The effect of methanol extract of soybean (Glycine max L. Merr.) on rat testicular steroid hormones

R Aryani¹, H Manurung¹, S Moeljopawiro², L H Nugroho³, and P Astuti⁴,*
¹Department of Biology, Faculty of Mathematics and Natural Sciences, Mulawarman University, Samarinda, Indonesia
²Department of Biochemistry, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia
³Department of Plant Anatomy, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia
⁴Department of Physiology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia

*Corresponding author: pastuti2@gmail.com

Abstract. Soybeans contain phytoestrogens whose structure resembles estrogen in the body. Its function can be estrogen and antioestrogens, affecting the metabolism of sex steroid hormones. This study aims to determine the effect of soybean methanol extract on testosterone and estrogen levels in testicular rats. This study aims to determine the effect of soybean methanol extract on testosteron and estrogen levels in testicular rats. Twenty male Wistar rats were divided into 4 groups namely: control and treatment group were given soybean extract (250 mg/kg BW, 500 mg/kg BW) and genistein (0.3 mg/kg BW), respectively, for 52 days. The results of this research indicate that the effects of soybean methanol extract caused weight gain and decreased testicular weight. In addition, it showed that 500 mg/kg BW of soybean methanol extract reduced the level of testicular estrogen. It can be concluded that soybean methanol extract significantly reduced testicular estrogen levels for 52 days.

1. Introduction
Soybean is one of Legumincea and has been consumed by humans in everyday life. Lately, many researches have been done on the effects of Legumincea. Research has been carried out because this plant has a number of compounds that have been isolated and has many important roles in human life. This is widely used as a prevention and treatment of diseases in pharmacology and medicine. Plants contain phytoestrogens, which can function as estrogen. Phytoestrogens consist of isoflavones, coumestants, and lignin. Isoflavones are the most common type of phytoestrogens found in soybeans. The main isoflavones in soybeans are genistein and daidzein [1]. Phytoestrogens not only have a variety of positive physiological effects; but it can also have a negative effect especially on the reproductive tract of most animal species. Many phytoestrogens can react as estrogen agonists or antagonists that affect the metabolism of sex hormones and are associated with biological activity.

During this time androgens are known as male sex hormones while estrogen is a female sex hormone. However, much evidence has shown that these hormones play an important role in regulating physiological function in men and women and in both sexes, androgens are metabolized to
estrogen. However, excessive exposure to estrogen can interfere with male reproductive function [2]. Estrogen (E2) is a pleiotropic hormone which is usually produced from testosterone (T) in the testes in mammals by the cytochrome P450 aromatase enzyme, which is synthesized in Sertoli, Leydig and germ cells [3]. E2 has a direct physiological effect on these cells to trigger complete and correct spermatogenesis. For example, E2 regulates T synthesis in Leydig cells [4] and ionic homeostasis in Sertoli cells [5], and it acts as a survival factor for germinal cells [6].

Recently, much public concern has increased that exposure to estrogenic chemicals in the environment can disrupt the endocrine and reproductive systems, for example, can reduce gonadal size, impair spermatogenesis, and reduce sperm count and quality. Previous studies have shown that isoflavones can cause male reproductive toxicity including disorders of sex hormone release [7], disruption of the puberty process [8], altering the structure of the corpus cavernosum penis, weakening erectile function [9], suppressing the activity of several steroidogenesis related enzymes [10], and reducing weight and high epithelial accessory sex organs [7]. Therefore, the purpose of this research was to determine the effect of soybean methanol extract on testosterone and estrogen levels in rat testicles.

2. Materials and methods

2.1. Materials
Soybeans var. Grobogan were obtained from Balitkabi (Balai Penelitian Aneka Tanaman Kacang dan Umbi) Malang, Indonesia.

2.2. Soybean extract preparation
Soybeans are mashed into powder. The soybean powder is then defatted by soaking in n-hexane (1:2). Non-fat soybean powder was dried and then extracted using 80% methanol for 2 days. The solution is filtered and then dried until a concentrated extract is obtained. Finally, the extract was stored for in vivo testing.

