

Bioassay of Functional Drink - KoTeJa (Coffee, Sea Cucumber, Ginger) Using Male Mice (*Mus musculus L.*)

Kurnia Harlina Dewi^{1,*}, Helmiyetti² and Ruca Ruci²

¹Department of Agricultural Industrial Technology, Faculty of Agricultural Technology, Andalas University, Padang, Indonesia

²Department of Biology, Faculty of Math and Natural Science, University of Bengkulu, Indonesia

E-mail: kurniaharlinadewi@ae.unand.ac.id*

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Abstract

*This study aimed to examine the effect of sea cucumber ginger coffee on the behaviors and libido of the Swiss Webster male mice (*Mus musculus L.*), which has given a functional drink product of sea cucumber ginger coffee (KoTeJa). This study consists of five treatments and each treatment was repeated 5 times repetition. These treatments in this study consisted of a negative control (P0); a positive control (P1) using methyl testosterone; a 2nd treatment (P2) using sea cucumber flour; (P3) for KoTeJa + sugar + creamer; and (P4) ginseng coffee. Observations of the libido were performed on days 0 to 5; on days 7 to 9 for locomotor activity; and on day 12 for the spermatozoa quality performed. The results showed that KoTeJa (P3) provides the best effect on the behavior of libido, with an average of 25 times introduction (on the 3rd day) and 39.25 times (on the 5th day), 6 times of average climbing (on the 3rd day), and 2 times of average coitus (on day 3), at an average of 86 times loops of locomotor activity (day 7), 108.66 loops (day to 8) and 124 loops (day 9), and an indicators of the quality of spermatozoa normality of 60.6%.*

Keywords: aphrodisiac; bioassay; KoTeJa; sea cucumber

1. INTRODUCTION

Indonesia is one of the countries with quite a lot of people who consume coffee. The coffee beverage is becoming well-liked because of its distinctive aroma which arouses the appetite of consumers. Coffee can cause dependence due to its caffeine content. Caffeine is one type of alkaloid that is widely found in coffee beans, tea leaves, and cocoa (Maramis et al., 2013).

The addition of ginger and sea cucumbers in coffee allegedly could improve body vitality. The content of secondary metabolites contained in ginger plant extracts is a class of flavonoids, phenols, terpenoids, and essential oils. Phenol derivatives such as gingerol and shogaol are the main constituent compounds of ginger oleoresin (Witantri et al., 2013). Another material believed to increase vitality is sea cucumber. The study results prove that sea cucumber contains testosterone [(Maramis et al., 2013), (Witantri et al., 2013), (Nurjanah, 2008)]. Flour of sea cucumber is reported to contain steroids, saponins, fat, protein, carbohydrates, essential amino acids, and minerals that are known to be useful for increasing libido in males (Nurjanah, 2008). Phytochemical investigations of a body wall sub-fraction of black sea cucumber (*H. atra*) resulted in two isolated compounds: one previously reported triterpene glycoside, deshlothurin B, and one triterpene glycoside isolated for the first time, 12-epi-deshlothurin B (Puspita, 2023). The extract was standardized with specific parameters, including organoleptic, water and ethanol soluble content, phytochemical screening, and protein content, and non-specific parameters, including drying shrinkage, water content, ash content, and acid insoluble ash content (Evascuasiy & Puradisastra, 2010).

Libido or sexual desire is the conscious feeling of the sex drive of the desire to have sex (Puspita, 2023). In the male high libido condition will affect locomotor activity. The higher the libido the higher the locomotor activity. Libido in males is very closely related to testosterone. Testosterone is a steroid hormone that is responsible for the establishment and development of sex organs and libido and can be used as an active ingredient aphrodisiac in humans (male). In the human body, the steroid hormone is produced by the Leydig cells within the testes. Leydig cells play a role in the biosynthesis of testosterone, thus allowing the process of spermatogenesis in the testes (Nurjanah, 2008). Spermatogenesis is the process of formation of spermatozoa from spermatogonium, through

the development of complex and orderly. Spermatogenesis in the seminiferous tubules occurs in the testes, through several processes, namely proliferation, differentiation, and transformation (Rusmini et al., 2023). Based on the above publication alleged that sea cucumber and ginger coffee (KoTeJa) will affect libido and the spermatogenesis process. So far, there have not been found publications on the effect of the combination of KoTeJa on the behavior of mice libido and sperm quality. Based on the description above, this study was conducted by observing the behavior of Swiss Webster male mice.

