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Toxicity profile of jamu sari rapet albumin and creatinine levels in female rats (*Rattus norvegicus*)

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ABSTRACT

Married Madurese women routinely consume jamu sari rapet to please their husbands. There is no information on the toxicity test of jamu sari rapet. The kidney is one of the organs that is targeted by toxic substances that enter the body. One of the parameters controlled is albumin and creatinine. The purpose of this study was to determine the effect on albumin and creatinine levels in white rats (*Rattus norvegicus*) given jamu sari rapet. This type of research is true experimental with a quantitative approach. This study was conducted for 28 days (subchronic). Data were analyzed using the Kruskal Wallis test. There were 4 groups, namely dose group 1 (1,56 g/gBB), dose group 2 (1,60 g/gBB), dose group 3 (1,66 g/gBB) and the control group. The results of this study showed no significant difference between albumin and creatinine levels compared to the control group. The conclusion shows that jamu sari rapet given to female rats (*Rattus norvegicus*) for 28 days has no significant effect on albumin and creatinine levels.

Keywords: Albumin, creatinine, herbs, kidneys function, sari rapet, toxicity test

INTRODUCTION

The habit of consuming herbal medicine is not new in Indonesia. Since ancient times, people have used herbal concoctions called jamu to maintain health (Kusumo et al., 2020). Javanese and Madurese communities are still famous for the use of herbal medicine to this day. Madurese women psychologically feel their bodies are healthier when consuming jamu (Rosana, 2015). Jamu sari rapet is an herbal medicine used by the Madurese community. Jamu sari rapet has the potential of astrungensia, which can cause a reduction in fluid in the female reproductive organs so that the vagina seems "rapet" (Gunawan, 1999). The composition of jamu sari rapet has beneficial properties.

Jamu sari rapet are herbs that have therapeutic qualities. Several simplices, including betel leaves, areca nut, ginger, cloves, key pepet, vetiver, tapak liman, and hedgehog waru leaves, are used in jamu sari rapet. Many phytochemical compounds are found in the composition of jamu sari rapet. The tannin component is one of those possessed by jamu sari rapet plants in the highest concentration. This substance has benefits for maintaining reproductive health. One of the foodstuffs that has a high content of tannin compounds is areca nut (Sulastri, 2009). As an antibacterial, tannins are very beneficial. Tannin compounds have the ability to shrink and damage cell walls while acting as antibacterials (Suhartati & Roziqin, 2018). The female reproductive organs need antibacterials to stay healthy, and one way to get them is by consuming traditional herbal medicine.

Madurese women routinely consume jamu sari rapet, but not every day. Jamu sari rapet makes Madurese women healthier, but there is still no toxicity test data on jamu sari rapet. Jamu sari rapet circulating in the community is still

limited to the benefits obtained, there is no information regarding toxicity tests on jamu sari rapet. There are several herbs that are still not in accordance with the standards of the Ministry of Health. Test requirements are needed on jamu sari rapet. Every new drug, including conventional drugs, must undergo toxicity tests on animals before being given to humans in accordance with international human rights standards (Maulia et al., 2021). The kidney is one of the organs of the body that is targeted by toxic substances. Harmful substances or compounds that enter the body will target the kidneys as the main target. The rest of the metabolism is excreted through the kidneys. If there is albumin in the urine, the kidney function decreases. High creatinine levels are the cause of one of the signs of impaired kidney function (Alfonso et al., 2016).

From these problems, this study tries to determine the albumin and creatinine levels of rats (*Rattus norvegicus*) given jamu sari rapet. Research conducted by (Sammad et al., 2017) and (Saryanto, 2015) did not find any relationship between serum albumin levels of rats with the administration of traditional herbs used to prevent various diseases. Creatinine levels of rats given dried extracts of temu putih rhizomes and Nigella sativa asthma herbs have no effect, according to research by (Sumarny & Parodi, 2006) and (Dollah & Izwan, 2013). This research is very important to do because there is no information related to toxicity test of albumin and creatinine levels in the kidneys of female rats (*Rattus norvegicus*) given jamu sari rapet. This research can be a reference for further research on the safety of herbal medicine, especially jamu sari rapet.

METHOD

This research is an experimental research with quantitative methods. This research uses True experimental, all external variables that affect the experimental process are under the control of the researcher. Posttest-Only Control design is the experimental design used.

Ethical clearance

This study received ethical approval from the Health Research Ethics Committee (KEPK), Faculty of Medicine, Universitas Muhammadiyah Malang, in ethical certificate number E.5.a/197/KEPK-UMM/IX/2022.

Preparation of jamu sari rapet powder

In making jamu sari rapet 20,4 g of jamu powder was weighed based on (Smaradhna et al., 2023) and 4 ml of hot water was added to each and then stirred until dissolved and filtered. The jamu sari rapet concoction does not yet have a brand and is obtained from the herbalist KSM of Annuqoyah Islamic boarding school, Sumenep, Madura.