2.3. Ethical clearance
The method used in this research has been approved by the Ethics Commission from the Integrated Research and Testing Laboratory, Gadjah Mada University, No. 279 / KEC-LPPT / VI / 2015.

2.4. Animal studies
Twenty male Wistar rats (Rattus norvegicus L.) from LPPT Unit IV UGM weighed 100–150 g. The animals were acclimatized for 1 week in laboratory conditions. Standard pellets and water are given in ad libitum. Rats were divided into 4 groups, namely control, soybean extract treatment group (250 mg/kg BW, 500 mg/kg BW) and genistein (0.3 mg/kg BW), for 52 days. On the last day, the animals were anesthetized, weighed, and dissected. The testes were weighed and stored at -80°C until analysis. The testes were mechanically crushed with a homogenizer and centrifuged at 5000 × g for 10 minutes at 4 °C. The supernatant was collected and stored in the cooling cabinet at -20°C until measurement of testosterone and estrogen-17β tissue.

2.5. Statistic analysis
Data were analyzed using SPSS statistical software and average data was compared using one-way ANOVA and Duncan test with a confidence level of 0.05.

3. Results and discussion

3.1. Weight of body and testis
Body weight, testis and epididymis, and the ratio of organ weight/body weight after 52 days of treatment of soybean methanol extract are shown in table 1. Table 1 shows that soybean methanol
extract affects the growth and development of male rat body weight. Changes in body weight indicate animal health status [11]. The body weight of the treatment group in the final study decreased significantly compared to control mice. According to Weber [12], estrogen is known to cause changes in eating behavior, body weight, and significantly improve the behavior of locomotion in rats. In addition, it is known that phytoestrogens have physicochemical and physiological characteristics that are similar to endogenous estrogens and affect brain tissue.

Table 1. Body weight, testicular weight, and organ / BW ratio of male rats after 52 days of treatment of soybean methanol extract

| Group (SE mg/kgBW) | Weight (gram) | Ratio of testis Weight/BW (%) |
|--------------------|--------------|-------------------------------|
|                    | Initial of BW | Final of BW | Testis |       |
| 0                  | 124.5 ± 0.76a | 283.9 ± 0.23a | 2.54 ± 0.03a | 0.9   |
| 250                | 125.6 ± 2.64a | 254.4 ± 3.18b | 2.42 ± 0.01b | 0.95  |
| 500                | 127.6 ± 2.08a | 254.9 ± 1.82b | 2.40 ± 0.01b | 0.94  |
| Genistein 0.3 mg/kgBW | 124.7 ± 2.84a | 254.1 ± 1.7b | 2.41 ± 0.08b | 0.95  |

The numbers followed by the different letter on each column are significantly different (n=5, p< 0.05). SE: Soy Extract

Table 1 also shows that there was a significant (P <0.05) decrease in testicular weight in the treatment group compared to the control group. This decrease is likely because the active compound in the extract causes abnormal spermatogenic activity. The number of spermatogenic cells affects the weight of the testis [13], possibly due to reduced number of germ cells, and inhibition of spermatogenesis and decreased spermatogenic enzyme activity [14]. The decrease in the number of spermatogenic cells found in the treatment group caused a decrease in testicular weight [15].

3.2. Effect of soybean methanol extract on testosterone and estrogen levels in testicular rats

The results of testosterone and estrogen levels in the testes with the treatment of soybean methanol extract in rats are listed in table 2. The testosterone level in the genistein treatment was the lowest among other treatments. While the soybean methanol extract 500 mg/kg BW showed the lowest estrogen level and a significant difference compared to other treatments. These results indicate that the treatment of soybean methanol extract has the greatest impact in reducing estrogen levels in the testes.