2. MATERIAL AND METHODS

A fiber cage measuring 22x16x14 cm and CCTV cameras were prepared for the observation of mice behavior. Materials used in this study are a 1001 brand coffee powder, sugar, creamer, sea cucumber powder, ginger powder, methyl testosterone, distilled water, feed mice, tissue, physiological solution (NaCl 0.9%), infusion solution, eosin of 2 %, and 0.5%.

2.1 Preparation of sea cucumber flour.

Sea cucumbers are cleaned from the entrails, then cut into small pieces, and then dried by using an oven at 160°C for \pm 2 hours. The dried material in the form of sheets was blended and then sieved with a sieve of \pm 60 mesh (Nurjanah, 2008).

2.2 Preparation of ginger flour.

The selected ginger was cleaned under running water, and then thinly sliced. Then the ginger slices dried using an oven at a temperature of 45°C for 30-40 hours. Once dry, then blended until finer and filtered with a sieve size of 60 mesh (Nurjanah, 2008).

2.3 Preparation of mice test animals.

Adult mice used in the range of 2.5-3 months of age. For the purposes of the study, a sample of 60 mice was taken randomly (by simple random). The number of male mice required is 30 and 30 female mice. Mice were acclimatized for one week.

2.4 Provision of functional drink.

Intake of KoTeJa product was conducted to obtain mice in conditions of high testosterone, by looking at the behavior shown by the high activity of libido. Methyl testosterone was given for the positive control. Intake of products and methyl testosterone was conducted at 02:00 pm as prescribed treatment using *gavage*.

This study uses a completely randomized design (CRD), with 5 treatments and 5 replications. The treatment consists of:

1. Treatment 0 (P0) as a negative control: mice without treatment KoTeJa, were only given food and drink ad libitum.
2. Treatment 1 (P1) as a positive control: mice were given methyl testosterone, at a dose of 0.0042 gr/bw in 1 ml of water.
3. Treatment 2 (P2), mice were given sea cucumbers powdered, at a dose of 0.0004 g/bw in 5 ml of water.
4. Treatment 3 (P3), mice were given KoTeJa + sugar + creamer, at the combination of all doses of 0.0074 gr/bw in 1 ml of water.
5. Treatment 4 (P4), mice were given ginseng coffee products, at a dose of 0.0108 g/bw in 1 ml of water.

The products are given once a day for 12 days orally using a *gavage*. Observations of libido performed on days 0, 1, 2, 3, 4, and 5 and its locomotor activity on days 7, 8, and 9. The observation was continued on the 12th day to see the quality of sperm of male mice.

3 RESULTS AND DISCUSSION

3.1 Change in locomotor activity behavior using a jogging ball.

Mice were put into the jogging ball. Observations were calculated based on wheel rotation within 15 minutes at intervals of 5 minutes. The time recorded since the mice were placed on a wheel (Evascuasiany & Puradisastra, 2010).

3.2 Observation of behavior change of Libido (Introduction).

Measurement of libido conduct by observing the behavior of male mice after 1 hour of treatment. One male mouse and two female mice were put in a fiber cage size of 22 x 16 x 14 cm. Cages lined for the bulkhead between male mice with females. Mice were adapted for 10 minutes, then the seal opened and observed their sexual behavior to see the introduction during the first 15 minutes, then male mice were issued to be rested. After 15 minutes made a second observation. Observations were conducted for six days, i.e. days 0, 1, 2, 3, 4, and 5. The behavior of libido was observed using CCTV cameras.

