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RESEARCH ARTICLE

The effects of saluang belum (*Luvunga sarmentosa*) root infusion on sperm motility, viability, and testicle histology in mice (*Mus musculus*)

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Abstract

Background: Men's infertility is frequently caused by a decrease in sperm quality. Herbal medicine, which is relatively cheap and easy to obtain, can treat this condition. People in Central Borneo frequently consume boiled Saluang Belum root (*Luvunga sarmentosa*). *L. sarmentosa* root has the potential to improve sperm quality. **Objective:** To investigate the effects of Saluang Belum root infusion on sperm cell survival and testicle histology in mice (*Mus musculus*). **Method:** *L. sarmentosa* root was extracted using an infusion method, analysed and administered to mice at 200 mg/kgBW, 400 mg/kgBW, and 600 mg/kgBW. Aquadest was used as a negative control for 15 days. On the 16th day, sperm motility and viability were assessed. The right testicles were removed and examined under a microscope at 100x and 400x magnification for five fields of view to examine mice testicle histology. **Result:** Infusion of *L. sarmentosa* contained triterpenoids, flavonoids, steroids, alkaloids, saponins, and phenols. Statistical tests of infused *L. sarmentosa* root on the motility, viability of sperm and spermatogenic potentials of mice indicated that the doses of 200mg/kgBW, 400mg/kgBW, and 600mg/kgBW are significantly increased than control. **Conclusion:** *L. sarmentosa* root infusion increased sperm motility and viability, as well as the spermatogenic cells and Leydig cells in mice.

Introduction

Infertility is a male and female reproductive disorder that results in pregnancy failure after 12 months without the use of sexual contraception. Millions of people of reproductive age worldwide suffer from infertility, which has a negative impact on their family's well-being. Infertility affects approximately 48 million couples and 186 million individuals (Boitrelle *et al.*, 2021). Based on data from the World Health Organisation's (WHO) Demographic and Health Surveys in 2004, it is estimated that there are 186 million women of reproductive age who have been married in developing countries and are infertile (WHO, 2024).

In Indonesia, there are 67 million couples of reproductive age, with 10-15%, or eight million, being

infertile (Halimah *et al.*, 2018; Boitrelle *et al.*, 2021). Infertility can affect both men and women. Around 40% of infertility is caused by women, 40% by men, and 20% by both men and women (Cui, 2020; Assidi, 2022). According to the WHO, men account for half of all cases of infertility (Assidi, 2022). Men's infertility is caused by a decrease in sperm quality, which includes sperm concentration, motility, and viability. Hormonal therapy is one of the remedies used to treat male infertility, but it is expensive. Many citizens consider herbal medication because it is relatively inexpensive, and the long-term side effects are unknown (Masterson *et al.*, 2021).

Many plants are used for alternative medicine in Indonesia, particularly Central Borneo. Saluang belum (*Luvunga sarmentosa*) is a well-known plant in Central

Borneo for increasing male stamina and fertility (Musfirah et al., 2016). According to previous research on the potential of saluang belum root infuse extracted using 70% ethanol, it contained compounds such as flavonoids and steroids that contribute to an increase in sperm quality and viability in mice (Anggriani, 2018; Syarif et al., 2016). The Dayaknese drank water infused with *L. sarmentosa* root (Musfirah et al., 2016).

The effect of the infused root of *Luvunga sarmentosa* on experimental animals to observe sperm quality and histology has not been studied yet. Hence, this study aims to observe the effect of the infused root of saluang belum (*Luvunga sarmentosa*) on *Mus musculus* sperm motility, viability and histology of its testicle.

Methods

Study design and sample size calculation

This was a true experimental study with a post-test-only group design. The samples were 24 mice divided into four groups of six rats each. The first group was the control group, which received aquadest. The second, third and fourth groups received saluang belum root infusion doses of 200mg/kgBW, 400mg/kgBW, and 600mg/kgBW, respectively. The study was conducted at the Wet Biomedical Laboratory, Faculty of Medicine, University of Palangka Raya.

Ethical approval

This study was approved by the Medical Research Ethics Committee of the Faculty of Medicine, the University of Palangka Raya under the number 22/UN24.9/LL/2022.