Table 2. The result of hormone testosterone (ng/mL) and estrogen level (pg/mL) testis

| Group                  | Testosteron (ng/mL) | Estrogen (pg/mL) |
|------------------------|---------------------|------------------|
|                        | Mean ± SD           | Mean ± SD        |
| Control                | 23.36 ± 2.19a       | 54.00 ± 2.08ab   |
| Soy Extract 250 mg/kg bw | 20.10 ± 3.59ab     | 55.31 ± 0.48a    |
| Soy Extract 500 mg/kg bw | 19.45 ± 3.64ab     | 47.32 ± 1.49c    |
| Genistein 0.3 mg/kg bw | 16.99 ± 2.31b       | 53.27 ± 0.81b    |

The numbers followed by the different letter on each column are significantly different (n=5, p< 0.05).

In the past few decades, male reproductive health has deteriorated in many countries. It is known that estrogen exposure or estrogenic environmental chemicals during the fetal period and childhood can explain the increased incidence of testicular disorders and testicular cancer. These compounds are included in the class of endocrine disrupting chemicals (EDC) which can interfere with synthesis and metabolism, or the action of natural hormones in the body that regulate homeostasis, reproduction, development, and/or behavior. Previous researchers have focused on changes in testosterone (T) in men and estrogen (E2) in women. Testosterone plays an important role in male reproduction and development. However, the results of recent studies in mice and humans have observed the role of
estrogen in male sexual differentiation, spermatogenesis, and steroidogenesis. Many exogenous chemicals, especially EDC, show estrogenicity in the body. EDCs can act to mimic or antagonize the action of endogenous hormones, block their receptors in target cells or alter hormone synthesis or metabolism and hormone receptors [16]. Thus, it is possible that the pathways of synthesis and metabolism of testosterone, estrogen and hormone receptors can be affected.

Decreasing testosterone in intratesticular can occur due to a decrease in steroidogenic enzyme activity in the testes, because this enzyme plays a role in regulating the biosynthesis of steroid hormones [17]. According to Pfaehler [18] isoflavones can suppress testosterone synthesis in Leydig cells by directly inhibiting the activity of 3hydroxysteroid dehydrogenase (3β HSD), and inducing secretion of adiponectin, which in turn can suppress StAR protein expression and reduce testosterone. Added according to Lehraiki [19] high-dose exposure to genistein has also been linked to male reproductive development disorders, both in vivo and in vitro models. Genistein has been shown to damage fetal testosterone production that works through ERα, inducing changes in prepubertal spermatogenesis, function and development of Leydig and Sertoli cells. A good balance between androgens and estrogen is fundamental to the development of normal testes and fertility in animals and humans. This balance can be disrupted by exposure to EDC [20]. StAR protein, enzyme P450scc, 3β-hydroxysteroid dehydrogenase (3β-HSD), 17α-hydroxylase/17,20-desmolase (P450c17) and 17β-HSD are the main enzymes involved in synthesis of testosterone [21]. T is converted to E2 by cytochrome P450 aromatase (CYP19), which is encoded by Cyp19 gene.

The intratesticular estrogen level in table 2 also shows that the highest reduction in testicular estrogen levels is 500 mg/kgBW soybean extract. Estrogen is a group of steroid compounds, which include estrone, estrogen, and estriol, which act as hormones that regulate development and function of reproductive. Although estrogen has historically been believed to be a female hormone, there is increasing evidence of the biological role of this steroid in male reproduction. E2 has a direct physiological effect on these cells to trigger complete and correct spermatogenesis. For example, E2 regulates T synthesis in Leydig cells [4] and ionic homeostasis in Sertoli cells [5], and it acts as a survival factor for germinal cells [6]. In addition, estrogen plays a role in negative feedback on the pituitary gland to regulate gonadotropin secretion, therefore the absence of estrogen and excessive estrogen exposure causes interference with the balance of the hypothalamic-pituitary-testicular axis [22].