3.3 Observation of behavior change of Climbing.

Climbing libido measurement was conducted by observing the behavior of male mice after 1 hour of treatment. One male mouse and two female mice were put in a fiber cage. Mice were adapted for 10 minutes, then the seal opened and observed their sexual behavior during the first 15 minutes. The observation was conducted on the stages that occur when male mice ride (climbing) female mice, and then male mice were issued to be rested. After 15 minutes made a second observation. Observations were conducted for six days, i.e. days 0, 1, 2, 3, 4, and 5. The behavior of libido was observed using CCTV cameras.

3.4 Observation of behavior change of Coitus.

Intercourse libido measurement (coitus) was also conducted by observing the behavior of male mice after 1 hour of treatment. One male mouse and two female mice were put in a fiber cage. Mice were adapted for 10 minutes, then the seal opened and observed their sexual behavior during the first 15 minutes. Observation was conducted on the stages that occur during intercourse, then male mice were issued to be rested. After 15 minutes made a second observation. Observations were conducted for six days, i.e. days 0, 1, 2, 3, 4, and 5. The behavior of libido was observed using CCTV cameras.

3.5 Observation of behavior change of locomotor activity.

Locomotor activity is an activity of motion as a result of changes in the electrical activity caused by changes in the postsynaptic membrane permeability and by the release of the transmitter by the presynaptic neurons in the central nervous system (Evascuasiany & Puradisastra, 2010). Figure 1 shows the results of the frequency of locomotor activity of mice. This graph shows that treatment with the provision of the product showed the presence of effects on locomotor activity of mice with higher average locomotor activity at each treatment compared to the control for 15 minutes. A given product affects locomotor activity in mice, and KoTeJa products given in treatment P3 give a better effect of all treatments since the average value of locomotor activity with the difference in numbers rise was not too large. It concluded that KoTeJa provided the most excellent effects on the locomotor activity of mice.

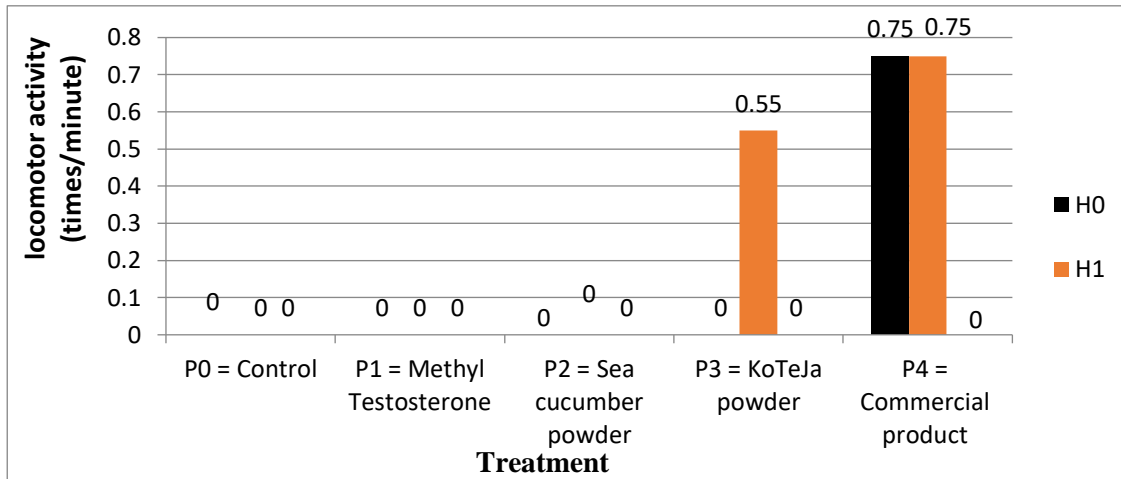


Figure 1. The average behavior of locomotor activity on 1 and 2nd day for 15 min of observation.

