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TOXICITY TEST OF INGGU (*Ruta Angustifolia (L))* ETHANOL LEAVES EXTRACT TO MALE WHITE MICE (*Mus Musculus*)

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Abstract:

Inggu leaf (Ruta angustifolia (L.) is one of the medicinal plants that is widely used by Indonesian people as traditional medicine for various diseases. One of the properties of the inggu plant can be used to treat fever, toothache, heartburn and ulcers. Toxicity testing ethanol extract of guinea leaf using a completely randomized design (CRD) with 7 treatments with stratified doses of 500, 1000, 2000, 4000, 8000, 16,000 mg/kg BW and control. Parameters observed after administration of the extract were diarrhea, changes in breathing, changes in aggressive behavior and decreased movement activity. Liver and kidney organs were taken to determine the organ weight ratio. The results showed that the ethanol extract of inggu leaves with graded doses up to a dose of 16,000 mg/kg BW in experimental animals did not cause death, which was included in the practically non-toxic category. Administration of ethanol extract of guinea leaves at a dose of 500 mg/kg BW to 16,000 mg/kg BW caused a decrease in locomotion activity in experimental animals during the 4-hour observation time. The ratio of organ weight of mice from the test results of the ratio of liver, right kidney and left kidney of mice was not different from that of control animals

Keywords : Inggu leaf (*Ruta angustifolia L*), extract ethanol, toxicity, phytochemical, male white mice (*Mus muculus*)

1. Introduction

The use of plants as traditional medicine to treat various diseases is believed by people in Indonesia. Traditional medicine has several advantages, one of which is that it can minimize reactions and effects (Safitri, 2016). One of the plants used as medicine is inggu leaf (*Ruta angustifolia (L.)*) empirically has long been trusted and used by the public as a medicine for various diseases. Diseases that are believed to be overcome with inggu leaf ingredients include dental disease, fever, seizures in children, heartburn, stimulate menstruation, suffocation, headaches and ulcers. The part most widely used as traditional medicine is in the leaves (Noer S and Pratiwi, 2016). Phytochemically the secondary metabolite content in inggu leaves is flavonoids as quarcetin , tannins and saponins and quinones that have the potential to be used as medicinal ingredients (Noer, S et al, 2017).The results of the analysis show that quantitative assays for the total content of flavonoids, tannins, and saponins in guinea leaf extract are 1, respectively. 67%, 7.04% and 2.13%.

Noer, S et al, 2017 have tested the antioxidant activity and antibacterial test of fusobacterium from ethanol extract of guinea pigs against dental disease caused by fusobacterium nucleatum bacteria. The test value showed IC50 of 100.99 ppm, the results showed that the antioxidant levels in inggu leaves had moderate activity with an inhibition zone of 40%. Based on research by Armansyah et al, (2017), plants containing flavonoid and tannin compounds in the ethanolic extract of malacca leaves (Phyllantus emblica) do not have the potential as acute toxicity compounds. Therefore, it is possible that inggu leaf extract (Ruta angustifolia (L.) also does not have a toxic effect.

One of the initial parameters needed to evaluate the safety of a drug is the potential acute toxicity of the drug or traditional herb. A compound can have the potential for toxicity in the body at

certain doses. Acute toxicity test is one of the toxicological evaluations of herbal extracts carried out before clinical trials (Sadashiv, 2010).

The purpose of acute toxicity tests is to detect the presence of toxicity of a substance, determine target organs, and their sensitivity to obtain hazard data after acute administration of a compound (Makiyah and Tresnayanti, 2017). Toxicity test is divided into two, namely general toxicity test and specific toxicity test. The general toxicity test consisted of an acute toxicity test which was carried out for 24 hours, a subchronic toxicity test which was carried out for 26 weeks and a chronic toxicity test which was carried out for 1 year. Specific toxicity tests consist of teratogenic tests or abnormalities in the fetus, mutagenic tests or tests carried out by changing DNA information and carcinogenic tests (Raina P, et al. 2015). Acute toxicity test is a pre-clinical test that aims to measure the degree of toxic effect of a compound within a certain time after giving a single dose (Syamsul et al, 2015).

The purpose of this study was to determine the LD50 of the ethanol extract of guinea pigs against white male mice. The LD50 test is used to determine the level of toxicity of natural ingredients. The LD50 value can provide information as a basis for considering the plant as a medicinal ingredient.

