



EFFECTS OF SOYBEAN JUICE (*Glycine max* L.) ON SPERMATOZOA FERTILITY AND TESTICULAR WEIGHT IN RATS (*Rattus norvegicus* L.)

Pengaruh Sari Kedelai (*Glycine max* L.) Terhadap Fertilitas Spermatozoa dan Berat Testis Tikus (*Rattus norvegicus* L.)

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ABSTRACT

Soybeans (*Glycine max* L.) contain isoflavone compounds which can disrupt hormonal balance through negative feedback on the hypothalamic-pituitary-gonadal axis causing inhibition of Leydig cell activity and Sertoli cell function. The aim of this research was to determine the effect of giving soybean juice on spermatozoa fertility and testicular weight in rats (*Rattus norvegicus* L.). This type of experimental research was structured based on a Completely Randomized Design (CRD) pattern consisting of 4 treatments and 6 replications, namely K (control/drinking water), P1 (7.1 g/kgBW/day), P2 (14.2 g/kgBW/day) and P3 (21.3 g/kgBW/day). Soybean extract was given for 49 days, and on the 50th day the rats were sacrificed and dissected to observe variables in the form of testicular weight and spermatozoa fertility (morphology, pH and viscosity). Data were analyzed using ANOVA and further BNT test $\alpha=0.05$. The results showed that administration of soybean extract caused a decrease in the percentage of testicular weight and spermatozoa fertility. Testicular weight is K=0.98 g, P1=1.08 g, P2=0.77g and P3=0.62 g. The percentage of abnormal spermatozoa morphology increased, namely K = 17.8%, P1 = 37.0%, P2 = 47.7%, P3 = 56.0%. The acidity of sperm pH increases, namely K= 7.50, P1=6.17, P2=5.67, and P3=5.83. Sperm-matozoa viscosity decreased, namely K=1.57, P1=1.58, P2= 0.76 and P3=0.77. The results of the study concluded that soybean juice caused a decrease in testicular weight and spermatozoa fertility in mice.

Keywords: *Glycine max*, *Rattus norvegicus*, *Spermatozoa*, *Testicles*

ABSTRAK

Kacang kedelai (*Glycine max* L.) mengandung senyawa isoflavon yang dapat mengganggu keseimbangan hormonal melalui umpan balik negatif terhadap poros hipotalamus-hipofisis-gonad menyebabkan hambatan aktivitas sel leydig dan fungsi sel sertoli. Tujuan penelitian ini untuk mengetahui pengaruh pemberian sari kacang kedelai terhadap fertilitas spermatozoa dan berat testis tikus (*Rattus norvegicus* L.). Jenis penelitian eksperimental disusun berdasarkan pola Rancangan Acak Lengkap (RAL) terdiri dari 4 perlakuan dan 6 ulangan yaitu K (kontrol/ air minum), P1(7,1 g/kgBB/hari), P2 (14,2 g/kgBB/hari) dan P3 (21,3 g/kgBB/hari). Sari kacang kedelai diberikan selama 49 hari, dan pada hari ke 50 tikus dikorbankan dan dibedah untuk diamati variabel berupa berat testis dan fertilitas spermatozoa (morfologi, pH dan viskositas). Data dianalisis menggunakan ANOVA dan uji lanjut BNT $\alpha=0.05$. Hasil penelitian menunjukkan bahwa pemberian sari kacang kedelai menyebabkan penurunan persentase berat testis dan fertilitas spermatozoa. Berat testis yaitu K=0,98 g, P1=1,08 g, P2=0,77g dan

P3=0,62 g. Persentase morfologi spermatozoa abnormal meningkat yaitu K = 17,8%, P1=37,0%, P2=47,7%, P3= 56,0%. Keasaman pH sperma meningkat yaitu K= 7,50, P1=6,17, P2=5,67, dan P3=5,83. Viskositas spermatozoa menurun yaitu K=1,57 P1=1,58, P2= 0,76 dan P3=0,77. Hasil penelitian disimpulkan bahwa sari kacang kedelai menyebabkan penurunan berat testis dan fertilitas spermatozoa tikus.

