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THE EFFICACY OF AVOCADO, MUNG BEAN SPROUTS, AND HOLY BASIL HERB COMBINATION (JAMU ATOKE) ON THE HEALTH AND REPRODUCTIVITY OF ADULT FEMALE RATS

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ABSTRACT

The purpose of this study was to examine the efficacy of a herbal combination of avocado, mung bean sprouts, and holy basil (Jamu ATOKE) in optimizing the reproductive health of adult female rats. Eighteen female Sprague Dawley rats aged 9-10 weeks old weighing around 180-250 g, and had not been pregnant previously were equally and randomly divided into 3 groups: one control group and two treatment groups (n= 6 rats per group). The treatment groups received Jamu ATOKE with a concentration of 2.5 and 5%, respectively. Jamu ATOKE was added to the drinking water and consumed by the rats for 30 days prior to pregnancy. Reproductivity (pregnancy success, estrogen and progesterone hormone concentrations), and health performance (body weight gain, food, and water consumption, motor activity, red blood cell (RBC) count, white blood cell (WBC) count and differentials, SGPT, SGOT, ureum, and creatinine) of rats were observed. The results showed that groups receiving the Jamu ATOKE had better reproductive and health performance compared to those of the control group. The administration of Jamu ATOKE can significantly increase the concentration of estrogen and progesterone in rats (P<0.05). It can also improve the fertility and health of the female rats prior to pregnancy.

Key words: female rats, health, Jamu ATOKE, pregnancy, reproductivity

ABSTRAK

Tujuan penelitian ini adalah mengetahui khasiat kombinasi alpukat, tauge, dan kemangi (Jamu ATOKE) dalam mengoptimalkan kesehatan reproduksi induk tikus betina. Delapan belas tikus betina Sprague Dawley (berusia 9-10 minggu, bobot badan 180-250 g) dibagi secara acak menjadi 3 kelompok (n= 6 tikus). Kelompok-kelompok tersebut terdiri atas kelompok kontrol dan kelompok yang diberi Jamu ATOKE dengan konsentrasi 2,5 dan 5%. Jamu ATOKE ditambahkan ke dalam air minum tikus dan dikonsumsi selama 30 hari sebelum kebuntingan. Reproduktivitas (keberhasilan kebuntingan, konsentrasi hormon estrogen dan progesteron), dan performa kesehatan (penambahan bobot badan, konsumsi pakan dan minum, aktivitas motorik, jumlah sel darah merah (RBC), jumlah dan perbedaan sel darah putih (WBC), SGPT, SGOT, ureum, dan kreatinin) tikus diamati. Kelompok yang diberi Jamu ATOKE memiliki kinerja reproduksi dan kesehatan yang lebih baik dibandingkan dengan kelompok kontrol. Pemberian ATOKE mampu secara signifikan meningkatkan konsentrasi estrogen dan progesteron pada tikus (P<0,05) dibandingkan dengan kontrol. Disimpulkan bahwa jamu ATOKE dapat meningkatkan kesuburan dan kesehatan induk tikus betina sebelum kebuntingan.

Kata kunci: tikus betina, kesehatan, Jamu ATOKE, kebuntingan, reproduktivitas

INTRODUCTION

Women's health has a profound effect on future generations (Onarheim *et al*. 2016). Improving women's reproductive health is important to building a healthy society (WHO 2011). However, health problems among women are still highly prevalent, especially among those living in developing countries. Women in developing countries with poor socio-economic status tend to have low body height (Bhalotra and Rawlings 2012). Low body height indicates a lack of adequate nutrition. Moreover, it was reported that 36% of women in developing countries experienced reproductive disorders during the productive period (Ekine *et al*. 2015). This is a serious issue because the health of girls and women before pregnancy affects the health in the maternal period, during the reproductive period, and after the reproductive period (Lattof *et al*. 2012).

