The Test of Improvement Activity on Kidney Function Lansau Etanol Extract by Muna Regency on Male Wistra White Rat

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Abstract

Background: Lansau is one of the traditional ingredients that consist of 44 types of plants that are believed by the local society as a nutritious herb to treat various diseases that exist in the society of Muna Southeast Sulawesi Province that has been used for hundreds of years for generations. Methods: The study was conducted to determine the effect of ethanol extract of Lansau on the improvement activity of kidney function that induced gentamicin-piroxicam. A total of 24 rats were used in which 20 rats were modeled with damage of renal function using gentamicin-piroxicam drug which was then grouped into 5 groups. The first group was treated using a Lansau extract with dose I, the second group was treated with Lansau extract dose II, the third group was treated with the Lansau extract dose III, the fourth group was treated with ketotestyl, the fifth group was no treatment only given Na. 1% CMC and the sixth group is the normal group. The Modeling was performed for 7 days and on the eighth day blood sampling was taken to measure creatinine and urea levels. As for therapy performed for 4 weeks and every end of the week also made blood taking for measurement of creatinin and urea. Results: The highest percentage of creatinin decrease for each group was negative group -8.92%, positive group control was 84.21%, dose group I 74.75%, dose group II 80.07%, and dose group III 84.08%. For the highest percentage of decrease of ureum level for each group that is negative group 8.92%, positive control group was 84.21%, dose group I 74.75%, dose group II 80.07%, dose group III 84.08%. Conclusion: The results showed that Lansau ethanol extract has the potential and effectiveness in the repair of kidney function damage.

Keywords: Lansau, Creatinine, Ureum, Gentamicin, Piroxicam

1. Introduction

The existence of 370 indigenous peoples in Indonesia with its diversity of customs and cultures also provides benefits for the treasures of ethnomedicine and the culture of the nation. The Differences of cultures and customs among the ethnic groups in Indonesia is a cultural treasure of the nation that is priceless. One of the wealthy of Indonesian ethnomedicine that is Lansau plant herb. Lansau is one of the traditional ingredients that is believed by the local society as a nutritious herb that exist in the community of Muna Region in Southeast Sulawesi Province that has been used for hundreds of years. The herb plant of Lansau consists of 44 types of plants that have a variety of properties to treat various diseases that have been trusted and used for generations. The Efficacy of Lansau traditional herbal medicine empirically has many benefits of prevention and treatment for disease. One of the efficacy of this Lansau plant herb is to treat kidney failure [1].

The chronic kidney disease is one of the diseases that has a high risk of morbidity and mortality in the world and increasing in number every year. The patients with chronic kidney disease each year had increased and cause more patients should do dialysis therapy[2]. The prevalence of CKD (Chronic kidney disease) is significant, ranging from 2.5% to 11.2% of adults in Europe, Asia, North America and Australia. In the United States the prevalence is reported to be 13.7% between 2007 and 2012 and the estimated prevalence will increase to 14.4% by 2020 and 16.7% by 2030 [17]. In Indonesia the prevalence of kidney failure based on data released by PT. Askes in 2010 the number of patients
with kidney failure is 17,507 people. Then it increased again by about five thousand more in 2011 to 23,261 patients. In 2012 there was an increase of 24,141 patients, increasing only 880 people. According to the Kidney Care Foundation (Yagudi), currently in Indonesia there are 40,000 patients with chronic renal failure (GGK). But of that number, only about 3000 patients who usually enjoy the service of dialysis or hemodialysis [2].

Several studies on experiment of improvements in kidney failure by utilizing plants have been done. The mechanisms that may be antioxidant, anti-inflammatory, vasodilating and diuretic activity in the Nephroprotective effect on Azima tetracantha root ethanol extract are phytochemical compounds such as flavonoids, terpenoids, tannins, glucosinolates and saponins [3]. Based on the results) it is known that in ingredients Lansau plant consists of 44 types of plants there are also compounds such as alkaloids, flavonoids, terpenoids and tannins [4]. Therefore, the researcher is interested to do the improvement activity of kidneys function of Lansau ethanol extract typical of Muna region on male wistar white rat induced by gentamicin and piroxicam.