Exogenous estrogen exposure causes male reproductive pathology in laboratory animals and men, especially during development. The function of efferent ducts and epididymis depends on estrogen signaling via ESR1, which loses ion transport and reabsorption of water, which causes abnormal sperm. Expression of GPER is very broad in the male channel, suggesting that the role of E2 signaling is also possible through these receptors in male reproduction [23]. Isoflavones are one of the most phytoestrogens in human food [24]. They can be classified as selective estrogen receptor modulators (SERMs) [25], where they can trigger estrogenic activity to act as agonists or antagonists [26]. However, the benefits of phytoestrogens are indirect and inconsistent. Exposure to estrogen compounds, causes disorders in the reproductive system [27]. Structurally, phytoestrogens are similar to endogenous estrogens and have an affinity for estrogen receptors. There are several types of phytoestrogens, including isoflavones, prenylated flavonoids and coumestans. Genistein and daidzein are the most common of these compounds [28]. Phytoestrogens produce a variety of physiological effects in humans and animals, where the types of phytoestrogens, concentrations and models studied affect their effects on the male reproductive system [29].

4. Conclusion
The results showed that exposure to soybean methanol extract in male rats at a dose of 500 mg/kgBW for 52 days can significantly reduce testicular estrogen.
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References
[1] Kim S H, Park M J 2012 Effects of phytoestrogen on sexual development. Review article Korean J. Pediatr. 55(8) pp 265-71
[2] Giwercman A 2011 Estrogens and phytoestrogens in male infertility Curr. Opin. Urol. 21(6) pp 519-26.
[3] Lardone M C, Castillo P, Valdevenito R, Ebensperger M, Ronco A M, Pommer R, Piottante A and Castro A 2010 P450-aromatase activity and expression in human testicular tissues with severe spermatogenic failure Int. J Androl. 33 pp 650–60
[4] Vaucher L, Funaro M G, Mehta A, Mielnik A, Bolyakov A, Prossnitz E R, Schlegel P N and Paduch D A 2014 Activation of GPER-1 estrogen receptor downregulates production of testosterone in isolated rat Leydig cells and adult human testis PLoS ONE 9 92425
[5] Bernardino R L, Costa A R, Martins A D, Silva J, Barros A, Sousa M, Sa R, Alves M G and Oliveira P F 2016 Estrogen modulates Na(+) –dependent HCO3 ( _) transporters altering intracellular pH and ion transport in human Sertoli cells: a role on male fertility? Biol Cell 108 pp 179–88
[6] Pentikainen V, Erkkila K, Suomalainen L, Parvinen M and Dunkel L 2000 Estrogen acts as a germ cell survival factor in the human testis in vitro J. Clin. Endocrinol. Metab. 85 pp 2057–67
[7] Yuan X X, Zhang B, Li L L, Xiao C W, Fan J X, Geng M M and Yin Y L 2012 Effects of soybean isoflavones on reproductive parameters in Chinese mini-pig boars J Anim Sci Biotechnol. 3 p 31
[8] Caceres S, Pena L, Moyano G, Martinez-Fernandez L, Monsalve B, Illera M J, Millan P, Illera J C and Silvan G 2015 Isoflavones and their effects on the onset of puberty in male Wistar rats Andrologia. 47 pp 1139-46
[9] Huang Y, Pan L, Xia X, Feng Y, Jiǎng C and Cui Y 2008 Long-term effects of phytoestrogen daidzein on penile cavernosal structures in adult rats Urology. 72 pp 220-4
[10] Hu G X, Zhao B H, Chu Y H, Zhou H Y, Akingbemi B T, Zheng Z Q and Ge R S 2010 Effects of genistein and equol on human and rat testicular 3 beta-hydroxysteroid dehydrogenase and 17beta-hydroxysteroid dehydrogenase 3 activities Asian J. Androl. 12 pp 519–26
[11] Hilaly J E, Issaili Z H, and Lyoussi B 2004 Acute and chronic toxicology studies of Agua jiva in experimental animals. J. of Ethnopharmacol. 91(1) pp 43-50
[12] Weber K S, Setchell K D R, Stocco D M, and Lephart E D 2001 Dietary soy-phytoestrogens decrease testosterone levels and prostate weight without altering LH, prostate 5α-reductase or testicular steroidogenic acute regulatory peptide levels in adult male Sprague-Dawley rats. J. of Endocrinol. 170 pp 591-9
[13] Kianifard D, Hasanzadeh S, and Kianifard L 2013 The study of time dependent administration of methylphenidate on the microscopic indices of spermatogenesis and sperm analysis in adults rats J.Exp. Integr.Med. 3(2) pp 121-5
[14] Sakr S A and Al-Amoudi W M 2012 Effect of Ginger extract on deltamethrin induced histomorphological and immunohistochemical changes in testes of albino rats Life Science Journal 9(1) pp 771-8
[15] Kachhawa J B S, Gupta R S, and Sharma K K 2012 Screening of isolated fractions of Dendrophthoe falcate methanol stem extract for its effects on reproductive function of male rats Int. J. of Pharm. Sci. and Drug Research 4(1) pp 84-7
[16] Hejmej A, Kotula-Balak M, and Bilinska B 2011 Antiandrogenic and Estrogenic Compounds: Effect on Development and Function of Male Reproductive System. In Steroid-Clinical Aspect, H A (ed). InTech: Rijeka, pp 51–82
[17] Jana K, Jana S, and Samantha P K 2006 Effects of chronic exposure to sodium arsenit on hypothalamo-pituitary-testicular activities in adult rats: possible an estrogenic mode of action Reprod. Biol. Endocrinol. 4 p 9