P0= negative control (food and drink), P1= positive control, P2: sea cucumbers flour (0.0004 g/kg bw in 5 ml of water), P3= KoTeJa (0.0074 gr/mm in 1 ml of water), and P4= ginseng coffee (0.0108 g/kg bw in 15 ml of water), P3= KoTeJa (0.0074 gr/mm in 1 ml of water), and P4= ginseng coffee (0.0108 g/kg bw in 1 ml of water).

3.6 Observation of behavior change of Libido (Introduction).

It's a stage that occurs when the male mice approached the female mice in the form of kissing mouth and kissing vagina. Figure 2 shows the behavior of frequency (introduction). Based on the frequency of introduction in Fig 2, the sea cucumber ginger coffee (KoTeJa) provides the best effect on the introduction behavior of male mice against the female mice. This influence is suspected because of the active compounds effect in the KoTeJa product. The results are consistent with the results of research conducted by references Widotama (2008) that the provision of flour trepang increases the frequency of sexual behavior (kissing vagina and mounting) in male mice. Sea cucumber flour contains steroids, saponins, fat, protein, carbohydrates, essential amino acids, and minerals that are very useful for increasing libido in male mice (Nurjanah, 2008).

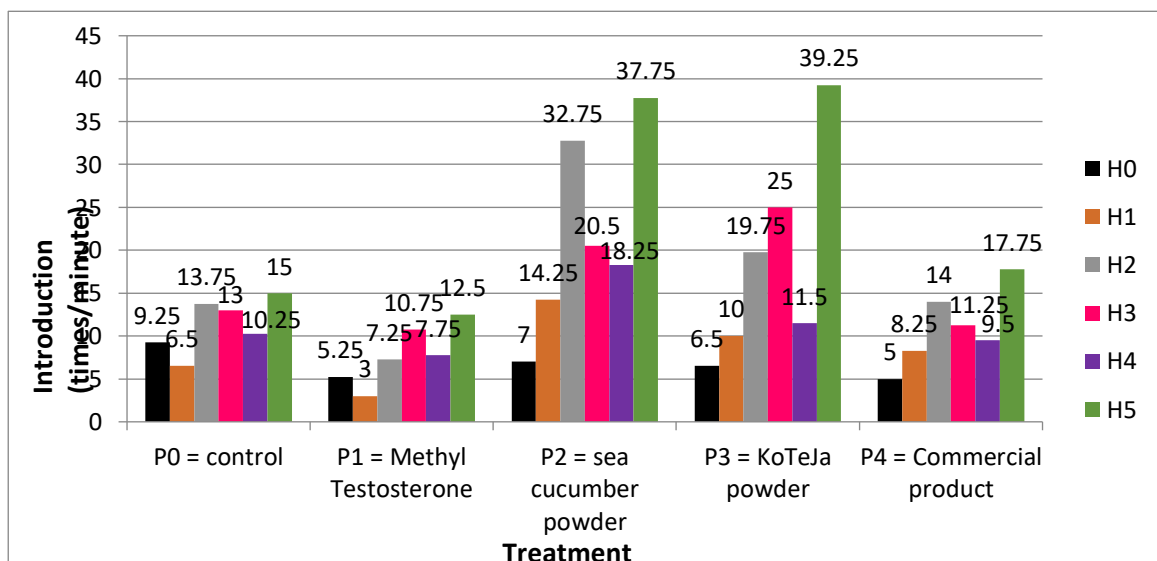


Figure 2. The average behavior of introduction on 0 - 5th day for 30 min of observation.

H0= 1st day, observation without treatment, H2= 2nd day, H3= 3rd day, H4= 4th day, H5= 5th day, P0= negative control (food and drink), P1= positive control, P2: sea cucumbers flour (0.0004 g/kg bw in 5 ml of water), P3= KoTeJa (0.0074 gr/mm in 1 ml of water), and P4= ginseng coffee (0.0108 g/kg bw in 1 ml of water).