2. Metode

This study has been approved by the research ethics commission of health, the institutes of research and devotion to society Halu Oleo University for health research using animal experiment as subject of research with number: 2123/UN29.20/PPM/2017.

2.1 Materials

The research material was obtained from Batalaiworu Subdistrict, Muna District, Southeast Sulawesi Province. The preparation is done by chopping samples that have been cleaned, then dried and mashed to become powder. The powder of each Lansau plant was weighed 500 grams and put into a 50 ml round flask each and then immersed with ethanol solvent of ± 700 mL. The separation of residue and filtrate is done by filtration. The filtrate was concentrated in evaporatory using a rotary vacuum evaporator at 50 °C until an ethanol extract of each Lansau plant was obtained and the rendamen value was calculated.

2.2 Acclimatization of Animal Experiment

The experiment of animal used was white male rat wistar strain obtained from rat breeder in Makassar city of South Sulawesi Province. Before treatment, the wistar rat were adapted for 7 days. During adaptation, wistar rat, given standard feed and aquadest drinking and observation of behavior and health conditions. Weighing is done at the beginning and end of the adaptation period [5].

2.3 Manufacture of an Lansau extract

The research material was obtained from Batalaiworu Subdistrict, Muna District, Southeast Sulawesi Province. The preparation is done by chopping samples that have been cleaned, then dried and mashed to become powder. The powder is then weighed and its weight is obtained. The powder of each Lansau plant was weighed 500 grams and put into a 50 ml round flask each and then immersed with ethanol solvent of ± 700 mL. The separation of residue and filtrate is done by filtration. The filtrate was concentrated in evaporatory using a rotary vacuum evaporator at 50 °C until an ethanol extract of each Lansau plant was obtained and the rendamen value was calculated.

2.4 Modeling of test animals

Modeling of kidney failure was performed in rat in excess amount of 30 rat. The animals test were induced by nephrotoxic drugs by combining an injection gentamicin drug with a dose of 100 mg / kg bw / day and a piroxicam oral suspension at a dose of 3.6 mg / kg bw / day until day 7 [16].

2.5 Grouping of animals test

The animals had tried to be grouped into 6 groups where each group consists of 5 experimental animals. The group consisted of a normal group, a negative control group, a positive control group, dose group I of Lansau (6.907 mg / kg bb), dose group II of Lansau (13.814 mg / kg bb), and dose group III of Lansau (27,628 mg / kg bb).
2.6 Test of improvement of kidney function

2.6.1 Blood Sampling

On the 8th day the induction was done by taking blood samples. Blood sampling is also performed at 24 hours after the 1st, 2nd, 3rd, and 4th week of therapy. The sample used for the evaluation of kidney function is a blood sample taken through the cutting edge of the rat tail. Rat are fed into the restrainer, then the tail of the rat to be cut, that given the alcohol. The rattail is cut approximately 5 mm long using scissors, then the blood is collected in an Ependorf tube. The blood is then centrifuged for 10 minutes at 10,000 rpm. The supernatant is a serum that separated from the bottom. The obtained serum was used to measure the levels of creatinine and urea using a specific reagent kit [6].

2.6.2 Measurement of serum creatinine levels

Serum creatinine levels were determined using the Jaffe kinetic method. A total of 25 μL serum was directed with 125 μL R1 reagent. Serum and R1 reagents are mixed and waited for up to 4 minutes 43 seconds. R2 reagent was then added as much as 125 μL. After homogeneous mixture, incubated for 24 seconds. Then measured the absorbance at 505 nm wavelength for 106 seconds [7].

2.6.3 Measurement of serum urea level

Serum urea level was measured by reacting R1 200 μL serum reagent with 50 μL R2 reagent. Then the mixture was incubated for 25 seconds at 37ºC, then added with serum of 2.5 μL. Absorbance is measured with a wavelength of 340 nm between 25 seconds and 75 seconds.

