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Impact of Soy Protein Isolate Supplementation on Testosterone Hormone Levels and Its Biosynthesis Pathway in Male Rats

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Abstract

Soy protein isolate (SPI) is a prevalent ingredient in various dairy, meat products, and health drinks. Concerns have been raised regarding potential adverse effects of soy consumption on male health, such as feminization and infertility. This concern is attributed to phytoestrogens, specifically isoflavones present in soy, acting as estrogen substitutes and modulators. This study aims to investigate the effects of soy protein isolate supplementation on testosterone hormone levels and the expression of genes associated with testosterone synthesis. Male rats (n = 18) were evenly divided into three groups: Group 1 (Normal Control) received oral administration of a saline vehicle. Group 2 (Low Dose) received 450 mg/kg body weight of soy protein isolate for 30 days. Group 3 (High Dose) received 900 mg/kg body weight of soy protein isolate for 30 days. All administrations were conducted intragastrically. Testosterone hormone levels were decreased with a disturbance in the mRNA expression of genes (Hsd17b6, SRD5A1, Akr1c3, Cyp17a1 and Hsd3b) involved in the biosynthesis of testosterone hormone in the low and high dose groups in in comparison to the control group. In the testes of the high dosage group, histopathological analysis showed a noticeable irregularity around the periphery of the seminiferous tubules. Our results have shown damage to testicular tissue and reduced genetic expression of certain genes responsible for the formation of testosterone hormones, followed by a reduction of testosterone hormones in the group of rats that were given a high dose of SPI for 30 days.

Key words: Soy Protein Isolate, Testosterone Hormone, Biosynthesis, Pathway

Introduction

Proteins play a pivotal role in human growth, nutrition, and health. While conventional wisdom has traditionally emphasized animal proteins as the primary dietary source, a notable shift in recent years has redirected focus towards plant-based proteins [1]. Globally, soybeans stand out as a principal crop, serving as a key source for protein and oil seed. They are utilized in the production of animal feed, various food ingredients, and non-food items. Approximately 40% of soybeans consist of proteins, 15% of mono- and/or poly-saccharides, 15% of fibers, and 20% of lipids [2]. Soy belongs to the legume family, sharing lineage with lentils, beans, and peas [3]. Soy is unique in that it has high-quality protein despite coming from plants. Various processing methods can be used to extract soy protein, producing a range of ingredients that can be used in different food preparations, including textured soy protein, soy flour, soy protein concentrate, and soy protein isolates [4].

A secondary metabolite found in soybean called phytoestrogens. It functions and is structurally similar to mammalian estrogens. The three main bioactive classes of phytoestrogens are lignans, coumestans, and isoflavones which can function as both an estrogen substitute and an estrogen activity modulator [5]. The complex mechanism of action of this compound involves its interaction with the nuclear estrogen receptor isoforms, ERα and ERβ, producing effects that are both estrogen agonist and antagonistic. Phytoestrogens can function as estrogen agonists or antagonists, depending on their concentration and bioavailability in different plant sources, such as soybean [6]. Isofavone, has been investigated for its physiological effects and role in nutrition [7]. The phenolic ring of isofavones has both antagonistic and agonistic effects on the ERβ and ERβ [8]. There have been worries expressed regarding the possible harm that soy consumption may cause to men, including feminization and infertility [9.10].

Sources of soy protein in concentrated form are the foundation for a wide variety of foods. According to Codex, the main components of soy protein are SPI, soy protein concentrate (SPC), and soy flour, that have respective protein contents of ≥ 90, 65–90, and 50–65% [11]. The distinct spatial structure of SPI is made up of various amino acids. Food additives containing SPI are frequently used to improve processing capabilities, including foaming, gelling, emulsifying, and structuring qualities [12]. SPI is the purest form of soybean protein and is frequently utilized in food processing to enhance the quality of food products because of its high protein content [13]. SPI are used extensively in the food industry, such as the main component of health drinks and as extenders in a variety of dairy and meat products. Different suppliers have varying amounts of isoflavone in SPI [14]. Consuming whole soy foods can

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result in higher intakes of isoflavones, whereas foods prepared with pure soy protein may contain 1.0–1.5 mg of isoflavones per gram of protein. [15].