Several studies reported that maternal health and endogenous secretion of pregnancy hormones (estrogen and progesterone) before mating and during pregnancy have a strong correlation with a child's birth weight, child mortality, breast milk production, and child's growth from birth to the weaning period (Andriyanto and Manalu 2011; Andriyanto and Manalu 2012; Andriyanto *et al*. 2015; Rahminiwati *et al*. 2017). Reproductive problems such as hormonal imbalance and the mother's poor nutrition contribute to the increasing rate of congenital malformations and developmental disorders such as autism (Cao *et al*. 2015; Baron-Cohen *et al*. 2019; Whitaker-Azmitia *et al*. 2015). Thus, to have healthy offspring, health care and adequate nutrition for women in the pre-pregnancy period should be the initial priority. Pre-pregnancy health can be used as an indicator to predict and increase pregnancy outcomes (Floyd *et al*. 2013).

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For generations, Indonesians have utilized nutritious plants in the form of herbal medicine or *jamu* for medical treatment and health maintenance. Primary Health Research reported that 49.53% of the Indonesian population consumed *jamu* both for health care and for illness treatment (National Institute of Health Research and Development 2010). Herbal ingredients such as avocado (*Persea americana*), mung bean sprouts (*Vigna radiata*), and holy basil (*Ocimum sanctum*) can be formulated as *jamu* to improve the health and reproductivity of female parents. Avocado contains folic acid which serves to maintain pregnancy. It also has various nutritional contents, such as monounsaturated fatty acids, fat soluble antioxidants, and various phytosterols that can improve the health of mothers, infants, and toddlers (Comerford *et al*. 2016). Mung bean sprouts contain vitamins C and E can reduce oxidative stress and lead to the more optimal secretion of estrogen (Fatmaningrum and Ningtyas 2019). Holy basil contains stigmasterol which plays a role in estrogen and progesterone synthesis (Kaur *et al*. 2011; Bano *et al*. 2017). This research was conducted as a basic study to evaluate the efficacy of Jamu ATOKE made of the avocado, mung bean sprouts, and holy basil combination in improving the health and fertility of adult female rats.

MATERIALS AND METHODS

Preparation of Jamu ATOKE Formulation

A combination of avocado, mung bean sprouts, and holy basil leaves (1:1:1/4) was used for the formulation of Jamu ATOKE. Prior to the formulation, the ingredients were sorted and cleaned. The ingredients were then mashed and mixed with the rats' drinking water in a ratio of 1:2. Next, the mixture was heated and stirred until the temperature reached 60° C. This step was repeated three times. The mixture was then cooled and filtered. The filtrate was put into a bottle and stored in a freezer/refrigerator at 4° C. Jamu ATOKE was ready to use.

Experimental Animals and Treatment Groups

The approval for this research was obtained from the animal ethics commission of Veterinary Medicine Faculty, IPB University, with the ethics number 14/KEH/SKE/VIII/2019. Eighteen adult female Sprague Dawley rats aged 8-10 weeks old, weighing around 180-250 g, and had not previously been pregnant were acclimatized for two weeks. Food and drink for the rats were administered *ad libitum*. The rats were then divided equally and randomly into three groups that consisted of one control group (receiving only solvent), and two treatment groups receiving Jamu ATOKE with a concentration of 2.5% and 5%, respectively, for 30 days before pregnancy.

Estrous Synchronization and Mating of Rats

After the administration period ended, the estrous of the female rats were synchronized using the whitening effect method by mixing every two female rats with one male rat (2:1) for 48 hours. Following the synchronized estrous, the female rats mated with male rats.

Data Collection in the Pre- and Post-Pregnancy Period

Jamu ATOKE was administered for 30 days. During the administration period, the food and drinking water of the rats were measured every day. The rats' body weight was also measured every five days to find out body weight gain. The success of mating was calculated using the percentage of pregnant rats. On the sixteenth day of post-pregnancy, the motor activity of the rats was observed using the swim test method by measuring their swimming duration. Before the swim test, the rats' blood was taken for red blood cell (RBC) profile analysis RBC count, hematocrit (Hct), hemoglobin (Hb)), white blood cell (WBC) count and differential (neutrophils, lymphocytes, monocytes, basophils, eosinophils), and serum for the measurement of estrogen and progesterone concentrations, serum glutamic pyruvic transaminase (SGPT), serum glutamic oxaloacetic transaminase (SGOT), ureum, and creatinine. The measurement of estrogen and progesterone concentrations was carried out using the enzyme-linked immunosorbent assay (ELISA) method while the analyses of SGPT, SGOT, ureum, and creatinine levels were performed using the UV spectrophotometer method. Dams mortality was also calculated during the study.