2.7 Analisis Data

To know the difference of influence of ethanol extract of Lansau with various dose to effect of kidney function improvement used by test of two way parametric analysis of Anova followed by LSD test and t-test

3. Result and Discussion

3.1 Modeling of animals test

To obtain animals test that have decreased kidney function, modeling of animals test by giving gentamicin drug combined with piroxicam. The dose of gentamicin used was 100 mg / kgbb while the dose of piroxicam used was 3.6 mg / kgbb. Before the animal test modeling was done, the measurement of creatinine and ureum was done by taking serum by blood. It aims to determine the baseline level of the animals test and to ascertain the presence or absence of kidney function in all rat. Measurement of creatinine and ureum levels as an indicator of decreased kidney function due to abnormal levels of ureal and creatinine indicates impaired kidney function [5]. Selection of creatinine and urea measurements through serum is the reason for the ease of sampling where blood collection is easier than urine is difficult to ascertain the time of expenditure. The results showed that all rat had normal creatinine and urea levels that could be used as animals test. Modelling is done by intraperitoneally administered gentamicin (i.p) and piroxicam given orally. Drug inducer is given once a day for 7 days. On the 8th day blood sampling is done to measure creatinine and urea levels. It aims to determine whether or not successful modelling is done by loading using gentamicin-piroxicam inducer. The result of the measurement showed that there was an increase of creatinine and uream level after modelling compared to baseline level where for the average creatinine level of 0.5 mg / dL while for the mean after average modelling 4.92 mg / dL and for the initial level of uream an average of 19.11 mg / dL while for the mean after modelling level was 56.48 mg / dL.

| Paired Differences | Mean | Std. Deviation | Std. Error Mean | F | Sig. (2-tailed) |
|-------------------|------|----------------|-----------------|---|----------------|
| Pair 1 | Kreatinin<sub>initial</sub> - Kreatinin<sub>induced</sub> | 4.47444 | 3.13529 | 0.73899 | 6.0336 | 6.055 | 7 | 000 |
| Pair 2 | Ureum<sub>initial</sub> - ureum<sub>induced</sub> | 37.3700 | 21.26020 | 5.01108 | 47.942 | 26.797 | 7 | 000 |
Increased levels of creatinin and urea after giving of gentamicin-piroxicam drugs were also proven by statistical testing using the t-test method (Appendix 8). The test result shows the sig: p ≤ 0.01 so it can be said that there is significant difference between the initial level and the level after the modeling. This indicates that the modeling was successful.

The decreased of kidney function can occur because gentamicin which is an aminoglycoside class antibiotic has effects of nephrotoxicity or irreversible ototoxicity. This can be due to the accumulation of gentamicin and the retention of the drug in the proximal tubule resulting in the ability of the tubules to excrete metabolic waste in the blood to decrease [8]. Piroxicam used also causes damage to kidney function. Piroxicam is a class of NSAID that inhibits the activity of COX enzyme (cyclooxygenase). If COX-1 is inhibited then homeostasis is impaired because prostaglandins are inhibited so that blood flow to the kidney decreases. The decreased blood flow causes kidney cells lack of oxygen supply (hypoxia) so that kidney cells will be degraded or damaged [9]. Therefore, the use of a combination of gentamicin-piroxicam as a nephrotoxic agent will increase the impairment of kidney function.

3.2 The examination activity of Lansau extract

The parameters that used in this study are creatinine and urea. In the test, variation of dose ie Lansau dose I (6.907 mg / kgbb), Lansau dose II (13.814 mg / kgbb), and Lansau dose III (27.628 mg / kgbb). As a positive control was given by ketostreril with a dose of 53.03 mg / kgbb and a negative control was given Na. CMC 1%. Giving extract in animal test conducted for 4 consecutive weeks orally once per day. Then the observations were performed 24 hours after the 1st, 2nd, 3rd, and 4th weeks of therapy by rat blood sampling in order to see whether there was any improvement in kidney function that occurred based on the measured creatinine and urea levels.

3.2.1 Creatinine

Creatinine is the end product of creatine's metabolism in muscle. Metabolically creatinine is an inactive component which then diffuses into the plasma and is excreted into the urine. The Increased levels of creatinine in the blood and the amount of creatinine in the urine can be used to estimate glomerular filtration rate. Therefore creatinine can be used as a parameter to determine the function and damage of the kidney [10]. Creatinine levels were measured weekly for 4 weeks of therapy. Here is the average data table of the results of measurement of creatinin levels during therapy.