3.7 Observation of behavior change of Climbing.

It’s a stage that occurs when the male mice ride (climbing) female mice. Figure 3 shows the behavior change of climbing. The treatment P4 shows the behavior of climbing with an average higher than P2 and P3. However, the treatment of P2 and P3 respectively shows the influence of product on the behavior of climbing, despite the low average daily. This happens because the mice have more activity introduction (recognition), so the mice become exhausted and do little climbing. From Figure 3, it can be seen that the products supplied to each treatment showed different effects depending on the active compound contained in the product.

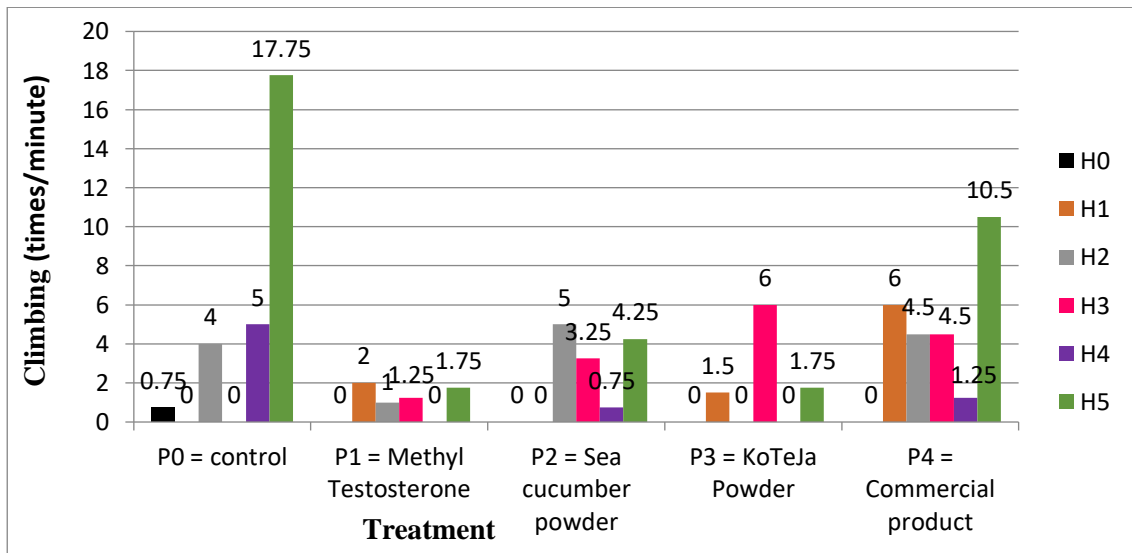


Figure 3. The average behavior changes of climbing on 0 - 5th day for 30 min of observation.

H0= 1st day, observation without treatment, H2= 2nd day, H3= 3rd day, H4= 4th day, H5= 5th day, P0= negative control (food and drink), P1= positive control, P2: sea cucumbers flour (0.0004 g/kg bw in 5 ml of water), P3= KoTeJa (0.0074 gr/mm in 1 ml of water), and P4= ginseng coffee (0.0108 g/kg bw in 1 ml of water)

3.8 Observation of behavior change of Coitus.

It’s the state when the male rides the female mice that lasts for 4-7 seconds to perform coitus (mating). Figure 4 shows the behavior of frequency of intercourse