When germ cells mature in the testicular seminiferous tubules, spermatogenesis is a sequential biological event that supports this maturation [16, 17]. Production of luteinizing hormone (LH) and follicle stimulating hormone (FSH) by the anterior pituitary gland, as a result of secretion of gonadotropin releasing hormone (GnRH) from the hypothalamus, is necessary for the development and maintenance of both qualitative and quantitative spermatogenesis. GnRH stimulates gonadotropin production and secretion by gonadotropic cells from the anterior pituitary in discrete pulses into the systemic circulation to regulate the development, maturation and function of the gonads [18]. LH stimulates the release of testosterone, which is thought to be essential for efficient spermatogenesis, secondary sexual traits and functions [19-21]. On the other hand, FSH keeps Sertoli cells' supporting role in spermatogenesis intact, which increases testosterone action. The dual roles of the adult testes in reproduction and virility depend on the interaction of these hormones [22]. FSH is known to influence both the quantity and quality of sperm, even though testosterone is the master switch of spermatogenesis [23, 24]. Testosterone is a crucial steroid hormone that supports male growth and development, fertility maintenance, and material metabolism [25-27]. Adolescent and young adult men in the USA had 25% lower testosterone levels in 2015–2016 compared to sixteen years earlier, according to a cross-sectional survey [28]. Male sexual dysfunction, accelerated organ aging, systemic disease onset, and other negative effects on quality of life are caused by low testosterone [29, 30]. Also, It is formed by Leydig cells, which are found in the testes' interstitial compartment and responsible to the production of sperm in the seminiferous tubules [31], the maintenance of accessory sex organs [32], sexual behavior in adulthood [33], sexual development and testes descent during the fetal period [34]. Leydig cells can be divided into two different populations: fetal and adult Leydig cells, Male reproductive tract development and testes descent depend on testosterone and Dihydrotestosterone (DHT) [34]. Testosterone, which is necessary for preserving spermatogenesis and male secondary sexual traits in adulthood, is produced by adult Leydig cells during puberty [35].

Testosterone biosynthesis involves at least four steroidogenic enzymes in Leydig cells, both in fetal and adult forms. Cholesterol is the first substrate used in testosterone biosynthesis. The cholesterol side chain cleavage enzyme (CYP11A1), which is found in the mitochondria's inner membrane, is the first steroidogenic enzyme. Three consecutive reactions from cholesterol to pregnenolone are catalyzed by the enzyme. From the mitochondria, pregnenolone diffuses into the surrounding smooth endoplasmic reticulum, which is home to three other steroidogenic enzymes: cytochrome P450 17α-hydroxylase/17,20-lyase (CYP17A1), 17β- hydroxysteroid dehydrogenase 3 (HSD17B3), and 3β-hydroxysteroid dehydrogenase (HSD3B). These three steroidogenic enzymes react sequentially to convert pregnenolone to testosterone. [36]. Rat testes was used to initially demonstrate the 4 pathway (pregnenolone → progesterone → androstenedione → testosterone) [37]. Once testosterone is formed, it is metabolized by various types of 5α-reductases (SRD5A1, 2 and 3) to more potent androgen, DHT in Leydig cells or peripheral tissues [38]. Male reproductive tract malformation can result from a mutation in the SRD5A2 gene [39, 40]. DHT stimulates the development of the prostate, sebaceous gland activity, male pattern baldness, and the growth of body, facial, and pubic hair throughout adolescence and adulthood [41].

This study aims to assess the impact of soy protein isolate supplementation, administered in both low and high doses over a 30-day period, on testosterone, FSH, and LH hormone levels, genes involved in the testosterone biosynthesis pathway, and histopathological changes in rat testes. Given the conflicting findings in previous researches, this investigation seeks to provide clarity on the effects of soy protein isolate in a controlled experimental setting.

2. Material and Methods

2.1. Chemicals and doses preparation

Soy protein isolate was used insulated from the market by IMTENAN Company, Cairo, Egypt. It has 88% protein. (Code: F050079- Sp19). Doses were prepared by adding SPI powder to water (20◦C) and stir it [42].