The above description of creatinine profile (Picture 2) shows that rat that have decreased kidney function at baseline have a high creatinine level. After the treatment that using an Lansau extract with variation in dose creatinine levels began to decrease. The decrease in creatinine levels that occur also vary for each group. This indicates that the given dose gives effect of different responses. Only in the negative control group the decrease was not significant. In the chart above can also be seen that in the first week until the third week of negative control group therapy decreased creatinine levels, although not given therapy. It describes the body's homeostatic process ie the condition when the body tries to restore itself to a state of balance.
Table 2. Average Data of rat Creatinin level measurement that decreased the kidney function in a therapy.

| Treatment Group | Average Creatinin level (mg/dL) every week |
|-----------------|------------------------------------------|
|                 | H1              | H2              | H3              | H4              |
| K(-)            | 3.84 ± 3.16     | 2.12 ± 0.41     | 1.95 ± 0.65     | 2.03 ± 0.47     |
| K(+)            | 4.18 ± 2.00     | 2.13 ± 0.47     | 1.43 ± 0.32     | 0.83 ± 0.08     |
| LS I            | 3.86 ± 0.53     | 1.97 ± 0.20     | 1.58 ± 0.42     | 1.40 ± 0.48     |
| LS II           | 4.02 ± 1.80     | 2.53 ± 0.79     | 1.66 ± 0.59     | 1.25 ± 0.40     |
| LS III          | 6.05 ± 2.40     | 4.15 ± 1.71     | 2.24 ± 0.68     | 1.21 ± 0.21     |

Explanation:
- H1 = Therapy Level on Week -1
- H2 = Therapy Level on Week -2
- H3 = Therapy Level on Week -3
- H4 = Therapy Level on Week -4

Other factors that can affect creatinine levels are gender, famine, and muscle tissue size. The use of rat of varying age also affects plasma creatinine levels. It is associated with increasing age and the number of glomeruli damaged normally also increases [5]. But in the fourth week of therapy the levels of creatinine rise again indicating that the homeostatic process of the body is no longer able to work. To prove the activity of ethanol extract Lansau in improving kidney function then performed statistical tests based on the percentage decrease in creatinine levels during therapy.

The collected data were then tested using SPSS two-way ANOVA to determine whether there was any difference in Lansau ethanol extract at various doses on percentage of creatinine decrease. The percentage data of creatinine degradation is as follows (Table 3).

Tabel 3. Percentage Data of Rat Creatinin Level Measurement that Decrease Kidney Function in Therapy

| Treatment Group | The Decrease of Creatinin Level (mg/dL) Every week (%) |
|-----------------|--------------------------------------------------------|
|                 | 1            | 2            | 3            | 4            |
| KN              | 7.63 ± 6.31  | 0.75 ± 10.66 | -0.68 ± 17.61| 8.50 ± 14.72 |
| K(-)            | -7.82 ± 41.19| -15.74 ± 104.55| -11.81 ± 100.35| -8.92 ± 90.28 |
| K(+)            | 27.82 ± 13.24| 59.53 ± 12.20| 73.23 ± 6.11 | 84.21 ± 4.54* |
| LS 1            | 27.23 ± 23.73| 63.55 ± 6.25 | 71.68 ± 0.96 | 74.75 ± 5.08* |
| LS 2            | 44.04 ± 15.96| 59.58 ± 4.73 | 73.74 ± 3.99 | 80.07 ± 2.00* |
| LS 3            | 24.35 ± 3.18 | 47.89 ± 7.24 | 71.05 ± 5.89 | 84.08 ± 3.05* |

(*) = Significantly different p<0.05 on negative control
The results showed that in the positive control group had a higher percentage of decrease that is equal to 84.21% compared with the negative control group that is equal to -8.92%. The LSD test also showed a significantly different positive control group (p < 0.05) with the negative control group. This proves that the method used in which the use of ketosteryl as a comparison is appropriate. Ketosteril or keto acid is a nitrogen-free analog of amino acids, and is transmitted to form amino acids in the body. Ketosteril is a common supplement to CRF (Chronic Renal Failure) patients [11].