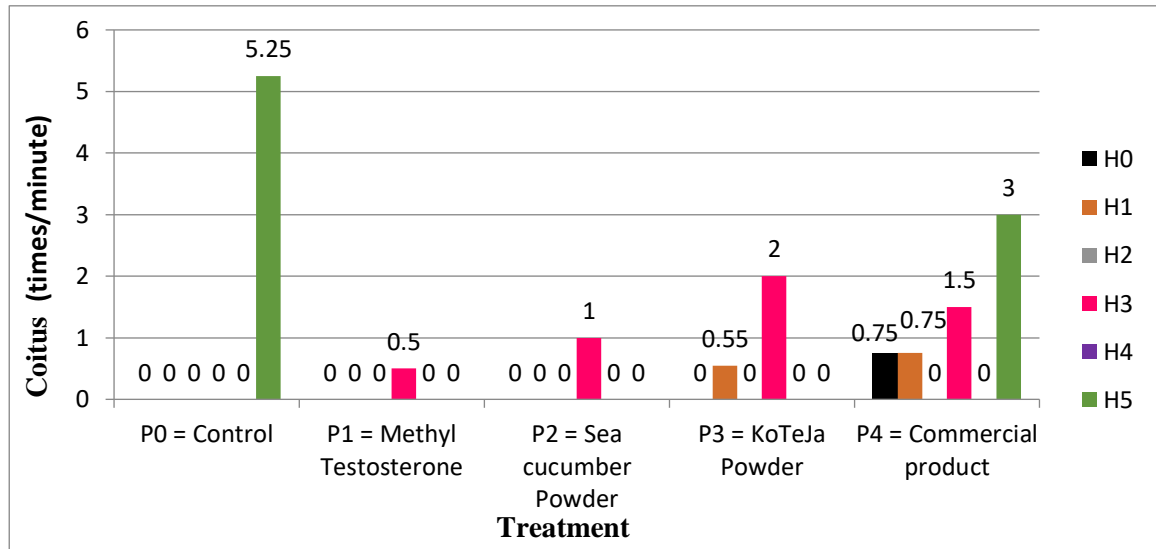


Figure 4. The average behavior changes of climbing on 0 - 5th day for 30 min of observation.

H0= 1st day, observation without treatment, H2= 2nd day, H3= 3rd day, H4= 4th day, H5= 5th day, P0= negative control (food and drink), P1= positive control, P2: sea cucumbers flour (0.0004 g/kg bw in 5 ml of water), P3= KoTeJa (0.0074 gr/mm in 1 ml of water), and P4= ginseng coffee (0.0108 g/kg bw in 1 ml of water).

After the introduction and climbing activity, usually mice will engage in sexual intercourse (coitus). Fig 4 shows that the average response to the behavior of the highest intercourse (coitus) on day five is indicated by P0 and P4. The mating/intercourse is preceded by climbing. The high percentage of intercourse behavior occurs because of a high riding percentage as well. Because at the time of going to engage in sexual intercourse, male mice will ride female mice first, and then just continue with mating that occurred in a few seconds. From these observations, it is stated that the mice had intercourse/mating when male mice rode the female mice for \pm 4-7 seconds and then down and mice immediately licked their genitals to clean the genitals.

In the treatment of P1 and P2, intercourse occurred only on day 3 with a low frequency. While on treatment of P3, intercourse behavior has been seen on the 2nd day and 3rd day, with an average higher than P1 and P2. Meanwhile, P4 treatment showed the best effect on the behavior of intercourse with a high average and intercourse took place almost daily. In the treatment of P1, P2, P3, and P4, the average difference in male mice performed coitus, is thought to occur because of the effect of any product that contains an active ingredient which is also different.

4. CONCLUSIONS

Based on the results of the study influence of sea cucumber ginger coffee (KoTeJa) to behavioral change of Swiss webster male mice libido (*Mus musculus* L.), it can be concluded that sea cucumber ginger coffee (KoTeJa) at a dose of 0.0074 gr/bw provides the best effect to changes in libido behaviors of mice, namely: Changes on behavior of libido introduction is the highest on day 5 (39.25 times). Change in the behavior of climbing is the highest on day 3 (6 times). Changes in the behavior of intercourse (coitus) are the highest on day 3 (2 times). Further study on the mechanism of KoTeJa as a natural aphrodisiac with several dose levels is necessary, so it can be seen the influence of KoTeJa based on the dose of provision to test animals. Further studies regarding KoTeJa as an aphrodisiac in mice on the process of spermatogenesis in histopathology.

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