Preparation and maintenance of animals test

The rats were adapted to laboratory conditions for 7 days prior to treatment, designed to avoid stress. During rearing, rats were placed in plastic cages with perforated wire covers measuring 30 cm x 5 cm x 15 cm. The number of rats per group contained 3-6 rats based on (Arifin & Zahiruddin, 2017). Each cage contained female rats (*Rattus norvegicus*) aged 2 - 3 months with a body weight of ± 200 grams and was placed at a room temperature of 20 - 25 °C and a 12-hour/12-hour light-dark cycle. The rat cage mat uses husks that are replaced twice a week. The daily consumption requirement of a rat was 40 grams per animal and was covered with pelleted food. Drinking rats were placed in drinking bottles with a daily requirement of 60 ml/rat.

Group division of animals test

The experimental animal groups were divided into four based on the formula (Federer, 1955) namely (1) Adult female rats that will be given a dose of 1,56 grams of jamu sari rapet, (2) Adult female rats that will be given a dose of 1,60 grams of jamu sari rapet, (3) Adult female rats that will be given a dose of 1,66 grams of jamu sari rapet, (4) Rats without treatment (Control).

Dose calculation

The determination of the human dose conversion factor by (Laurence & Bacharach, 1964) weighing 70 kg to white rats (*Rattus norvegicus*) with a body weight of 200 g is 0.018. The herbal medicine was given according to the dose. The administration of herbal medicine is done orally using a syringe that has been modified with a sonde tip. The toxicity test doses of jamu sari rapet used in this study were doses of 1,56 g; 1,60 g; and 1,66 g per body weight of the test animals.

Animal testing

Total of 12 rats (*Rattus novergicus*) were grouped into 4 groups of 3 rats. Each group was given jamu sari rapet. Giving jamu sari rapet is done every day after animal acclimatization is complete and carried out for 28 days. The administration of herbal medicine is adjusted to the predetermined dose and weight in each group, according to the formula for calculating the dose per body weight (Athijah et al., 2011).

Administration of jamu sari rapet to animals test

The administration of jamu sari rapet medicine was carried out every day after animal acclimatization was completed and carried out for 28 days. Jamu sari rapet was given in accordance with the dose described in the Treatment of Test Animals section, giving the jamu is done orally using a syringe that has been modified with a sonde tip. Jamu sari rapet was injected into the mouth of the rats.

Dissection and blood sampling of animals test

The surgical stage begins after anesthesia is carried out to the test animals by putting the rats into a glass jar that is given cotton that has been mixed with chloroform. Anesthesia is carried out until the rat faints, then counted for 20 seconds, after which the rat is moved for surgery. After the test animals were anesthetized, they were placed on a wax table and the four legs of the test animals were fixed to the wax table using needles. Then, using a scapel, dissection was performed on the abdomen to the neck of the rat. Blood was drawn from the heart using a 5 ml syringe and put into a vacutainer. Blood was then centrifuged at 3000 rpm for 10 minutes. Serum was then taken and stored in the freezer.

Measurement and calculation of albumin and creatinine levels

a. Measurement and calculation of albumin levels

Albumin levels were measured using the BCG Dye Method, End Point. This method uses BCG reagent which consists of acetate buffer 50 mmol/l pH 4.2, bromcresol green 0.1 mmol/l and albumin standard 3.8 g/dl. In acidic conditions the reaction of albumin and BCG will produce a color complex and then measured using spectrophotometry. Measurement of albumin levels is based on (Jiang et al., 2005) by mixing 1 mL of BCG reagent with 10 µl of sample and the color change that occurs is observed using a spectrophotometer at a wavelength of 620 nm.

Albumin levels were measured using BCG (Bromocresol Green) reagent and samples were mixed, then incubated for 1 minute at 37°C. Absorbance readings of the standard solution (As) and sample (Ax) against the reagent blank were taken. After the absorbance measurement procedure was completed, the albumin concentration (g/dl) in the sample was calculated using the formula.

b. Measurement and calculation of creatinine levels

Measurement of creatinine levels was carried out using Jaffe's method, Initial Rate. Measurement of creatinine levels was carried out by mixing 500 µl of reagent 1, 500 µl of reagent 2 with 100 µl of sample. The color change that occurs is observed with a spectrophotometer at a wavelength of 500 - 520 nm.

Calculation of creatinine levels was carried out by mixing reagent 1, reagent 2, and samples. Incubation was carried out for 60 seconds at 37°C. The absorbance (A1) was recorded at minute 0 and the absorbance (A2) was recorded at minute 60. The creatinine concentration (mg/dl) in the sample was calculated using the

formula after the absorbance measurement procedure was completed.

Data analysis technique

The data obtained were statistically analyzed using SPSS. Testing the normality of albumin and creatinine using the Shapiro wilk test. The distribution of abnormal data was analyzed using the Kruskal Wallis test which aims to test the comparison of mean values between the four treatment groups.