Kata kunci: *Glycine max*, *Rattus norvegicus*, Spermatozoa, Testis

INTRODUCTION

Soybean (*Glycine max* L.) is a plant from the Fabaceae family which is widely used in society as a food ingredient as well as a natural supplement (Mishra et al., 2025). Soybeans are known to have high nutritional value, contain quality vegetable protein, unsaturated fats, and are rich in vitamins (such as vitamin B complex) and minerals (such as iron, calcium and magnesium) as well as other compounds such as isoflavones (Guilherme et al., 2022). These contents make soybeans useful for maintaining health, including heart function and metabolism (Alleza et al., 2025). Soybeans consumed excessively and in high quantities can have negative effects, because they contain isoflavones which resemble the hormone estrogen. This compound has the potential to affect the hormonal system, especially in men, with the risk of disrupting fertility (Sousa, 2021).

Phytoestrogens in soybeans, especially isoflavones such as genistein and daidzein, can imitate the action of the hormone estrogen in the body because of their similar structure, so that when consumed in high amounts (Kim, 2021), these phytoestrogens can bind to estrogen receptors in the male reproductive system, disrupting the balance of testosterone and estrogen hormones (Petrine & Bianco-borges, 2020). This hormonal imbalance can affect the spermatogenesis process in the testicles, reducing the production and quality of spermatozoa (Li et al., 2024). Excessive phytoestrogen exposure is also associated with increased oxidative stress in testicular tissue, which can damage sperm-producing cells and worsen overall sperm quality (Assan et al., 2018).

The isoflavone content in soybeans reaches 2-4 mg/g dry soybeans and this amount will vary in other processed

products (Foodstuffs et al., 2023). This isoflavone content is known to inhibit the enzyme 17- β -hydroxysteroid-oxidoreductase, an enzyme used to synthesize testosterone, which results in a decrease in testosterone levels in Leydig cells (Jin et al., 2020). Another bad impact of decreasing testosterone is a decrease in the quality of spermatozoa, resulting in the perception that the presence of isoflavone phytoestrogens in soybeans has a bad effect on male fertility (Khaled et al., 2021). The isoflavone content in soybean seeds varies depending on the soybean variety, environment, plant growing conditions and post-harvest handling (Baek, 2020).

Infertility is a disorder of the reproductive system, this condition is characterized by the inability to produce offspring naturally for a married couple (Mansour, 2023). Infertility is still a world health problem, globally there are cases of infertility in couples of childbearing age (PUS), around 50-80 million infertile couples in the world (Liang et al., 2025). Infertility in Indonesia according to Central Statistics Data in 2012, 10-15% or around 4-6 million of them are infertile and this is increasing every year. The failure of married couples to have children, 40% of difficulties in having children are found in women, 40% in men and 20% in both (Babakhanzadeh et al., 2020).

Infertility factors in men are divided into internal factors and external factors (Bräuner et al., 2020). Internal factors include DNA abnormalities in the formation of special proteins which function in the formation of male reproductive tissue and the spermatogenesis process, while external factors occur due to exposure to substances in the surrounding environment such as heavy metals, radiation, cigarette smoke, alcohol and drugs (Cannarella et al., 2020). Other disturbing substances can be found naturally in food that can affect male fertility,

one of which is consumption of soybeans which can interfere with the spermatogenesis process. Several studies state that consuming soybeans can cause reproductive defects and infertility (Pecora et al., 2023). The isoflavone content of soybeans is high enough so that it can be used as research material in the field of reproduction to see the effect of isoflavones on the male reproductive system (Khaled et al., 2021).

Previous research conducted by (Nita & Kunci, 2015), which used soybean extract on the quality and quantity of male rat (*Rattus norvegicus*) spermatozoa using varying doses of 2.52 mg, 3.78 mg and 5.04 mg of soybean extract given for 48 days, found that there was a significant effect on decreasing motility and decreasing viability of male rat spermatozoa, so the researchers were interested in trying to use soy milk (*Glycine max* L.) as a test material. Based on this, it is important to carry out this research to see the effect of soybean extract (*Glycine max* L.) on testicular weight and spermatozoa cell fertility. It is hoped that this research can provide information to the public regarding the effects of soybeans on reproduction.

