose given, the greater the hepatic damage that occurs in experimental animals.

In this study also found parasites in the form of *T. taeniaeformis* worms. The *T. taeniaeformis* worm is a type of cestode worm that can live in the hepar of rats at the larval stage and *T. taeniaeformis* forms cysticerci inside or on the surface of the hepar (Lovitasari et al., 2021). Some of the factors that cause this to happen are, Contamination of food, shelter, and water can cause pathogenic infections in laboratory animals, especially rats.

To control *T. Taeniaeformis* infection, animal quarantine programs for laboratory experiments, animal health surveillance programs, and transportation safety considerations are necessary (Chairunnisa et al., 2019).

4. Conclusion and Suggestion

Conclusion

Based on the research that has been done, conclusions and suggestions can be drawn:

1. Not found the right dose variation of burdock root extract (*Arctium lappa L*) as a hepatoprotector.
2. The preparation of burdock root extract (*Arctium lappa L*) is toxic and seen from the results of microscopic observations of hepatic organs, and burdock root extract has tannin content where rodents will experience stress when consuming foods that have high tannin content. And the main factor for the extract to be toxic is that the dose is too high. But there are other confounding factors, namely the parasitic worm *Taenia taeniaeformis* found so that the interpretation of the results is disturbed and biased.

Suggestion

From the conclusions obtained from the research conducted, there are several suggestions, such as:

1. Looking for more dose variations to get the right dose.
2. Researchers must pay attention to the process of making extracts and the condition of experimental animals during treatment.
3. Further research needs to be done using biochemical parameters to determine the

therapeutic effect of burdock root extract as well as conducting chemical parameters to see transaminase enzyme levels and antioxidant enzyme levels so that more complete data is obtained about the hepatoprotector function of burdock root ethanol extract.
4. Further testing needs to be done with a longer period of time to ensure that burdock roots are safe for consumption in the long term.

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Confused You have used either an imprecise word or an incorrect word.

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Word Error Did you type **the** instead of **they**, or have you left out a word?

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