Extraction

Luvunga sarmentosa root (4 kg) was obtained from Desa Buhut, Muara Teweh, Central Borneo. The root was washed and cut into 1 cm pieces before drying for 14 days. The dried root was then ground into a 2 kg net-weight powder. Saluang belum root infusion was made in an infusion pan heated to 90°C and kept warm for 15 minutes. Figure 1 shows the root of *Luvunga sarmentosa*.



Figure 1: Saluang belum (*Luvunga sarmentosa*)

Phytochemical test

Quantitative phytochemical analysis was carried out to identify the contents of saluang belum root simplicia. The analysis was conducted to screen for flavonoids, terpenoids, phenolics, saponins, alkaloids, steroids, and tannins.

Effects of saluang belum root infusion on sperm motility, viability, and testicle histology in *Mus musculus*

The treatment was applied to the four groups of mice for a total of 15 days. On the 15th day, anaesthesia was administered to the mice, followed by termination with cervical dislocation. The cauda epididymis was then removed via an operation that involved cutting the proximal and distal vas deferens. The cauda epididymis was then chopped and placed in a petri dish with 1 ml of NaCl 0.9%. To analyse the sperm motility, 10 µL sperm suspension was observed under a microscope using 400x magnification. The motility assessment was divided into four criteria; A: moving fast and straight, B: moving slowly, C: moving on the same spot, and D: not moving., the formula for calculating the motility is:

$$\% \text{ Motility} = \frac{A + B}{100} \times 100 \%$$

Then for the sperm viability, the suspension was added to 2% eosin and then placed on prepared microscope slides. The viability of the spermatozoa was examined at 400x magnification. The calculations were based on 200 sperm cells, colourless living spermatozoa, and red dead spermatozoa from its head. The viability observation result was given in percentages (Permatasari et al., 2023).

$$\% \text{ Viability} = \frac{\text{Total of Live Sperm}}{\text{Total of Live Sperm} + \text{Dead (200)}} \times 100 \%$$

Mice histology test was done using the right testicle which was placed in a container of 10% Neutral Buffer Formalin. A histology slide was then created using Hematoxylin and Eosin. Image Raster (Micronos) was used to calculate the average amount of spermatocytes and spermatids in one seminiferous tubule and Leydig cell for five fields of view observation (Listyorini *et al.*, 2021). All data were analysed using one-way ANOVA and multiple comparisons with a post hoc LSD $p < 0.05$.

Results

This study did a quantitative phytochemical test on the boiled root of saluang belum (*Luvunga sarmentosa*) and discovered some compounds such as triterpenoids, flavonoids, phenols, saponins, steroids, alkaloids, and tannins. Table I shows the results of the compound analysis using quantitative phytochemical tests.

Table I: Active compounds in the root of saluang belum (*Luvunga sarmentosa*)

Active compound	Root of saluang belum content
Triterpenoid (mg/ml)	112.133±0.577
Flavonoid (mg/ml QE)	53.583±0.144
Phenol (mg/ml)	38.244±0.077
Steroid (mg/ml)	19.401±0.126
Saponin (%)	9.273±0.130
Alkaloid (%)	7.817±0.081
Tannin (mg/mL GAE)	0.042±0.017

The results show that boiled *Luvunga sarmentosa* root significantly affects sperm motility and viability ($p < 0.05$). The average percentage of motile and viability sperm in the negative group, 200mg/kgBW group, and 600mg/kgBW is shown in Table II. The average result of infused saluang belum (*Luvunga sarmentosa*) root on *Mus musculus* testicle histology of spermatogenic and Leydig cell amount is shown in Table III.

Table II: The average result of infused saluang belum root on *Mus musculus* sperm motility and viability

Group	Sperm motility Mean ± DS	Sperm viability (%) Mean ± DS
Control	52.0±2.09 ^a	52.5±6.15 ^a
200 mg/Kg BW	57.0±1.89 ^b	60.67±5.92 ^b
400 mg/Kg BW	67.17±3.60 ^c	70.5±5.94 ^c
600 mg/Kg BW	77.33±2.07 ^d	81.67±5.71 ^d

Different superscripts on the same column indicate a significant difference ($p < 0.05$). ± is a standard data deviation. DS = Deviation standard.