2.2. Animals

At the Animal House of the National Research Centre in Giza, Egypt, eighteen male Wistar rats (8 weeks old) weighing 180 ± 10 g were provided. Before the experiments started, the rats were kept in cages made of polypropylene and given three days to get used to a particular set of pathogen-free surroundings. Under a 12-hour light/dark cycle, the rats were kept at 24 ± 1 °C and between 55 and 65 percent humidity. A standard rodent diet consisting of 17.48% protein, 6.85% fat, 62.99% carbohydrates, 4.08% ash, and 2.16% minerals and vitamins was fed to the rats along with unlimited water. The National Research Center's Medical Research Ethical Committee's ethical guidelines for animal handling were followed, and the procedure was approved with the number "09410923".

2.3. Experimental setting

After the time for acclimatization, the rats (n = 18) were weighed and divided equally into three groups.

Group 1: Normal control group (*n* = 6), administered saline orally.

Group 2: Low dose soy protein isolate group (*n* = 6), orally administered with 450 mg/kg body weight soy protein isolate (0.09 gm/2.5 ml water) for 30 days intragastrically [43, 44].

Group3: High dose soy protein isolate group (*n* = 6).), group, orally administered with 900 mg/kg body weight soy protein isolate (0.18 gm/2.5 ml water) for 30 days intragastrically [43, 44].

2.4. Samples collection

Using clean and dry centrifuge tubes, blood samples were removed from the retro-orbital plexus of rats and allowed to clot in order to separate the sera. Centrifugation was used to separate serum samples at 4000 r/min for 10 min at 4°C. For additional hormonal assay determination, serum aliquots were frozen and kept at -20 °C. Testes were removed, and the right testes were preserved for histological examination in 10% phosphate-buffered formalin (pH 7.4), while the left testes were removed right away, blotted, and then kept at -80 ◦C until they were needed for the analysis of Hsd17b6, SRD5A1, Akr1c3, Cyp17a1, and Hsd3b gene expression.

2.5. Methods

2.5.1. Hormonal assays

Enzyme-linked immunosorbent assay (ELISA) kits were used to measure the levels of testosterone hormone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) for rats (Shanghai Korain Company, bioassay technology laboratory (BT-Lab)),
Cat. No. E0259Ra, EA0015Ra and EA0013Ra respectively, following the manufacturer's instructions.

2.5.2. RNA extraction and cDNA synthesis

Testes specimens weighing approximately 30 mg were homogenized using a mortar and pestle in liquid nitrogen, derived from stored test tissue samples from all animals. Employing the Thermo Scientific Gene JET RNA Purification Kit, total RNA was isolated from tissues. Utilizing a spectrophotometer (ND-1000; NanoDrop), RNA was quantified through spectrophotometry. RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific) was used to extract cDNA from a 1 mg sample of tissue-derived RNA (high capacity cDNA), and the gradient thermal cycler (Bio-Rad) was used for the incubation.

2.5.3. Quantitative real-time gene expression analysis (qRT-PCR)

Using a gilent Technologies Stratagene Mx3000P Real-Time PCR System, quantitative real-time gene expression analysis was performed. Maxima SYBR Green qPCR Master Mix from Thermo Scientific was used to conduct the qRT-PCR in duplicate for every sample. Ten microliters of SYBR Green Master Mix, four microliters of cDNA (100 ng/mL), and twenty microliters of nuclease-free water made up the reaction mixture, which had a total volume of twenty-five microliters. Using GAPDH, a housekeeping gene, each gene's expression was adjusted. The primer sequences for the various genes (Hsd3b, SRD5A1, Akr1c3, Hsd17b6, and Cyp17a1) are shown in Table 1. The program for the thermal cycler was as follows: 94 °C for 15 s in 40 cycles, 95 °C for 5 min, 60 s of annealing (depending on the melting point that works best for each primer set), and 10 s of extension at 72 °C. The fold difference in gene expression was calculated using the 2-ΔΔCT formula, which is a relative quantification method of mRNA [45].