Tests performed showed that the three groups of dose I, dose II, and dose III groups had significant differences (p < 0.05) compared to the negative control group. This can be seen from the percentage decrease in creatinine levels of each test group that began to occur in week 1 of therapy for both positive control groups and for dose groups. But the week of therapy to achieve the highest percentage of decrease in creatinine levels for each group varied where the group dose I percentage decrease in creatinine levels was highest at the 4th week reached 74.75%, in group dose II the highest percentage of creatinine decrease occurred in week to-4 reached 80.07%, while in the dose group III the highest percentage of creatinine decrease occurred at week 4 reached 84.08%. This indicates that *lansau* ethanol extract has a potential to improve kidney function.

The effectiveness was also shown from the three dosage groups in which the percentage of creatinine degradation for the three dosage groups did not differ significantly with the positive control group. This indicates that all three doses of groups have the same effectiveness as positive controls in the improvement of kidney function. Ketosteryl is used as a positive control is a nitrogen-free analog of amino acids, and its transmitted to form amino acids in the body. The action mechanism of ketosteril is similar to compounds that are antioxidants.

The results of research that has been done suggests that possible mechanisms may be antioxidant, anti-inflammatory, vasodilating and diuretic activity in the Nephroprotective effect of phytochemical compounds such as flavonoids, terpenoids, tannins, glucosinolates and saponins [3]. Based on the results of research it is known that in ingredients *Lansau* plant consists of 44 types of plants there are also compounds such as alkaloids, flavonoids, terpenoids and tannins [4].

The above compounds that work to repair damage to kidney function so that it can decrease creatinine levels. This is probably due to the antioxidant flavonoid compounds have a high role in preventing oxygen radical species to cause cytotoxicity and damage in humans. Similarly, for alkaloid compounds that provide antioxidant effects. As for tannin compounds have activities that resemble astringen so it can cause precipitation of proteins in the cell membranes that form the barrier to prevent the occurrence of attacks by free radicals.

### 3.2.2 Ureum

Beside the measurement of creatinine levels, another indicator that can be used to see kidney function is through measurement of urea levels. Ureum is a small molecule that is produced in the liver and is normally excreted through the kidney so that high urea levels indicate abnormal or decreased kidney function [12]. Here is a table of measurements of urea content per week for 4 weeks of therapy.
**Table 5.** Average Data of Rat Ureum Level Measurement Decreased The Kidney Function on Therapy

| Treatment Group | H1          | H2          | H3          | H4          |
|-----------------|-------------|-------------|-------------|-------------|
| K(-)            | 44.93 ± 8.14| 58.47 ± 13.35| 57.3 ± 15.14| 64.37 ± 10.93 |
| K(+)            | 72.52 ± 19.46| 47.49 ± 5.25 | 39.24 ± 2.16 | 31.28 ± 1.14  |
| LS 1            | 66.33 ± 21.18| 45.3 ± 12.87 | 47.87 ± 5.70 | 31.28 ± 1.14  |
| LS 2            | 66.97 ± 8.09 | 62.2 ± 11.10 | 49.12 ± 8.20 | 42.33 ± 1.48  |
| LS 3            | 73.5 ± 6.17  | 70.67 ± 8.71 | 55.67 ± 11.48| 41.8 ± 3.87   |

Explanations:
- H1 = Therapy Level on Week -1
- H2 = Therapy Level on Week -2
- H3 = Therapy Level on Week -3
- H4 = Therapy Level on Week -4

Average Profil of Ureum Level on Therapy can be see from the graphic above (Picture 4).

![Picture 4. Average Profil Chart on Ureum Level](image)

Description of the profile of urea content (Picture 4) shows that at the beginning of therapy the levels of urea are quite high. After treatment using the Lansau extract with variations in dosage urea levels began to decrease. Only in the negative control group the decrease was not significant. This is because in the negative control group is not treated but only given Na. CMC 1%. Furthermore, the data that has been collected is done by using statistical test using SPSS two-way ANOVA as well as on creatinine level test which aims to know whether there is difference of ethanol ethanol extract with various doses to decrease percentage of ureum level. The percentage table of decrease in ureum content is as follows (Table 6).

