Effect of Repeated Exposure of Soy Flour on Testicular Histology and Morphology of Mice Spermatozoa

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ABSTRACT

Isoflavones are active compounds in soybean seeds with a chemical structure resembling 17β-estradiol and are able to bind to estrogen receptors on Leydig cells. The chemical structure and physiological activity of isoflavones are similar to estrogen. This research was conducted to evaluate changes in the structure of testicular seminiferous tubule tissue and spermatozoa morphology. Male Balb/c mice, 9 weeks old, 20–25 g, 24 mice, grouped into 4 treatments: 6 mice in control group (P0), 6 mice given 0.369 g/kg soy flour (P1), 6 mice given 0.369 g/kg soy flour 0.74 g/kg (P2), 6 mice given soybean flour 1.47 g/kg (P3) treatment for 36 days. Surgery and removal of testicular organs were carried out on the 37th day, and HE staining and preparation of spermatozoa smears were made. Data analysis parameters are changes in the structure of the testicular seminiferous tubule tissue and testicular germ cells (spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and spermatozoa). Spermatozoa abnormalities include the head, neck, and tail. The results showed that the administration of soy flour caused the widening of the testicular tubule lumen; no spermatozoa were found in the tubule lumen. Spermatozoa abnormalities in treatment P1 was 18%, P2 was 29%, and P3 was 38%.

Keywords: Isoflavones, soybeans, spermatozoa abnormalities, testicular seminiferous tubules.

1. INTRODUCTION

Soybean (Glycine max Merr.) is a Leguminocae plant; the properties and nutritional content of soybeans are increasingly popular for consumption throughout the world at all ages (Caceres et al., 2023). Soybeans are widely used in the form of processed products in the form of flour, fermented products and solutions. Countries in Asia consume a lot of soybeans in fermented soybean products, such as soy sauce, yoghurt, tofu, and tempeh, and tauco (Surono, 2016; Mah et al., 2019; Kim, 2021). The use of soybeans is not only a food product but also a health product (Dukariya et al., 2020; Gawęda et al., 2020; Agomoh et al., 2021; Qin et al., 2022).

The complexities of soybean compounds in various processed products are used as food ingredients (O’Keele et al., 2015; Ganesan & Xu, 2017; Varnosfaderani et al., 2019; Król-Grzymała & Amarowicz, 2020). Isoflavone is one of the secondary metabolite compounds in soybeans and is widely used for health (Danciu et al., 2017; Popa & Rusu, 2017; Das et al., 2020; Chen & Chen, 2021; Huang et al., 2022; Wang et al., 2022).

Isoflavone has a chemical structure similar to 17β-estradiol (Barnes, 2010; Nanashima et al., 2017; Miklos et al., 2018) and has the ability to bind to estrogen receptors (Vitale et al., 2013; Nanashima et al., 2017). Isoflavones, including the flavonoid group, have potential estrogenic activity (Andres et al., 2015; de Ávila et al., 2018). Isoflavones, as estrogenic compounds from plants, are often referred to as phytoestrogens (Pilzáková et al., 2010; Krčžová et al., 2019). Daily intake of soybeans causes accumulation and influences various biological processes in the body (Zaheer & Humayoun Akhtar, 2017; Pabich & Materska, 2019; Kim, 2021). The estrogenic activity of isoflavones affects all systems in the body, including the male reproductive system (Vitale et al., 2013; Lecomte et al., 2017; Sleiman et al., 2021).

Isoflavone activity as a secondary metabolite compound of soybeans is able to act like the hormone estrogen, often affecting the endocrine system (Garg et al., 2020; Gawe et al., 2022).
Research results have been published that show the good influence of soybeans on the body system, but there is a negative impact of soybeans on the male reproductive system by disrupting spermatogenesis, sperm capacitation, and fertility (Cederroth et al., 2010; Sleiman et al., 2021).

Soybean isoflavone compounds are thought to have antifertility capabilities (Canivenc-Lavier & Bennetau-Pelissero, 2023). Isoflavones reduce spermatogenesis, the number of spermatozoa, reduce the size of the seminal vesicles (Sleiman et al., 2021). Administration of isoflavones can delay the onset of puberty in male mice (Caceres et al., 2015). Administration of low and high doses of isoflavones causes a decrease in androgen levels, decreased sperm quality, decreased testicular weight, and decreased diameter of the seminiferous tubules (Mostafa et al., 2021; Caceres et al., 2023).

2. Materials and Method

2.1. Making Soy Flour
The local Anjasmoro variety of soybeans is obtained from agricultural products in Bancar Village, Ponorogo Regency, East Java, and Indonesia. Soybean seeds were identified in the plant taxonomy laboratory at Universitas PGRI Madiun Indonesia with identification number 017b/Taxo-Plant/Biology/III/2022. Soybeans were dried in an oven at 50 °C, ground using a blender. Fine soybean powder was sifted using a 60 mesh sieve. Soybean powder was weighed based on the size given to each treatment, namely 0.369, 0.74, and 1.47 g/kg.

2.2. Experimental Animals
Male Balb/c strain mice (Mus musculus), 9 weeks old, 20–25 g, 24 mice. Mice were maintained at room temperature (±27 °C), relative humidity 50%–60% and a 12-hour light cycle. One week of acclimatization in the rearing cage. Mice were grouped into 4 treatments, namely 6 mice in the control group (P0), 6 mice given 0.369 g/kg soy flour (P1), 6 mice given 0.74 g/kg soy flour (P2), and 6 mice given 1.47 g soy flour, g/kg (P3).