2. Methods

a. Types of research

This research is an experimental laboratory using a completely randomized design (CRD). Seven treatments (K, P1, P2, P3, P4, P5, and P6) with 3 repetitions, each replication unit consisted of 2 male white mice.

b. Research procedure

Simplicia Powder Making

Making simplicia that goes through the stages of wet sorting aims to separate dirt or foreign materials that stick. Then it was washed with running water to remove impurities that were still attached to the wet-sorted material. Then dried using an oven at a temperature of 50 ° C until completely dry and dry sorting will be carried out to separate the dirt that is still attached. Furthermore, the simplicia which was completely dry was ground using a grinder to obtain a powder.

Making Inggu Leaf Extract

Extracts were made from dry simplicia powder by maceration using 70% ethanol. A total of 672 g of simplicia was macerated with ethanol for 24 hours and repeated 2 times until an ethanolic extract was obtained. The macerate was collected and evaporated with a rotary vacuum evaporator to obtain a thick extract.

Characterization of Ingu Leaf Ethanol Extract

Extract characterization was carried out including examination of non-specific parameters (drying shrinkage, ash content) and phytochemical screening.

Preparation of 0.5% Na-CMC Colloidal Solution

Prepared Na-CMC then weighed as much as 0.5 g and then developed in distilled water heated at 60 °C as much as 10 ml (20 times the weight of Na-CMC) for approximately 15 minutes then homogenized and the solution made up to 100 mL.

Test Animal Grouping

Mice were divided into 7 treatment groups, each group consisted of 6 mice with 3 repetitions. Animals in 1 group are placed together in 1 cage. Group 1 as control, group 2-6 as treatment group. Then in groups 2 to 6 were given ethanol extract of inggu leaves orally according to the dose levels, namely:

Control group (K) : given 0.5% Na-CMC
Treatment group 1 (P1) : ethanol extract of guinea leaves 500 mg/kg BW
Treatment group 2 (P2) : ethanol extract of guinea leaves 1000 mg/kg BW
Treatment group 3 (P3) : ethanol extract of guinea leaves 2000 mg/kg BW
Treatment group 5 (P4) : ethanol extract of guinea leaves 4000 mg/kg BW
Treatment group 5 (P5) : ethanol extract of ginggu leaves 8000 mg/kg BW
Treatment group 6 (P6) : ethanol extract of guinea leaves 16,000 mg/kg BW

Acute Toxicity Testing on Test Animals

Determination of LD50 and observation of toxicity of ethanol extract of inggu leaf (*Ruta angustifolia (L.)* in test animals that have been grouped given suspension and extract orally with a single predetermined dose. The volume of administration is 1% of the animal's body weight. Toxic effects were observed. compared with control. The observation time was 5 minutes, 10 minutes, 15 minutes, 30 minutes, 60 minutes, 120 minutes, 180 minutes and 240 minutes. So the total observation time was 4 hours. The criteria for observing toxic effects included: aggressive behavior, diarrhea, changes in breathing, urination, excessive salivation, and decreased movement activity.The LD50 value was calculated based on the number of mice that died in each treatment group for a span of 7 days.

Determination of Organ Weight Ratio

Test animals were sacrificed after treatment after 7 days and then vertically dissected on the abdomen. The liver and kidneys were taken and cleaned with filter paper and then weighed. Furthermore, the ratio of organ weight to body weight is determined using the equation.

3. Result and Discussion

The process of extracting inggu leaves obtained a thick extract of 120 g with a yield value of 28%. The ethanol extract of guinea leaf was then analyzed for its specific and non-specific parameters. Analysis of non-specific parameters determined is the determination of drying shrinkage and determination of ash content. Determination of drying shrinkage aims to show how many compounds are contained in the extract and are lost or easily evaporated in the drying process. The results of the shrinkage weight of the ethanol extract of inggu leaves (Ruta angustifolia (L) is 30.76%. The determination of the ash content aims to provide an overview of the internal and external mineral content and inorganic content from the initial process until the extract is formed (Depkes RI, 2000). The value of the ash content obtained is 12.04%. The ash content depends on the type of material, the method of ashing, the time and temperature used during drying. The results of the non-specific characterization of the ethanol extract can be seen in Table 1.

Table 1. Non-specific parameters of guinea leaf ethanol extract

Parameter	Value (%)
Drying shrinkage	30,7
Ash content	12

Chemical Compounds Ethanol Extract of Ingu Leaves (Ruta angustifolia (L.)