Data Analysis

The data were analyzed with one-way ANOVA, Duncan's test at a 95% accuracy level, and a t-test. Microsoft Excel 2013 and Minitab 16 were used to perform data analysis.

RESULTS AND DISCUSSION

Efficacy of Jamu ATOKE on the Health and Reproductive Performance

Jamu ATOKE can be used to improve maternal health conditions before pregnancy. The efficacy of Jamu ATOKE on the health and reproductive performance of the female rats is presented in Table 1.

The rats receiving 5% of Jamu ATOKE had a higher increase in body weight per five days, daily food consumption, and daily water consumption of drinking compared to those of the control group ($P<0.05$). However, there was no significant difference found in the same parameters among rats in the control group and rats in the 2.5% Jamu ATOKE group. The administration of Jamu ATOKE with a concentration of 5% increased the rats' appetite and water consumption, as indicated by the weight gain. The number of pregnant rats, pregnancy rates, and mortality rates of rats in all groups were similar. Motor activity of the pregnant rats consuming 2.5% and 5% of Jamu ATOKE was significantly higher than that of the control group ($P<0.05$). The treatment groups also had longer swimming durations, which were 193 and 230

seconds, respectively (± twice as long). The estrogen and progesterone secretion of the female rats given Jamu ATOKE were also significantly higher compared to those of the control group (P<0.05), accounting for 48.33 and 41.55 pg/mL for the 2.5% ATOKE group and 51.50 and 51.59 ng/mL for the 5% ATOKE group. During the study, the rats in all treatment groups had a 0% percentage of mortality.

Efficacy of Jamu ATOKE on the Rats' Haematology and Blood Chemistry Profile

The effects of Jamu ATOKE on the RBC profile, WBC count and differential, liver function, and kidney function of the female rats are presented in Table 2. The number of RBC, Hct, and Hb of rats consuming Jamu ATOKE with a concentration of 2.5 and 5% was higher compared to that of the control (P<0.05). This showed that the administration of Jamu ATOKE can increase the production of RBC components. Furthermore, rats treated with ATOKE had a higher WBC count compared to rats in the control group (P<0.05). Higher WBC components were found in the 5% ATOKE group consisting of lymphocytes, monocytes, and basophils. However, in the 2.5% ATOKE group, only lymphocytes were higher. Neutrophil count, neutrophil/lymphocyte ratio, SGPT, SGOT, ureum, and creatinine of the rats given ATOKE were similar to those in the control group (P>0.05).

Jamu ATOKE is effective in improving the reproductivity and health performance of mother rats, including their body weight. Previous studies have reported that the weight and body mass index of the mothers prior to pregnancy reflect the nutritional status and readiness of the mother rats to conceive as well as influence their pregnancy (Han *et al*. 2011; Yu *et al*. 2013; Soltani *et al*. 2017). Mother rats who have proper nutrition before pregnancy tend to have better early placental and embryonic development and better distribution of nutrients between mother and fetus (King 2016). In this study, it was found that the combination of avocado, mung bean sprouts, and holy basil in Jamu ATOKE can increase the rats' food intake and body weight. Good appetite correlates with a low stress level. Avocado has antihypertensive activity (Adelina 2015) which can reduce stress and depression. Basils were reported to have antidepressant compounds (Tewari 2014). In addition, Yeap *et al*. (2014) revealed that mung bean sprouts contain antidepressant and antioxidant compounds. Antidepressants in the ingredients can increase the appetite by regulating the hunger center mechanism in the hypothalamus (Serretti and Mandelli 2010).

Table 1. Efficacy of Jamu ATOKE on the health and reproductive performance of adult female rats

Parameters	Control	Concentration of ATOKE (%)		p-value
		2.5	5	
Number of rats (head)	6	6	6	-
Average weight gain per five days (g)	2.65±0.84^{b}	4.00±2.28^{ab}	6.33±3.45^{a}	0.06
Average daily food consumption (g/day)	50.57±11.72^{b}	55.62±13.38^{b}	70.01±10.43^{a}	0.00
Average daily drinking consumption (mL/day)	24.54±0.86^{b}	22.33±0.92^{b}	29.83±4.74^{a}	0.00
Number of pregnant rats (head)	4	4	3	-
Pregnancy percentage (%)	67 (4/6)	67 (4/6)	50 (3/6)	-
Mortality (%)	0 (0/6)	0 (0/6)	0 (0/6)	-
Pregnant rat motor activity(s)	112.70±40.9^{b}	193.20±52.60^{a}	237.30±36.20^{a}	0.00
Estrogen concentration in rats' blood serum (pg/mL)	37.00±3.78^{b}	48.33±1.47^{a}	51.50±1.68^{a}	0.00
Progesterone concentration in rats' blood serum (ng/mL)	18.75±1.84^{c}	41.55±2.68^{b}	51.59±2.09^{a}	0.00