RESULTS AND DISCUSSION

The results of albumin and creatinine tests of rats (*Rattus norvegicus*) given jamu sari rapet compared to normal albumin and creatinine levels are shown in Table 1. The results of the Kruskal Wallis test analysis showed that rats given jamu sari rapet for 28 days with different treatments were 1,56 g; 1,60g; 1,66 g per body weight of the animals test. There is no significant difference between the rats of groups P1, P2, P3 with control albumin and creatinine levels of rats (*Rattus norvegicus*), $p > 0.05$ in Table 2.

Three doses were used in the test of albumin and creatinine levels of rats (*Rattus norvegicus*) given jamu sari rapet, namely 1,56 g; 1,60 g; 1,66 g per body weight of the animals test. The stronger the therapeutic impact, the higher the dose given (Wimott et al., 2018). Minimal Effective Concentration (MEC) is the lowest concentration of herbal medicine needed to produce herbal medicine effects and toxic effects.

Table 1. Test results of albumin and creatinine levels of rats given jamu sari rapet.

Group	Count of Rats	Average		BPOM	Standard
		Albumin	Creatinin	Albumin	Creatinin
P1	3	0,002	5,8	3,3 – 4,2 g/dL	0,3 – 0,9 mg/dL
P2	3	0,002	18,3		
P3	3	0,003	11,6		
Control	3	0,003	17,4		

The liver synthesizes albumin to carry drugs. Synthesis rate, breakdown rate, and extravascular and intravascular distribution are factors that influence albumin levels (Setiawan et al., 2019). The rate of synthesis is influenced by various factors such as nutrition, oncotic pressure, inflammation, and hormonal conditions. Oncotic pressure is an important factor in determining albumin production in healthy animals. Albumin synthesis increases with decreasing oncotic pressure and decreases with increasing oncotic pressure (Suharjono et al., 2016).

Table 2. Kruskal Wallis test results of albumin and creatinine levels.

	Albumin	Creatinin
Kruskal Wallis	5.019	3.720
Df	3	3
Asymp. Sig.	.170	.293

Low albumin levels indicate impaired kidney function (Putri et al., 2016). The kidneys will filter back albumin from the glomerulus. Decreased kidney function makes albumin unable to be filtered out of the body through the kidneys, so albumin is present in the urine (Saputra et al., 2019). Albumin levels in this study were not significantly different from the control, $p < 0.05$ (Table 2). The decrease in albumin in this study is possible due to the infiltration of inflammatory cells into the kidney organs due to consuming jamu sari rapet which can damage the kidneys. There is a decrease in albumin synthesis production when inflammation occurs (Oka, 2018).

Creatinine is the end product of creatine phosphate metabolism, which is a compound used to store energy in skeletal muscle. The creatinine reabsorption system is not possessed by the body and most of the creatinine synthesized in the muscles is excreted through the kidneys (Dasgupta & Wahed, 2021). Creatinine levels increase and creatinine filtration capacity decreases in renal failure (Chadijah & Wirawanni, 2013).

Rats (*Rattus norvegicus*) in this study may experience stress, which affects the decrease in albumin levels in groups P1, P2, and P3 group albumin is the same as the control, while creatinine levels increase in rats in groups P1, P2 and P3. Rats become stressed and agitated during herbal medicine rounds, such as when holding

rats. Stress hormones can inhibit the secretion of growth hormone. Growth hormone is an anabolic hormone that is important for protein synthesis, including albumin. Stress hormones can inhibit albumin synthesis by the liver (Rejeki & Kuswardhani, 2019). Jamu sari rapet contains several bioactive compounds.

The composition of jamu sari rapet contains tannin and flavonoid compounds. Tannin compounds function as antibacterial and flavonoid compounds have antioxidant activity. Glutathione levels in kidney tissue can increase due to flavonoid compounds, flavonoid compounds are antioxidants. Oxidative stress can cause free radicals. Damage to the kidneys can occur due to free radical reactions (Pellegrino et al., 2019). Antioxidants can stabilize free radicals in the body. The ingredients of jamu sari rapet may contain active substances that can interfere with glomerular function.

The glomerulus has kidney pores that measure 8 nm (Guyton & Hall, 2016). Jamu sari rapet is a solute that may have a size greater than 8 nm, so that glomerular function is impaired. The size and charge of molecules to be filtered are determined selectively during glomerular filtration (Klein, 2013).

CONCLUSION

There is no significant difference in albumin and creatinine levels of rats (*Rattus norvegicus*), but there is a tendency in the group to decrease albumin levels in groups P1 and P2 against the normal group. Giving jamu sari rapet with a dose of 1,50 gr; 1,60 gr; 1,66 gr in rats (*Rattus norvegicus*) has no significant effect on albumin and creatinine levels.

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