MATERIALS AND METHODS

Place and Time of Research

This research was carried out in the Biology Laboratory of the Zoology Unit and the mouse cage of the Faculty of Mathematics and Natural Sciences, Halu Oleo University, Kendari Southeast Sulawesi from April to June 2025.

Material

Materials used include: rats (*Rattus norvegicus* L.) Wistar strain, drinking water, commercial pellets (BP11-BRAVO) produced by PT. Charoen Pokphand Indonesia, chloroform, husk, tissue paper, label paper, NaCl 0.9%, distilled water, Eosin-nigrosin dye, 20% Giemsa dye, and 96% methanol.

Method

A total of 24 healthy rats aged 2 months, body weight \pm 200 grams were divided into 4 treatments and 6 replications. Rats were acclimated, their body weight was weighed and fasted for 24 hours before

treatment. Making soybean juice is done by drying the soybeans using an oven at 60°C for 48 hours and powdering them to then make a dose according to the treatment, namely Control (drinking water); soybean juice on P1 (7.1 gr/kgBW/day); P2 (14.2 gr/kgBW/day) and P3 (21.3 gr/kgBW/day). Soybean juice was given orally using a syringe with a volume of 2 mL. Rats were treated for 49 days, on the 50th day the rats were sacrificed by anesthesia using 10% chloroform, then dissected and testicles were taken to observe the weight of the testicles and cauda epididymis to observe sperm fertility parameters.

Observation of testicular weight is carried out by weighing the testicular organs using analytical scales. The pH of spermatozoa was measured by dripping semen on pH paper with a scale of 5.5-9.0 and compared with controls. The viscosity of spermatozoa is measured by dipping a glass stick into the cement, then lifting it slowly to see the distance the semen drips to form a thread. Cement threads that form >2 cm before breaking are said to have high viscosity. The morphology of spermatozoa is observed by: One drop of spermatozoa suspension is placed on a glass object, then a smear is made by moving another glass object above it at an angle of 45°. The preparations were air-dried for 15 minutes, then fixed with 96% methanol for 5 minutes, stained with 20% Giemsa solution for 30 minutes, and rinsed with distilled water. Counts were carried out under a microscope with 100X magnification on 100 spermatozoa cells using a hand counter. The percentage of spermatozoa morphology is calculated based on the formula according to Muhammad Ja'far Luthfi & Mahanem Mat Noor (2023) as follows:

Abnormal spermatozoa morphology

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

Information:

A = Normal morphology

B = Abnormal morphology

Ethical Consideration

The ethical clearance was obtained from the Faculty of Natural Sciences and Mathematics with Letter Number UN.29.17.5/SK/EC/2025.

Data Analysis

The research data were analyzed using Analysis of Variance (ANOVA) and a further BNT test with a confidence level of 95% to determine the mean differences between treatments (Ibrahim & Abdullahi, 2023).

RESULTS AND DISCUSSION

Spermatozoa Fertility

The results of observations of spermatozoa fertility parameters in the form of pH viscosity and spermatozoa morphology are shown in Table 1.

Table 1. Mean Percentage of Spermatozoa Fertility Between Soybean Milk Treatments

Treatment	Viscosity (%) \pm SD	pH \pm SD	Abnormal Morphology (%) \pm SD
K	1.57 \pm 0.08 ^a	7.50 \pm 0.55 ^a	17.8 \pm 2.8 ^a
P1	1.57 \pm 0.08 ^a	6.17 \pm 0.75 ^b	37.0 \pm 3.6 ^b
P2	0.76 \pm 0.08 ^b	5.67 \pm 0.82 ^b	47.7 \pm 3.6 ^c
P3	0.77 \pm 0.08 ^b	5.83 \pm 0,75 ^b	56.0 \pm 4.5 ^d

Note: Numbers followed by different letters indicate significant differences based on the BNT test ($\alpha=0.05$)