Phytochemical test results are used to determine the secondary metabolites contained in a sample extract. The phytochemical results of inggu leaf ethanol extract can be seen in Table 2. From the secondary metabolite analysis of inggu leaf ethanol extract, it can be seen that inggu leaf ethanol extract contains secondary metabolites such as flavonoids, saponins, tannins, steroids, alkaloids and phenolics. The secondary metabolite results obtained are the same as those reported by Noer, S., 2017.

Table 2: Phytochemical screening of ethanol extract of guinea leaves.

Phytochemical Test	Reactor	Result	Description
Flavanoid	Concentrated Mg+ + HCl Powder	Formation of red, yellow or orange color	Positive
Saponin	Hot Water + HCl 2N	Formed a layer of foam 1 cm	Positive
Tanin	Aquades + $FeCl_3$	Formed a blackish green color	Positive
Steroid	Acetic acid + sulfuric acid	Formed a blackish green color	Positive
Alkaloid	Mayer Dragen droff	A white precipitate is formed	Positive

Phenolic	Mayer	A white precipitate is formed	Positive

Acute Toxicity Testing Ethanol Extract of Inggu Leaves (Ruta angustifolia (L.)

Toxicity test is a test to determine the toxic effect of a substance on a biological system and to obtain typical dose response data from the test preparation. The data obtained can be used to provide information about the degree of danger of the test preparation in the event of exposure to humans. Toxicity test using test animals as a useful model to see the presence of biochemical, physiological and pathological reactions in humans to a test preparation, the results of the toxicity test cannot be used absolutely to prove the safety of a substance or preparation in humans, but can provide an indication of the relative toxicity and help identify toxic effects in the event of human exposure (BPOM, 2014).

In acute toxicity testing, the test animals used were Swiss Webster strain mice (Mus musculus) because they have several advantages, namely, being more economical, small in size, and on a physiological basis close to humans, which are both mammals. The mice used were mice with an age of 2-3 months and a weight of 20-30 g. The mice used were healthy, naive, aggressive mice with normal behavior during acclimatization time.

Mice Acclimatization

The results of the measurement of the average body weight of mice at the beginning (before acclimatization) and day 7 (after acclimatization) are given in Table 3. From the results of acclimatization of mice for 7 days, it can be seen that there was an increase in the average body weight of mice after acclimatization. The purpose of acclimatization is to uniform the way of life and diet of mice used in the study. The same results were also obtained by Sudjana et al (2020) which showed that 5 days of acclimatization there was an increase in body weight with the same profile in all dose groups of test animals.

Determination of Acute Toxicity

According to Cotoras et al, 2014, regulation of toxicity testing with experimental animals must pay attention to animal welfare, using as few animals as possible. Acute toxicity testing is carried out to obtain information or data on the toxicity of a compound in test animals. The results of the percentage of deaths of test animals after administration of inggu leaf ethanol extract can be seen in Table 4. In addition to observing the number of animals that died in the acute toxicity test of inggu leaf ethanol extract (Ruta angustifolia (L)) observations were also carried out by looking at qualitative parameters such as aggressive behavior, diarrhea, changes in breathing, urination, salivation, and decreased movement activity.

Table 3. Average Body Weight of Mice During Acclimatization.

Group	Weight Average of mice		
	Before Acclimatization	After Acclimatization	Weight (%)
K	20,66	23,16	12,10%
P1	23	25,5	10,80%
P2	22,83	25,66	12,30%
P3	22,16	25	12,80%
P4	22,16	24,66	11,20%
P5	23,33	25,83	10,70%
P6	21,83	24,83	13,70%

Table 4. Results of Percentage of Animal Mortality

Group	Number of Animals	Dose (mg/20g BB)	Number of Dead Animals	% Death
Control	6	0	0	0
P1	6	10	0	0
P2	6	20	0	0
P3	6	40	0	0
P4	6	80	0	0
P5	6	160	0	0
P6	6	320	0	0

Aggressive behavior is behavior that is carried out to hurt or against objects that are around both physical and non-physical. According to Iwan et al, aggressive behavior is caused by stimulation of the central nervous system or the sympathetic nervous system and if there is a decrease in depression, if the behavior looks aggressive, this indicates the presence of central stimulation (CNS) or sympathetic stimulation. In the acute toxicity test of the ethanol extract of guinea pigs (*Ruta angustifolia (L*) it is listed in Table 5 above that in the control group and the treatment group with graded doses with an observation time of 4 hours there was no reaction that occurred in aggressive behavior in test animals, this shows that the ethanol extract of guinea leaves did not affect the test animals on the central nervous system

Table 5. Number of Mice Experiencing Aggressive Behavior.