Table 1. Specific primer sequences

Gene	Forward Sequence	Reverse Sequence
GAPDH	5 ` -AGTGCCAGCCTCGTCTCATA-3`	5 ` -GATGGTGATGGGTTTCCCGT-3`
Cyp17a1	5 ` -TCAAAGCCTCTTGTCGGACC-3`	5 ` TGTCCGTCAGGCTGGAGATA-3`
Hsd3b	5 ` -CAGGGCCCAACTCCTACAAG-3`	5 ` -AAGGCAAGCCAGTAGAGCAG-3`
Akr1c3	5 ` -GGTCAACTTCCCATCGTCCA-3`	5 `- GCTGGAGTCCGCTTGTACTT-3`
Hsd17b6	5 `- GACTGCTCCCTGACTTGCAT-3`	5 `- AGTCACCTGTGCCAAACCAA-3`
SRD5A1	5 `- GAGTTGGATGAGCTGTGCCT-3`	5 `- CTCCACGAGCTCCCCAAAAT-3`

2.5.4. Histological procedures

Testes specimens from all animals were removed as soon as it died and preserved for at least 12 hours in a 10% neutral-buffered formalin solution. Following a thirty-minute soak in tap water, each specimen was dehydrated using increasing alcohol grades, cleared in xylene, and then embedded in paraffin. Haematoxylin and eosin was used to cut and stain serial sections that were 3 µm thick for histopathological analysis [46]. Picture analysis system with Olympus CX41 light microscope and SC100 video camera connected to PC was used in the pathology lab at the National Research Centre to take pictures. Version 8.0 of Adobe Photoshop was used to process photomicrographs that were taken at various magnifications.

2.5.5. Statistical analysis

The Prism 8.0.1 software for Windows was used to conduct the analysis. The displayed data was mean ± SEM. Utilizing one-way analysis of variance (ANOVA) and Tukey's test, differences between low dose and high dose groups with respect to the control group were examined for statistical significance. In every analysis of the data, $P < 0.05$ was considered significant.

3. Results

3.1. Effects of SPI supplementation on testosterone, FSH and LH hormones concentrations male rats

A shown in figure (1), serum testosterone levels decreased significantly for low and high doses groups of SPI relative to the control group ($P<0.05$) but in figure (2) and (3), serum FSH, and LH levels did not show remarkable differences between groups with low and high doses of SPI when compared with control group.

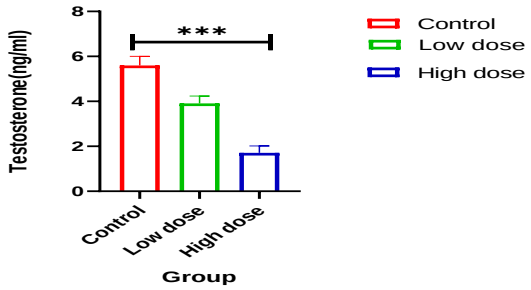

Figure (1): Effect of soy protein isolate supplementation (low and high doses) on testosterone hormone of male rats compared with control group. Data are represented as mean ± SEM (n = 6). * P < 0.05.

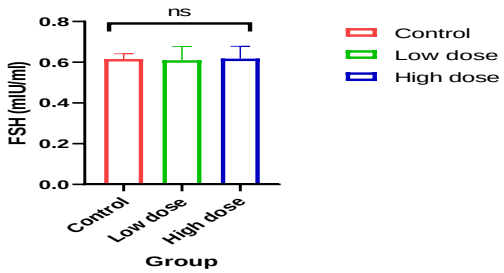

Figure (2): Effect of soy protein isolate supplementation (low and high doses) on FSH hormone of male rats compared with control group. Data are represented as mean ± SEM (n = 6). * P < 0.05.

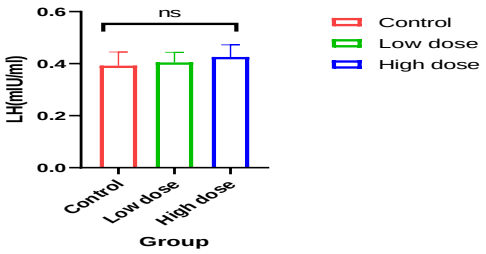

Figure (3): Effect of Effect of soy protein isolate supplementation (low and high doses) on LH hormone of male rats compared with control group. Data are represented as mean ± SEM (n = 6). * P < 0.05.