Table 1 shows that spermatozoa viscosity decreased from 1.57 in controls to 0.76 (P2) and 0.77 (P3). Low viscosity reflects impaired secretion from accessory glands such as the seminal vesicles and prostate gland. This disorder is most likely triggered by a decrease in testosterone levels which regulate the function and secretion of this organ. Semen fluid that is thin and less viscous means that spermatozoa do not have an adequate medium to maintain their integrity and direction of movement. Spermatozoa at low viscosity become more susceptible to membrane damage. This condition directly affects the viability and motility of spermatozoa. A decrease in viscosity is also associated with an increase in the number of abnormal spermatozoa that are unable to adapt to unstable environmental conditions. Imperfect spermatozoa structure, such as a distorted head or bent tail, inhibits the progressive movement ability needed to reach and fertilize the ovum. Low viscosity accelerates the disintegration of spermatozoa, reduces the potential for fertilization, and increases the death rate of germ cells due to not being protected by a sufficiently thick and stable seminal layer.

Table 1 shows that the mean pH value of spermatozoa in the treatments showed a decrease. A decrease in pH can cause disruption of the spermatozoa plasma membrane and reduce the integrity of cell structure (Safitri & Isnaini, 2021). Changes in the acidic environment accelerate the protein

denaturation process, reduce enzyme activity in the sperm head, and damage the acrosome which is important in the fertilization process. Mitochondria in the midpiece are not able to produce energy optimally in low pH conditions, so that the movement of spermatozoa is hampered (Durairajanayagam & Henkel, 2020). This combination of structural damage and metabolic disorders causes decreased motility, viability and increased morphological abnormalities of spermatozoa (Oehninger & Kruger, 2020). These results indicate that a pH that is too low is one of the main indicators of damage to semen quality due to exposure to phytoestrogens from soy milk.

Abnormal spermatozoa morphology increased in mice given soybean extract compared to controls. The percentage of abnormal spermatozoa increased with increasing treatment dose, from 17.8% in controls to 56.0% in P3. These results indicate that increasing the dose of soybean juice has a direct impact on reducing sperm quality and the morphological structure of sperm cells. The presence of morphological abnormalities indicates disturbances in the sperm formation stage due to hormonal imbalance or exposure to toxic compounds such as phytoestrogens in soybean juice (Tawfik et al., 2025). The spermatogenesis process requires balanced hormonal conditions so that the sperm produced has perfect shape and structure. Hormonal balance is disturbed, the sperm that is formed can experience

morphological abnormalities, such as a deformed head, a bent neck, or an abnormal tail (Oehninger & Kruger, 2020).

Phytoestrogens in soybean juice cause an increase in free radicals in testicular tissue, thereby worsening the condition of Sertoli and germ cells through the process of apoptosis and cytoskeleton dysfunction. High exposure to isoflavones also

triggers a decrease in the structural integrity of the seminiferous tubules where spermatogenesis takes place. Sertoli cells lose their ability to support nutrition and protection for germ cells, causing DNA fragmentation in spermatids. The morphology of the abnormal spermatozoa found can be seen in Figure 1.