Group	Treatment	Minute							
		5	10	15	30	60	120	180	240
K	Na CMC	0	0	0	0	0	0	0	0
P1	Dose 500 mg/kgBB	0	0	0	0	0	0	0	0
P2	Dose 1000 mg/kgBB	0	0	0	0	0	0	0	0
P3	Dose 2000 mg/kgBB	0	0	0	0	0	0	0	0
P4	Dose 4000 mg/kgBB	0	0	0	0	0	0	0	0
P5	Dose 8000 mg/kgBB	0	0	0	0	0	0	0	0
P6	Dose 16.000 mg/kgBB	0	0	0	0	0	0	0	0

In the acute toxicity test of the ethanol extract of inggu leaves with qualitative observation data, namely respiratory changes did not experience respiratory problems after treatment to the highest dose of 16,000 mg/kgBW with an observation period of up to 320 minutes or 4 hours, the test animals did not show this behavior, it can be said that the guinea leaf extract does not affect the test animal to show or interfere with the breathing of the test animal. According to Ikeda et al, 2018, the regulation of breathing is located in the medulla oblongata and the lower part of the brainstem and spinal cord. So that by giving ethanol extract of ginggu leaves with graded or varying doses, the extract may not have an effect on the medulla oblongata and brain stem and spinal cord so that the results given do not affect breathing so that there is no change in breathing in the test animals. The results of testing the number of mice experiencing respiratory changes can be seen in Table 6.

Table 6. Number of Mice Experiencing Respiratory Changes

Group	Treatment	Minutes-							
		5	10	15	30	60	120	180	240
K	Na CMC	0	0	0	0	0	0	0	0
P1	Dose 500 mg/kgBB	0	0	0	0	0	0	0	0
P2	Dose 1000 mg/kgBB	0	0	0	0	0	0	0	0
P3	Dose 2000 mg/kgBB	0	0	0	0	0	0	0	0
P4	Dose 4000 mg/kgBB	0	0	0	0	0	0	0	0
P5	Dose 8000 mg/kgBB	0	0	0	0	0	0	0	0
P6	Dose 16.000 mg/kgBB	0	0	0	0	0	0	0	0

The next observation was to determine the number of mice that experienced a decrease in movement activity at a certain time. The results of determining the number of mice that experienced a decrease in movement activity can be seen in Table 7.

Table 7 shows that the mice experienced a decrease in movement activity. The decrease in movement activity with a dose of 500 mg/kg BW occurred at the 30th and 60th minutes, at 120 minutes the mice did not show a decrease in movement activity behavior then at 180 and 240 minutes there was a decrease in movement activity. At a dose of 1000 mg/kg BW toxic symptoms were observed in the 30th minute the number of mice increased per minute with a decrease in movement activity up to the 240th minute. Furthermore, at a dose of 2000 mg/kg BW, the mice also began to experience a decrease in locomotion activity at the 30th minute. symptoms increased to 3 mice, previously only 1 mouse, the decrease in activity continued until the 240th minute. Then at a dose of 4000 mg/kg BW, a decrease in movement activity was observed at the 30th minute and continued to increase until the 240th minute. At a dose of 8000 mg/kgBW there was a decrease movement activity at minute 5, the longer the observation of the mice decreased movement activity until the 240th minute. And at a dose of 16,000 mg/kgBB seen at 5 minutes and an increase in the number of animals experiencing toxic symptoms at 5 minutes, then 10 minutes It can be seen that 6 mice experienced a decrease in locomotion activity and then almost all animals experienced a decrease in locomotion activity until the minute to 240 pa and a dose of 16,000 mg/kg BW. Symptoms of decreased movement activity is a manifestation of sedative activity, central nervous depressants, muscle relaxants, paralysis or anesthesia. Decreased movement activity in mice can occur due to disturbances in the nervous system that controls movements such as hands and feet. This allows the ethanol extract of inggu leaf to have a calming effect, it is shown that the higher the dose given, the faster the sedative effect caused by the inggu leaf extract to experimental animals.