$^{a, b}$Different superscripts within the same row indicated significant differences (P<0.05)

Table 2. RBC profile, WBC count and differential, liver function, and kidney function of the female rats receiving Jamu ATOKE

Parameters	Control	Concentration of jamu ATOKE (%)		P-value
		2.5	5	
RBC count (× $10^{6}/\mu$L)	5.83±0.07b	7.15±0.32^{a}	7.93±0.72^{a}	0.00
Hct (%)	29.60±0.54^{b}	35.80±4.26^{a}	36.60±2.88^{a}	0.00
Hb (g%)	11.48±1.04^{b}	14.24±0.93^{a}	15.08±0.30^{a}	0.00
WBC cell count (× $10^{3}/\mu$L)	4.99±0.43^{b}	6.55±1.53^{a}	7.28±0.73^{a}	0.04
Neutrophils (x $10^{3}/\mu$L)	1.58±0.49^{a}	1.81±0.43^{a}	1.91±0.47^{a}	0.47
Lymphocytes (x $10^{3}/\mu$L)	3.02±0.38^{b}	4.39±1.13^{a}	4.74±0.56^{a}	0.00
Monocytes (x $10^{3}/\mu$L)	0.29±0.03^{b}	0.27±0.18^{b}	0.46±0.10^{a}	0.06
Eosinophils (x $10^{3}/\mu$L)	0.08±0.03^{a}	0.08±0.05^{a}	0.06±0.03^{a}	0.65
Basophils (x $10^{3}/\mu$L)	0.02±0.03^{b}	0.00±0.00^{b}	0.06±0.03^{a}	0.00
Neutrophil/lymphocyte ratio	0.54±0.19^{a}	0.42±0.07^{a}	0.41±0.13^{a}	0.24
SGPT (Unit/L)	50.55±6.72^{a}	53.30±4.53^{a}	53.2±4.38^{a}	0.85
SGOT (Unit/L)	83.7±6.36^{a}	77.95±3.61^{a}	81.85±3.75^{a}	0.54
Ureum (mg/dL)	46.35±4.17^{a}	46.85±1.77^{a}	45.65±2.19^{a}	0.92
Creatinine (mg/dL)	0.56±0.04^{a}	0.54±0.02^{a}	0.57±0.04^{a}	0.75

$^{a, b}$Different superscripts within the same row indicated significant differences (P<0.05)

A forced swim test on rats is a method to test the effectiveness of antidepressants (Can *et al*. 2012). When rats experience stress, the secretion of corticosterone will increase, which can affect the rats' motor performance, such as abnormal arm and leg movements (Metz 2007). The administration of 2.5 and 5% of Jamu ATOKE can lessen rats' stress and lengthen the swimming period. The results of this test correspond with the previous paragraph which mentioned that the combination of avocado, mung bean sprouts and holy basil can reduce stress and depression levels although the mechanism of improving motor activity is not yet known.

Rats given Jamu ATOKE had significantly higher concentrations of estrogen and progesterone compared to rats in the control group. This is due to cholesterol and monounsaturated fatty acids, especially oleic acid contained in avocado (Alkalf *et al*. 2018). Cholesterol plays an important role in the biosynthesis of steroid hormones, especially estrogen and progesterone which are important for reproduction (Hu *et al*. 2010). Isoflavones, one of the phytoestrogen in mung bean sprouts, interact and are bound to ERα and ERβ receptors of estrogen, which will optimize the estrogen function (Pilsakova *et al*. 2010; Tang *et al*. 2017). Stigmastrerol in holy basil also plays a role in estrogen and progesterone synthesis (Kaur *et al*. 2011; Bano *et al*. 2017). Optimizing the secretion of the two hormones is important in preparing for and maintaining pregnancy. Estrogen stimulates endometrial differentiation and thickening, whereas progesterone increases the success of embryo implantation and mammary gland proliferation (Costa *et al*. 2015).