Table III: The average result of infused saluang belum root on *Mus musculus* testicle histology of spermatogenic and Leydig cell amount

Group	Spermatocyte Mean ± DS	Spermatid Mean ± DS	Leydig cell Mean ± DS
Control	54.3±8.64 ^a	77.0±23.43 ^a	12.33±2.81 ^a
200 mg/Kg BW	64.33±9.58 ^b	107.17±9.28 ^b	20.83.±3.06 ^b
400 mg/Kg BW	74.0±4.60 ^c	170.67±10.93 ^c	27.17±3.76 ^c
600 mg/Kg BW	83.33±6.83 ^d	201.83±18.82 ^d	40.33±6.47 ^d

Different superscripts on the same column indicate a significant difference ($p < 0.05$). ± is a standard data deviation. DS = Deviation standard.

The average number of spermatocytes and spermatids in mice seminiferous tubule histology increases significantly with increasing dose (Figure 2). The

average number of Leydig cells in mouse testicle histology increases significantly as the dose increases (Figure 3).

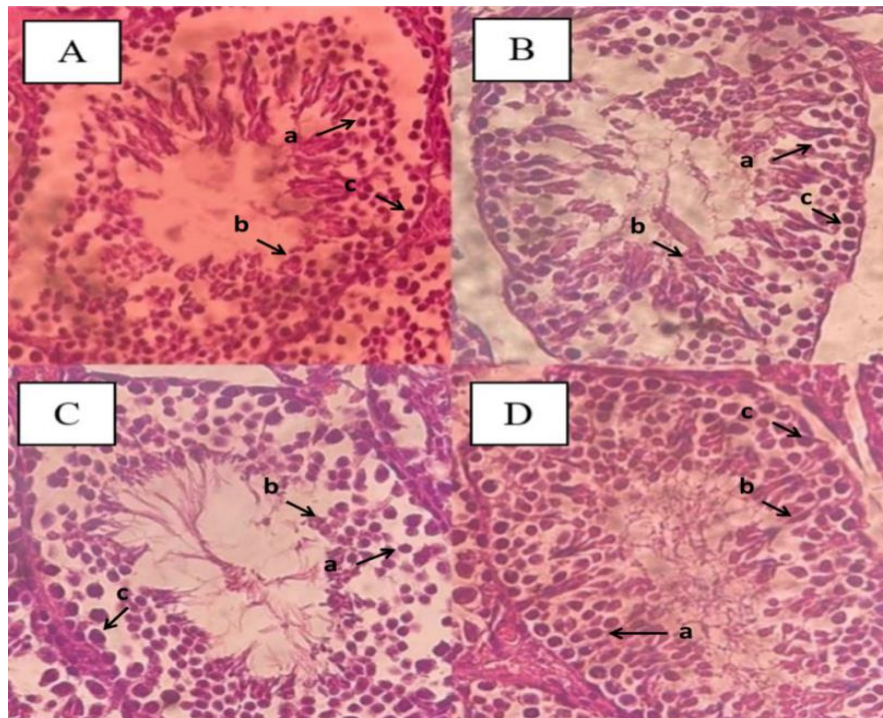


Figure 2: Histology of spermatogenic cell in mice (*Mus musculus*) seminiferous tubule. A: negative control (aquadest), B: dosage of 200mg/KgBW, C: dosage of 400mg/KgBW, D: dosage of 600mg/KgBW. a: spermatocyte, b: spermatid, c: spermatogonium. HE staining, magnification of 400x.