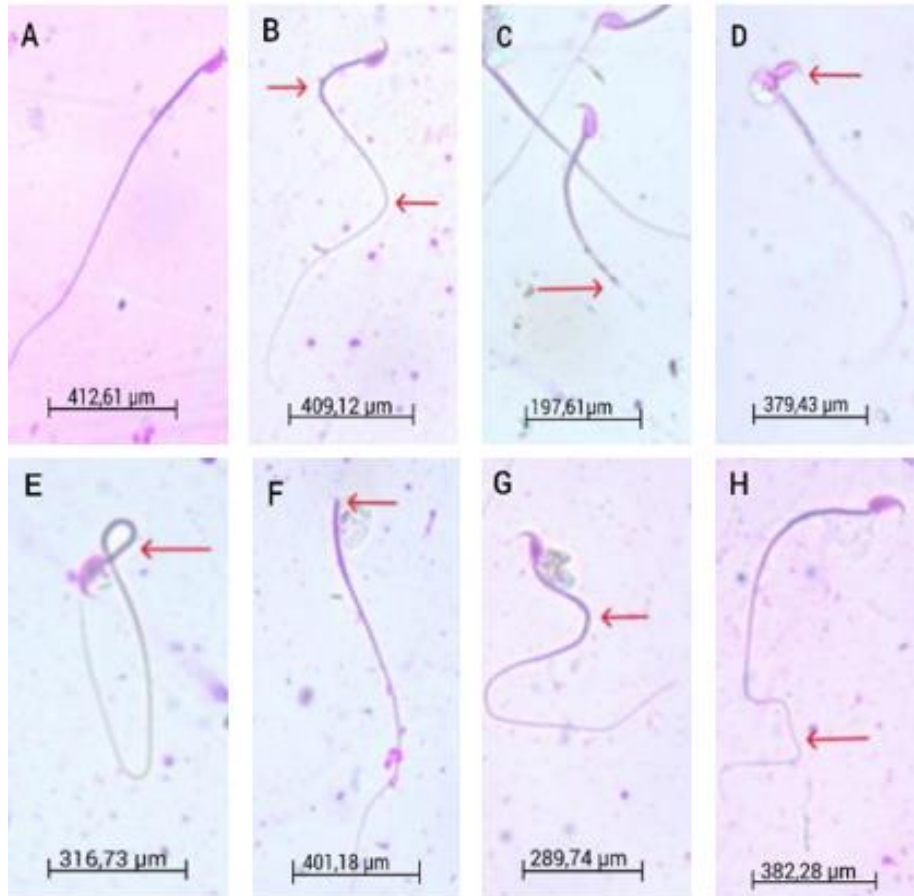


Figure 1. Various abnormal forms of spermatozoa found after administering soybean juice. A Normal spermatozoa; B. Zigzag body spermatozoa; C. Spermatozoa without tails; D. Double-headed spermatozoa; E. Spermatozoa body rolled up wavy tail; F. Head body spermatozoa; G. Wavy spermatozoa; H. Wavy tail spermatozoa

Testicular Weight

The results of observing the average weight of rat testicles between treatments can be seen in Table 2.

Table 2. Average weight of rat testicles between soybean juice treatments

Treatment	Average ± SD
K	0.98±0,11 ^a
P1	1.08±0,12 ^a
P2	0.77±0,04 ^b
P3	0.62±0,04 ^c

Note: Numbers followed by different letters indicate significant differences based on the BNT test (α=0.05)

Table 2 shows that soybean extract treatment had a significant effect on reducing testicular weight. The testicular weight of the control treatment was 0.98 g, while P1 increased to 1.08 g. P2 decreased to 0.77 g and P3 was even lower, namely 0.62 g. This value indicates the effect of soybean extract on testicular degeneration. Isoflavones in soybeans are estrogenic because they are able to bind to estrogen receptors (ER α and ER β) in target tissues, including the testes and hypothalamus. Isoflavone activity in soybeans causes disruption of hormonal balance through negative feedback on the hypothalamus-pituitary-gonad axis (Zhao et al., 2025). Gonadotropin-releasing hormone (GnRH) secretion decreases, causing a decrease in luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Decreased LH inhibits Leydig cell activity in testosterone production, while low FSH reduces Sertoli cell function. Decreased testosterone levels have an impact on disrupting spermatogenic cell maturation and weakening the structure of testicular tissue. This process explains the decrease in testicular weight and damage to the male reproductive system as a whole (Dutta et al., 2021).

CONCLUSION

The conclusion of this study is that soybean juice at a dose of 14.2 mg/g BW (P2) and a dose of 21.3 mg/gBW (P3) significantly reduces normal testicular weight and reduces spermatozoa fertilization with an increase in the percentage of abnormal spermatozoa morphology, a decrease in pH value, and a decrease in the viscosity of semen. The results of this study provide information about other effects of soybeans on reducing male reproductive function.

AUTHOR'S CONTRIBUTION

The research team's contributions to this research are: Wa Ode Harlis: contributed to data collection, data analysis and writing of the paper. Nurhayu Malik: contribution was data analysis and revision of the paper draft. Isdayanti: contribution is data collection and data analysis. Takdir Saili: contributed to discussing research data and

Resman: contribution is revision according to the review correction.

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