Pregnancy affects the physiological state of mothers. There will be a greater increase in plasma volume rate compared to the rate of RBC formation, causing the RBC count, Hct, and Hb values to decline. However, it does not affect the mean of corpuscular volume (MCV) and the mean of corpuscular hemoglobin concentration (MCHC) (Soma-Pillay *et al*. 2016). A pregnancy that is not supported with adequate intake of folic acid, vitamin A, vitamin B12, and iron can lead to anemia (Stephen *et al*. 2018). This is indicated by the RBC profile of the rats in the control group which was below normal (Kim *et al*. 2000). Rats given Jamu ATOKE with concentrations of 2.5 and 5% had RBC profiles that were generally closer to normal values except for the hematocrit. The RBC counts of rats receiving Jamu ATOKE were also above normal, which indicates the efficacy of Jamu ATOKE in improving the rats' RBC profile. Folic acid and cobalamin (vitamin B12) in avocados can affect the production of RBC (Pacheco *et al*. 2011). Mung bean sprouts contain antianemia compounds, such as iron or Fe (Manikandaselvi *et al*. 2015). A previous study by Sawariya *et al*. (2018) discovered that ethanol extract from holy basil can boost the amount of RBC and Hct of rats. Folic acid and vitamin B12 are needed for erythroblast differentiation, whereas Fe is essential in the Hb synthesis (Koury and Ponka 2004).

The results of rats' WBC evaluation showed that the WBC counts of the rats in the 2.5 and 5% ATOKE groups during pregnancy were higher than that of the control group although the values were not yet close to normal according to Kim *et al*. (2000) and LaBorde *et al*. (1999). Jamu ATOKE can increase the production of WBC which will promote a better immune system for the rats accordingly. Holy basil contains flavonoids and terpenoids which can act as immunomodulators by increasing the number of WBC (Jeba *et al*. 2011). Folic acid and vitamin B6 present in avocado can also help boost the immune system by increasing antibody production (Singh 2015). The neutrophil/lymphocyte ratio can be an indicator of stress, especially at the chronic level (Swan and Hickman 2014). Both 2.5 and 5% ATOKE groups had a lower neutrophil/ lymphocyte ratio than that of the control group. This suggests that rats treated with ATOKE had a lower stress level. This is in line with the previous statement that Jamu ATOKE is effective in reducing stress levels.

It was also revealed that the administration of Jamu ATOKE did not cause liver damage, as shown by the SGPT and SGOT values of the Jamu ATOKE rat group which were not significantly different from the control group. Avocado, mung bean sprouts, and holy basil were reported to be able to maintain the values of SGPT and SGOT after the experimental animals were induced with certain toxic substances (Mahmoed and Rezq 2013; Manikandaselvi *et al*. 2015; Satapathy *et al*. 2017). Avocado and holy basil have hepatoprotective effects (Andriyanto *et al*. 2014; Ranade *et al*. 2015). Additionally, alkaloids, flavonoids, triterpenoids, minerals, and vitamins present in avocados can act as antioxidants (Rao *et al*. 2011). Antioxidative properties are present in mung bean sprouts because they contain protein, phenols, and flavonoids (Ganesan and Xu 2018). Jamu ATOKE does not cause kidney damage to the dams, as suggested by the ureum and creatinine values of ATOKE rats that were not significantly different from the control group. These results are consistent with those in the previous studies, in which the three ingredients helped to maintain kidney function compared to groups exposed to toxic materials. A study revealed that avocados can limit rats' urea and creatinine levels induced by meloxicam (Anshari *et al*. 2018). Mung bean sprouts can decrease the ureum level of rats with type 2 diabetes (Yao *et al*. 2008), whereas holy basil can deplete the ureum and creatinine levels of rats induced by cisplatin (Manigauha and Patankar 2017). In other words, Jamu ATOKE helped improve the health of female rats and promoted a healthier pregnancy.

CONCLUSION

The result showed that Jamu ATOKE had positive effects on the observed parameters. It can improve the health of female rats and promote a healthier pregnancy.

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