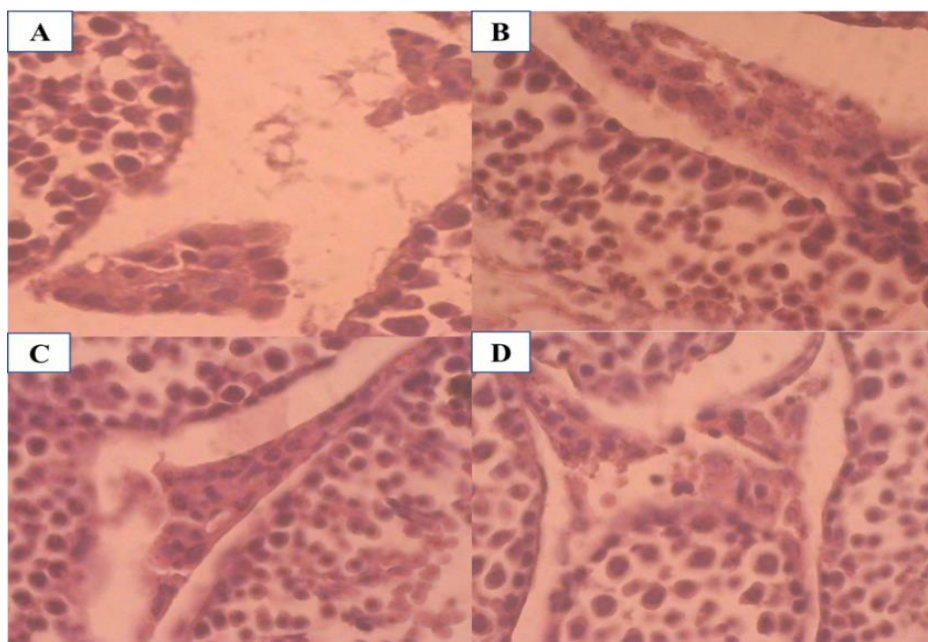


Figure 3: Histology of Leydig cell in mice (*Mus musculus*) seminiferous tubule. A: negative control (aquadest), B: dosage of 200mg/KgBW, C: dosage of 400mg/KgBW, D: dosage of 600mg/KgBW. HE staining, magnification of 100x.

Discussion

The phytochemical test results showed that the infused root of *Luvunga sarmentosa* contained triterpenoids,

flavonoids, phenols, saponins, steroids, alkaloids, and tannins. According to the previous study's findings, saluang belum root ethanol extract contained steroids and flavonoids (Anggriani, 2018). The compounds in the

infused root of *Luvunga sarmentosa* affected sperm quality and quantity in mice, as evidenced by the condition of the seminiferous tubule and an increase in Leydig cells. The steroid binds to androgen receptors. It enters the cytoplasm of the Leydig cells, penetrating a membrane and causing testosterone production. Testosterone is a hormone that can stimulate and maintain spermatogenesis, resulting in functional sperm with good motility and viability (Syarif et al., 2016).

Saponins and alkaloids can stimulate testosterone production (Munyali et al., 2020). Saponin is involved in the biosynthesis of dehydroepiandrosterone (DHEA), which increases testosterone levels in the body (El Hazzam et al., 2020). Saponin can bind with triterpenoid as a steroid precursor, synthesising testosterone (Munyali et al., 2020). Triterpenoid stimulates spermatogenesis and maturation of sperm. Triterpenoid also improves sperm motility, viability, and plasma membrane integrity and protects the acrosome in the sperm cell (Cox-Georgian et al., 2019; Wijayanti et al., 2020). Flavonoids and phenols are two other compounds found in the infused root of *Luvunga sarmentosa*, and both act as antioxidants (Arifin & Ibrahim, 2018; Jofré et al., 2019). Flavonoids can neutralise free radicals in the mitochondria to protect and maximise ATP production needed to optimise sperm production. Additionally, ATP can be transferred to the sperm tail to aid in its motility (Syarif et al., 2016; Permatasari et al., 2023).

When compared to the negative control, an administered dose of *Luvunga sarmentosa* infusion of 200 mg/kgBW resulted in significantly higher sperm quality and quantity, and this trend continued with higher doses. Based on this finding, it is demonstrated that increasing infusion concentration has a dose-dependent effect. Musfirah and colleagues 2016 supported this finding by demonstrating that a 70% ethanol extract can increase spermatogenesis in mice. However, because of the various solvents used, phytochemical screening has some differences. According to Musfirah and colleagues in 2016, an experimental group receiving a dose of 400 mg/kgBW had a decrease in testosterone due to the high steroid content in the 70% ethanol extract, which initiated the negative feedback that suppressed Luteinizing hormone (LH) and Follicle-stimulating hormone (FSH) (Sherwood, 2016; Masterson et al., 2021).

Conclusion

saluang belum (*Luvunga sarmentosa*) root infusion increased sperm viability and motility in mice, as well

as the spermatogenic amount and Leydig cell based on testicle histology, with the highest dose being 600 mg/kg BW. Alkaloids, saponins, triterpenoids, flavonoids, phenolics, and steroids are among the compounds in saluang belum (*Luvunga sarmentosa*) root infusion.

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