Table 7. Number of Mice with Decreased Movement Activity

Group	Treatment	Minutes							
		5	10	15	30	60	120	180	240
K	Na CMC	0	0	0	0	0	0	0	0
P1	Dose 500 mg/kgBB	0	0	0	1	1	0	2	1
P2	Dose 1000 mg/kgBB	0	0	0	1	3	5	5	6
P3	Dose 2000 mg/kgBB	0	0	0	3	5	3	5	5
P4	Dose 4000 mg/kgBB	0	0	0	3	4	4	5	6
P5	Dose 8000 mg/kgBB	1	2	4	4	5	5	5	6
P6	Dose 16.000 mg/kgBB	3	6	5	5	4	6	6	6

The next observation was to see the weight of the mice before taking the liver and kidneys. Before sacrificing or taking the liver and kidneys of mice, the weight of the mice was first weighed or the weight of the mice, the results of the weight obtained were used as a calculation of the ratio of the

weight of the liver, right and left kidneys of mice. The results of the weight of the mice before the organs were taken can be seen in Table 8 and the average results of the weight of the liver, right and left kidneys can be seen in Table 9.

Table 8. Body weight of mice before liver and kidneys were taken

Group	Weight Avarage of Mice (g)
K	27,6
P1	28,83
P2	29,66
P3	26,66
P4	26
P5	27,16
P6	27,5

Table 9. The results of the average weight ratio of the liver, right kidney and left kidney

Treatment Group	RRBO Heart ±SE	RRBO Kidney (Right)±SE	RRBO Kidney (Left)±SE
K	0,0583a±0,0013585	0,0069a±0,0007277	
P1	0,0616a±0,0024983	0,0075a±0,0004312	0,0068a±0,0006292
P2	0,0644a±0,0027759	0,0074a±0,0002724	0,0076a±0,0003497
P3	0,0599a±0,0004885	0,0072a±0,0008444	0,0066a±0,0003244
P4	0,0646a±0,0011139	0,0079a±0,0006006	0,0073a±0,0007308
P5	0,0609a±0,0043567	0,0069a±0,0007725	0,0067a±0,0004422
P6	0,0658a±0,0019854	0,0072a±0,0007106	0,0069a±0,0004888

REBO = Average ratio of liver weight; SE = Standard error

In the quantitative data of acute toxicity test, namely determining the ratio of the weight of the liver, right kidney and left kidney, experimental animals were sacrificed 7 days after treatment by observing delayed toxicity then the test animals were dissected and liver and kidney organs were taken to see the ratio of organ weight. The liver is the main site of drug metabolism, the largest being located under the ribs. According to Tappi et al, 2013, the liver is an important organ for the body and is the center of the body's metabolism, the liver's metabolic process will detoxify toxic materials, but this process can produce more toxic metabolites. The liver is often the target organ because most of the toxicants enter the body through the gastrointestinal system and after being absorbed, the toxicants are carried by the portal vein to the liver, about 80% of the blood in the liver comes from the portal vein, so the liver becomes the target organ for toxic compounds in the body. The average weight of the liver in mice ranges from 1-2% of the total body weight. Kidneys are the site of excretion in the body through which large amounts of blood pass. Therefore, the kidneys are one of the target organs in determining acute toxicity tests. Kidneys are vital organs that play an important role in maintaining a stable environment in the body. The main function of the kidney is to produce urine as a pathway for the excretion of most toxic compounds, so that the kidney is the target organ for toxic effects. The kidneys regulate the body's fluid, acid-base balance by filtering blood, electrolytes and non-electrolytes and excreting excess as urine. The average weight of the kidney organs in mice ranges from 0.1-0.2 g. The results of the research by Gautam and Goel., (2014) also showed that there were changes in renal histology and serum creatinine levels in mice given 50% ethanol extract of basil leaves at doses of 200, 600, and 2000 mg/KgBW. Changes in the histopathological picture of the kidneys on exposure to ethanol extract of basil leaves can be caused by phytochemical compounds, including flavonoids. Flavonoid compounds are compounds that have high antioxidant properties so that they can slow down or prevent molecular oxidation (Shirzad et al., 2011).

4. Conclusion

1. Administration of ethanol extract of guinea leaves up to a dose of 16,000 mg/kg BW did not cause death in experimental animals so that the LD50 value obtained was greater than 16,000 mg/kg BW and was included in the non-toxic category.
2. Administration of ethanolic extract of guinea pigs with a gradual dose of 500-16,000 mg/kg BW can cause a decrease in motor or movement in experimental animals which is characterized by a depressant effect and has no effect on the ratio of liver, right kidney and left kidney weight in mice.

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