[18] Pfaehler A, Nanjappa M K, Coleman E S, Mansour M, Wanders D, Plaisance E P, Judd R L, and Akingbemi B T 2012 Regulation of adiponecin secretion by soy isoflavones has implication for endocrine function of the testis Toxicol. Lett. 209 pp 78-85

[19] Lehraiki A, Chamaillard C, Krust A, Habert R, and Levacher C 2011 Genistein impairs early testosterone production in fetal mouse testis via estrogen receptor alpha Toxicol. In Vitro. 25 pp 1542–47

[20] Hu G X, Lian Q Q, Ge R S, Hardy D O, Li X K 2009 Phthalate-induced testicular dysgenesis syndrome: Leydig cell influence Trends in Endocrinology, Metabolism. 20 pp 139–145

[21] Scott H M, Mason J I, Sharpe R M 2009 Steroidogenesis in the Fetal Testis and Its Susceptibility to Disruption by Exogenous Compounds Endocrine Reviews 30 pp 883–925

[22] O’Donnell L, Robertson K M, Jones M E, and Simpson E R 2001 Estrogen and spermatogenesis Endocr. Rev. 22(3) pp 289-318

[23] Cooke P S, Nanjappa M K, Ko C, Prins G S, and Hess R A 2017 Estrogens In Male Physiology. Physiol. Rev. 97 pp 995–1043

[24] Kelany O E, Khaled H E, Amal M, Abdelrazek H M, and Abdel-Daim M M 2017 Hepatoprotective and metabolic effects of dietary soy phytoestrogens against hypercaloric diet in cyclic female albino rats is mediated through estrogen receptors beta Biomed. Pharmacol. J. 10 pp 1061–9

[25] Oseni T, Patel R, Pyle J, and Jordan V C 2008 Selective estrogen receptor modulators and phytoestrogens Planta. Med. 74 pp 1656–65

[26] Shanle E K and Xu W 2011 Endocrine disrupting chemicals targeting estrogen receptor signaling: identification and mechanisms of action Chem. Res. Toxicol. 24 pp 6–19

[27] Bar-El D S and Reifen R 2010 Soy as an endocrine disruptor: cause for caution? J. Pediatr. Endocrinol. Metab. 23(9) pp 855-61

[28] Khani B, Mehrabian F, Khaesi E, and Eshraghi A 2011 Effect of soy phytoestrogen on metabolic and hormonal disturbance of women with polycystic ovary syndrome J. Res. Med. Sci. 16(3) pp 297-302

[29] Zoidou E, Magiatis P, Constantinou M, and Skaltsounis A 2008 Oleuropein as a bioactive constituent of functional milk and yogurt Planta Medica 74 p 62