3.2. Effect of SPI supplementation on Hsd17b6 and SRD5A1, Akr1c3, Cyp17a1 and Hsd3b mRNA expression of male rats

As shown in figure 4, there was a significant (P < 0.05) down- regulation in mRNA expressions of SRD5A1 and Hsd17b6 genes in groups with low and high doses of SPI in comparison with control group. In figure 5, a significant (P < 0.05) down- regulation in mRNA expressions of Akr1c3 gene by fold change (0.44) in high dose group of SPI compared with control group was observed (Fig5). On the contrary, comparative gene expression analysis by RT-PCR in figure 6 revealed that there was significant (P < 0.05) up-regulation in mRNA expressions of Cyp17a1 by fold change (1.65), and (4.8) in low and high dose SPI groups, respectively compared to normal control. While there was insignificant up- regulation in Hsd3b gene in low and high dose SPI groups compared to control group. In figure 7, data indicated that the T level negatively correlated with Cyp17a1gene. On the other hand, testosterone is positively correlated with HSda7b6 and SRD5A1 genes.

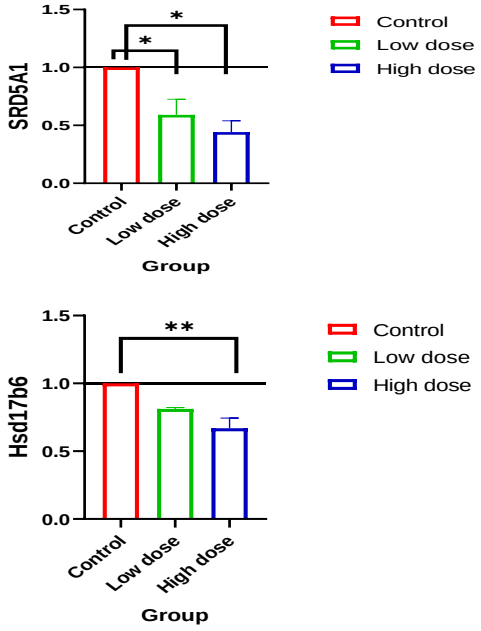

Figure (4): Effect of soy protein isolate supplementation (low and high doses) on the mRNA expression levels expression of SRD5A1 and Hsd17b6 genes. The fold change of mRNA in low dose and high dose groups is compared to control group. Data are represented as mean ± SEM (n = 6). * P < 0.05.

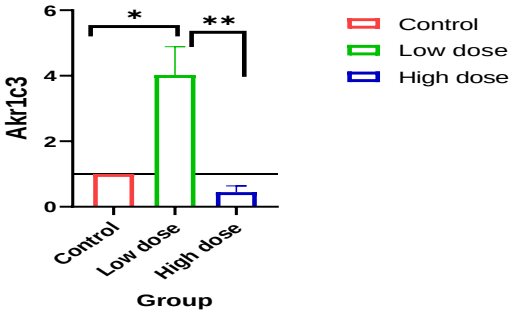

Figure (5): Effect of soy protein isolate supplementation (low and high doses) on mRNA expression levels of Akr1c3 gene. The fold change of mRNA in low dose and high dose groups is compared to control group. Data are represented as mean ± SEM (n = 6). * P < 0.05.

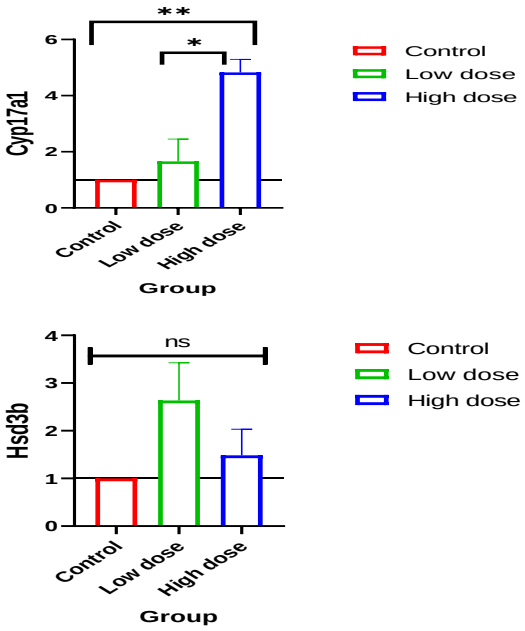

Figure (6): Effect of soy protein isolate supplementation (low and high doses) on mRNA expression levels of Cyp17a1 and Hsd3b genes. The fold change of mRNA in low dose and high dose groups is compared to control group. Data are represented as mean ± SEM (n = 6). * P < 0.05.