**Table 6.** Percentage Data on Rat Creatinin Level Measurement decreased the Kidney Function on Therapy

| Treatment Group | H1          | H2          | H3          | H4          |
|-----------------|-------------|-------------|-------------|-------------|
| Normal Control  | -4.73 ± 11.40| -1.57 ± 7.25| 4.26 ± 3.88 | -1.22 ± 9.29 |
| Negative Control| 13.96 ± 20.52| -20.74 ± 57.67| -18.90 ± 58.95| -31.49 ± 57.75 |
| Positive Control| -12.11 ± 8.95| 22.54 ± 23.40| 37.09 ± 12.28| 49.59 ± 11.41* |
| Dose I Group    | -2.33 ± 33.88| 28.11 ± 38.85| 40.53 ± 25.00| 26.21 ± 7.85* |
| Dose II Group   | -14.34 ± 25.76| -8.52 ± 41.29| 16.43 ± 19.26| 26.79 ± 20.17* |
| Dose III Group  | 0.20 ± 17.53  | 3.89 ± 20.00 | 24.98 ± 14.56| 43.32 ± 9.43* |

(*) = Significantly different p<0.05 on Negative Control Group
As with the creatinine levels, similar results are also shown in testing urea levels. The results showed that the negative control group had the lowest percentage of decrease while the highest in the positive control group, then dose III, dose II, and dose I. From the results of statistical tests also showed that the three groups of test dose group I, group dose II, and the dose III group had a significant difference (P <0.05) than the negative control group. Whereas in the dose I group the decrease in urea level began to occur at week 2 of therapy and the highest decrease occurred at 3rd week that is equal to 40.53%, whereas in group of dose II decrease of urea level started to happen at 3rd week of therapy and decrease the highest occurred at week 4 that is equal to 26.79%, and for group of dose III decrease of urea level started to happen at week 1 therapy and the highest decrease happened at week 4 that is equal to 43.32%. This indicates that lansau ethanol extract has the potential to improve kidney function.

The effectiveness was also shown in the three dosage groups where the percentage decrease of urea level for the three dosage groups did not differ significantly (p> 0.05) with positive control group which in the positive control group decreased urea level started at week 2 and the highest decrease at 4th week of therapy that is equal to 49.59%. This indicates that all three doses of the group have the same effectiveness in improving kidney function.

The results of research that has been done by Konda et al. (2016) suggests that possible mechanisms may be antioxidant, anti-inflammatory, vasodilating and diuretic activity in the Nephroprotective effect of phytochemical compounds such as flavonoids, terpenoids, tannins, glucosinolates and saponins [3]. Based on the results of the research, it is known that in ingredients of Lansau plant consists of 44 types of plants there are also compounds such as alkaloids, flavonoids, terpenoids and tannins [4].

The above compounds that work to repair damage to kidney function so as to decrease urea levels. This is probably due to the antioxidant flavonoid compounds have a high role in preventing oxygen radical species to cause cytotoxicity and damage in humans [13. Similarly, for alkaloid compounds that provide antioxidant effects [14]. As for tannin compounds have activities that resemble astringent so it can cause precipitation of proteins in the cell membranes that form the barrier to prevent the occurrence of attacks by free radicals [15].

4. Conclusion

The typical of Lansau ethanol extract of Muna Region has the potential to improve kidney function in male wistar white rat based on the LSD test in which the percentage of creatinine and urea decrease in the LS I, LS II and LS III groups was significantly different (p<0.05) negative controls. Percentage of creatinine decrease for LS I group 74.75%, LS II 80.07% and LS III group 84.08% and percentage decrease of ureum group LS I 40.53%, LS II group 26.79% and group LS III 43.32%. The typical of Lansau ethanol extract of Muna Region at doses of 6.907 mg/kgbb, doses of 13.814 mg/kgbb, and doses of 27.628 mg/kgbb had the same effectiveness as ketosteryl 53.03 mg/kgbb in improving kidney function in induced male wistar white rat gentamicin-piroxicam based on the LSD test.
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