2.3. Experimental Animal Treatment Techniques
Soy flour was administered by induction into the stomachs of mice using a gavage tube once a day for 36 days.

2.4. Surgery, Testes Removal, and HE Staining
Surgery was carried out by dislocating the neck on the 37th day, taking the testes for making histology preparations and staining with Hematoxyline Eosin (HE).

2.5. Making Sperm Preparations
Take the epididymis and give 5 ml of 0.9% NaCl until a suspension is formed. Then add 4 drops of eosin and 2 drops of negrosin, and stir until evenly mixed. Take the solution and then place it on a glass object to make smear preparations.

2.6. Data Analysis
Data on changes in the histological structure of the testes were analyzed descriptively based on changes that occurred in the seminiferous tubules, including germ cells, including spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and spermatozoa and tubule lumen. Morphological analysis of spermatozoa is based on observation and percentage calculation using a haemocytometer.

3. Result
Based on the results of observations on the histology of the testicular seminiferous tubules, results were obtained that there were changes in spermatogenesis in the germ cells in the testicular seminiferous tubules, stratum basale, stratum myodeum, and stratum fibrosum (Fig. 1).

The results of observations of spermatozoa smear preparations showed changes in the morphological structure of spermatozoa (Fig. 2). Fig. 2 shows the different morphological structures of spermatozoa after treatment in mice.

4. Discussion
Histological analysis of mouse testes after treatment with soybean flour (Fig. 1). Gives results that the testicular seminiferous tubules have not changed. Spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa are clearly visible. The tubule lumen is full of spermatozoa wide, not many spermatozoa are found in the tubule lumen, D. formation of spermatozoa, primary spermatocytes and secondary spermatocytes, the tubule lumen is very wide, spermatozoa morphogenesis is reduced so that no spermatozoa are found in the tubule lumen.

Giving soy flour contains isoflavone compounds, which are compounds similar to the hormone estrogen. Isoflavone has the activity of being able to bind to estrogen receptors on ERα and ERβ (Kuiper et al., 1996; Uifălean et al., 2015; Bairi et al., 2022). The binding of isoflavones to estrogen receptors causes physiological mechanisms resembling the hormone estrogen (Rando et al., 2009; Andres et al., 2015). The physiological activity
of isoflavones affects the male reproductive system. The estrogenic ability of isoflavones shows its ability to bind to estrogen receptors on Leydig cells (Kuiper et al., 1996; Sherrill et al., 2010; Lozi et al., 2021).

Estrogenic compounds (phytoestrogens) such as isoflavones can interfere with spermatogenesis because these compounds inhibit the action of the enzyme 17β-hydroxysteroidoxidoreductase, an enzyme needed for testosterone synthesis (Le Bail et al., 1998; Lu et al., 2000; West et al., 2005; Swart et al., 2019; Rochester & Millam, 2009). The antiandrogenic effect of phytoestrogens can inhibit the secretion of Luteinizing Hormone and Follicle Stimulating Hormone from the pituitary which results in a decrease in testosterone secretion levels in Leydig cells. Decreased testosterone levels cause spermatogenesis not to run optimally, which ultimately reduces sperm quality, including spermatozoa morphology (Pasqualotto et al., 2005; Chavario et al., 2008; Mitra et al., 2012; Meena & Reddy, 2014; Panpan et al., 2023).

Giving soy flour to treatments P1, P2, and P3 contained abnormal spermatozoa (Fig. 3). The percentage of sperm abnormalities in the control treatment (P0) was 2%, P1 was 18%, P2 was 29%, and P3 was 38%. Giving P3 treated soybean flour for 36 days showed a response to an increase in spermatozoa morphology abnormalities. Phytoestrogen accumulation at a dose of 1.47 g/kg was relatively high so that soybean flour entering the bodies of mice (Mus musculus, L.) caused abnormalities in spermatozoa morphology. The results of observations of spermatozoa morphological abnormalities include head, neck, and tail abnormalities. The abnormalities in mice spermatozoa treated with soy flour were forked tails, normal spermatozoa, double/forked heads and tails, round heads without tails, head abnormalities, long curved tails, kidney-shaped heads, macrocephalic and triple tails, macrocephalic, curved bodies and tails, double head, straight body and tail, flat head, macrocephalic and erect tail, circular body, curvy tail, curved body, no head, no neck and tail, curved middle body (Fig. 2).

Spermatogonia are unable to develop into primary spermatocytes, secondary spermatocytes, and spermatids (Fig. 1C). This occurs because isoflavones as estrogenic compounds similar to the hormone estrogen cause deficiency of the aromatase enzyme as an enzyme that converts testosterone into estradiol (Rambhatla et al., 2016; Schuster et al., 2016; Hammes & Levin, 2019; Guercio et al., 2020). As a result of aromatase deficiency, spermatid damage occurs, and spermatid maturation decreases (Schlegel, 2012; Hammes and Levin, 2019). Administration of high concentrations of phytoestrogens causes apoptosis of germ cells in the seminiferous tubules before and after the meiotic phase (Assinder et al., 2007; Kale et al., 2022).

5. Conclusion

The results showed that exposure to soy flour during the spermatogenesis period had an effect on spermatozoa morphology. The percentage of spermatozoa morphology abnormalities in P3 with a dose of 1.47 g/kg soy flour was 38%.

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Conflict of Interest

Authors declare that they do not have any conflict of interest.

References