Figure (7): Correlation coefficients and significance values between testosterone and gene expression of

Cyp17a1, Hsd3b, Akr1c3, HSda7b6, and SRD5A1. Correlation is significant at P < 0.05.

3.3. Effect of SPI supplementation on testicular histology in male rats comparable to control group

Fig 8 shows the histological cross-sections (Photomicrography) of testes tissue of various groups to investigate overall morphological changes. The control group without SPI (Figure 8.A) and the group with low dose of SPI (Figure 8.B) showing normal shape of seminiferous tubules and clear lumen (ST Lumen) with normal arrangement of spermatogonia and Sertoli cells resting on intact basement membrane (Black arrows) and separated with average Leyding cells (L) where the group with high dose of SPI (Figure 8.C) showing destructed seminiferous tubules (Blue arrow) with some of spermatogonia and Sertoli cells showing pyknotic degeneration (Brown arrows) they are widely separated (Black star).

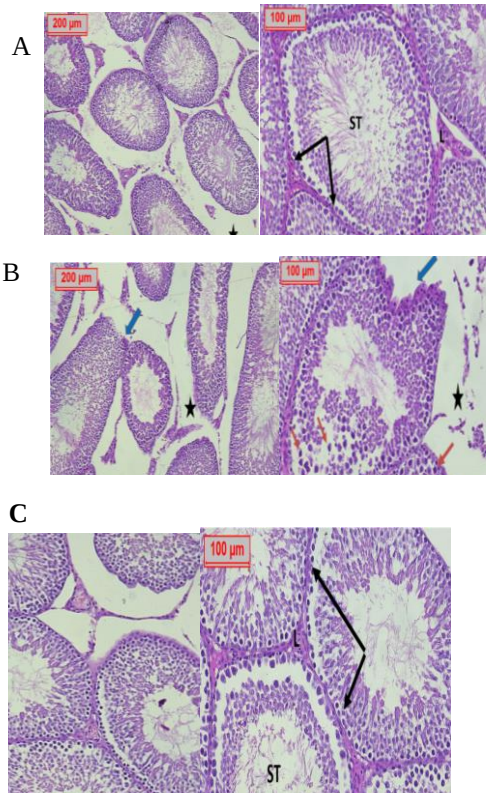

Figure (8): (A): A Photomicrography of testes tissue, (G1: Control testes). (B): A Photomicrography of testes tissue, (G2: Low dose testes). (C): A Photomicrography of testes tissue, (G3: High dose testes) (H&E 100x& 200x)

4. Discussion

Isoflavones are bioactive phytoestrogenic compounds, commonly known as plant estrogens, predominantly found in soybeans [47, 48]. The three primary isoflavones in soybeans are genistein, daidzein, and glycitein. According to Křížová et al. [49], isoflavones exhibit a structural and/or functional resemblance to steroidal estrogens in mammals. Due to this similarity, isoflavones can influence various estrogen-regulated systems, including the reproductive system, leading to estrogenic or anti-estrogenic effects [50, 51]. The goal of this study was to investigate the impact of SPI supplementation, a soy product rich in phytoestrogens, on the fertility of male rats. This was assessed by measuring testosterone, FSH, and LH hormone levels, examining the expression of genes responsible for testosterone hormone biosynthesis, and evaluating the histopathology of testes tissues across groups of male rats. The results were compared between groups administered low and high doses of SPI and the control group.

Dietary intake of soy may significantly affect the growth and function of male reproductive tissues [52]. In adult male rats fed a high phytoestrogen diet, there was a notable increase in seminiferous tubule lumen and testes diameter [53]. However, conflicting findings indicate that tubular diameter and interstitial spaces were reduced in size in other studies [54]. Soy consumption in rodents resulted in cellular debris in seminiferous tubules, sloughing of germ cells, and the absence of maturing spermatids [55, 56], along with hyperplasia of Leydig cells [57]. Genistein and soy, in vivo, decreased sperm counts in testes [54]. While soy isoflavones (SIF) were found to promote spermatogenesis in diet-induced obese male rats [58], they also decreased serum testosterone levels in rodents [59, 60]. Phytoestrogens were reported to cause hormonal imbalances in men [61, 62]. Conversely, some studies indicated that consuming soy protein or isoflavones had no impact on serum testosterone levels in rats [63].

In accordance with the findings of Badger et al. [64, 65], SPI emerges as a prevalent constituent in processed food items, meat substitutes, and soy-based infant formulas. Beyond its isoflavone content, notably genistein and daidzein, which are intricately associated with potentially bioactive proteins and peptides, SPI constitutes a multifaceted amalgamation of phytochemicals [66]. Estrogen receptors (ERs) can be bound and activated by isoflavones in conjunction with the metabolite equol, which is derived from the microbiota and is a dietary supplement. Given that they have the ability to impede fertility and reproductive development, these substances fall under the category of endocrine disrupting chemicals (EDCs) [67].

It is commonly known that testosterone plays a crucial role in the development of spermatids and germ cells. Reduced levels of this hormone cause spermatids to undergo apoptosis, which in turn causes sperm to undergo apoptosis [68, 69].

In male rats exposed to varying doses of SPI, a noteworthy decrease in serum testosterone levels was observed compared to the control group. This finding aligns with prior research on rodents [54, 70-77]. The reduced serum testosterone levels are linked to the estrogenic activity of isoflavones present in SPI and the inhibition of steroidogenic enzymes [59]. Isoflavones may impede the activity of enzymes involved in the steroidogenic pathway by disrupting the metabolism of steroid hormones [78]. Studies have indicated that isoflavones elevate the production of sex hormone binding globulin in the liver, leading to the binding of biologically active testosterone and a subsequent reduction in both testosterone levels and its bioavailability to target cells [61, 79]. Conversely, the levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) remained unchanged when comparing the results of groups receiving low and high doses of SPI with the control group. This outcome is consistent with the findings of Ohno et al.

[73] and suggests that there is no adverse impact of SPI on the hypothalamic-pituitary-adrenal (HPA) axis.

Several steroidogenic enzymes, including cytochrome P450 17_-hydroxylase/20-lyase (CYP17a1), 3_-hydroxysteroid dehydrogenase (Hsd3b1 in rodents and Hsd3b2 in humans), and 17_-hydroxysteroid dehydrogenase type 3 (Hsd17b3), play crucial roles in the cascade of hydroxylation, cleavage, dehydrogenation, and isomerization reactions necessary for the synthesis of testosterone from cholesterol [80]. Testicular Leydig cells possess the capability to biosynthesize testosterone from cholesterol. Testosterone and its metabolically activated product dihydrotestosterone are essential for the development of the male reproductive system and spermatogenesis. The production of testosterone involves at least four steroidogenic enzymes: cholesterol side chain cleavage enzyme (CYP11a1), which converts cholesterol into pregnenolone inside the mitochondria; 3β-hydroxysteroid dehydrogenase (Hsd3b), which transforms pregnenolone into progesterone; 17α-hydroxylase/17,20-lyase (Cyp17a1), which transforms progesterone into androstenedione; and 17β-hydroxysteroid dehydrogenase (Hsd17b6), which converts androstenedione into testosterone [81].

Additionally, in Leydig cells and peripheral tissues, two isoforms of 5α-reductase 1 (SRD5A1) and 2 (SRD5A2) metabolically activate testosterone to more potent androgen dihydrotestosterone. Numerous endocrine disruptors function as antiandrogens by directly blocking one or more enzymes involved in metabolic activation and testosterone biosynthesis. According to Leping et al. [35], these substances include industrial materials (perfluoroalkyl compounds, phthalates, bisphenol A, and benzophenone) as well as pesticides and biocides (methoxychlor, organotins, 1, 2-dibromo-3-chloropropane, prochloraz, and genistein). According to Liu et al. [82], isofavones may also change the activity of enzymes involved in the synthesis and metabolism of hormones. In our study m.RNA expression of Akr1c3, Hsd17b6, SRD5A1, Cyp17a1 and Hsd3b genes were carried out.

Akr1c3, also known as (Hsd17b5) , is the primary enzyme in the prostate that carries out the conversion of androstenedione to testosterone [83]. The expression of Akr1c3 in group with high dose of SPI was significantly down regulated when compared with the control group. This result means that when Akr1c3 gene is expressed in low level, the conversion of androstenedione to testosterone will be affected and the testosterone production was reduced in group of high dose of SPI. As shown in our results of testosterone hormone concentration, it was decreased in group of low and high doses of SPI.

Dihydrotestosterone (DHT) and other androgens are critical for male sexual maturation, masculinization, and fertility. The canonical androgen production pathway is used to produce testosterone, which is necessary for both normal testicular function and masculinization. A series of enzymatic reactions known as androgen biosynthesis transform steroid precursors into the more potent DHT and testosterone, the two biologically active androgens. The first step needed to create androgens is cholesterol, just like with all steroid hormones. This means that the Leydig cells require a steady supply. The Leydig cells can start the androgen synthesis process by obtaining free cholesterol. The conversion of cholesterol to pregnenolone, which takes place in the inner membrane of the mitochondria of Leydig cells, is the initial stage of steroidogenesis [84, 85]. The luteinizing hormone/choriogonadotropin receptor (LHCGR) phosphorylates the steroidogenic acute regulatory (STAR) protein more when luteinizing hormone (LH) binds to it. This process controls the transfer of cholesterol to the inner mitochondrial membrane. The enzyme CYP11A1, which catalyzes cholesterol side-chain cleavage, subsequently transforms cholesterol into pregnenolone [85]. Pregnenolon exits the mitochondria by passive diffusion, and the smooth endoplasmic reticulum of the cell is the site of all ensuing androgen biosynthesis processes [86].

In our investigation, we conducted mRNA expression analyses of several genes involved in testosterone biosynthesis. Notably, we observed a significant down-regulation in the expression of Hsd17b6 and SRD5A1 genes, encoding 17b-hydroxysteroid dehydrogenase type 6 and Steroid 5α-reductase type I enzymes, respectively, in both low and high doses of SPI compared to the control group. These findings align with the work of Evans et al. [87], who demonstrated that isoflavone components, specifically genistein and daidzein present in SPI, exhibit inhibitory effects on 5α-reductase activity. This inhibition leads to a decrease in levels of DHT in the plasma of male rats. Additionally, our results are consistent with the findings of Amanda et al. [88], who investigated the impact of dietary SPI supplementation on the androgen biosynthesis pathway in men. Significantly reduced levels of dehydroepiandrosterone sulfate (DHEAS) were observed with SPI preparation compared to soy alone. This implies that the isoflavones present in SPI may influence the steroidogenic pathway or the sulphation/desulfation of dehydroepiandrosterone (DHEA). Our results corroborate and extend these previous findings, highlighting the potential adverse effects of SPI on the conversion of testosterone to dihydrotestosterone, a more potent androgen crucial for male sexual maturation, masculinization, and fertility.

On the other hands, our study showed a significant up-regulation of the expression of Cyp17a1gene and non-significant up-regulation of HSD3B gene in low and high SPI dose groups compared to control group which is consistent with Sherrill et al. [89]. It was discovered that exposing male rats to soy isoflavones during their early development led to the proliferation of Leydig cells and elevated levels of Cyp17a1 and HSD3B, which counteracted the decline in Hsd17b3 steroidogenic enzyme activity. Furthermore, compared to male rats fed a normal diet, male rodents exposed to genistein during perinatal development had decreased testicular mass, lower testosterone serum levels, and shorter anogenital distances [90, 91].

The current histological analysis of the testes in our study revealed that rats given a high dose of SPI for 30 days destroyed their seminiferous tubules, and some of their spermatogonia and Sertoli cells displayed pyknotic degeneration, which is characterized by their wide separation. This result is in line with previous studies that have demonstrated that isoflavone (genistein) in SPI delays spermatogenesis in male rats (55, 56, 62, 92). The presented data indicated that the testosterone level negatively correlated with Cyp17a1gene. On the other hand, testosterone is positively correlated with HSda7b6 and SRD5A1 genes.

5. Conclusion

The findings of our study have demonstrated a deleterious impact on testicular tissue in male rats subjected to elevated doses of soy protein isolate (SPI), characterized by a down regulation in the genetic expression of specific genes associated with testosterone hormone synthesis, subsequently leading to a reduction in testosterone levels. Prolonged and high-dose administration of soy protein isolate is implicated in potential adverse effects on male fertility. Caution is warranted in its usage due to the presence of phytoestrogens, particularly isoflavones, which exhibit structural similarities to endogenous female estrogen.

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