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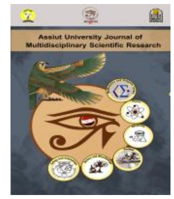

Dietary soybean sauce impact testicular tissue of rats in dose and duration dependent

Mona M. Atia[3]*, [2]Hanaa M. Yahia, [3]Hanem S. Abdeltwab[3], [1,4]Hossam EL-din M. Omar

[1&2] Laboratories of Physiology, [3]Laboratory of Histology and *Molecular Cell Biology, Zoology and Entomology Department, Faculty of Science, Assiut University, 71516, Egypt, [4] School of Bitotechnology, Badr University in Assiut

*Corresponding Author: e-mail: monatia@aun.edu.eg

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ABSTRACT

Soy sauce has been traditionally used as both a condiment and health food with antioxidant, anti-mutagenic, and antitumor activities. The present work aims to study the effect of soya sauce supplementation on the testis of male rats. Twenty-four male albino rats were divided into 3 equal groups. Group I; control males, group II; received low doses for consecutive 30 days, and group III; received a high dose of soy sauce for consecutive 15 days then recovered for another 15 days. Soy sauce in low doses decreases the amount of total white blood cells, red blood cells, hemoglobin, lymphocytes, and platelets levels than high doses while increasing neutrophils, and monocytes in high doses of soya. Histopathological examination showed that the testis of the low dose group had marked irregularity of seminiferous tubules periphery and degeneration of Leydig cells. Spermatogenesis arrest at the spermatid stage and degenerated spermatozoa were noticed in the center of tubules. Also, a significant increase in collagen fiber content of the testis was found in comparison with controls. However, rats treated with high doses showed restoration of the normal testis architecture. Furthermore, free testosterone hormones, lipid peroxidation, and nitric oxide level were decreased in the low-dose group than in the high-dose group. In contrast, Glutathione level was increased in rats supplemented with a high-dose than a low-dose of soya sauce. Supplementation of rats with Soya sauce is impact testicular tissue in dose and duration time-dependent.

INTRODUCTION

Soy sauce is commonly used throughout East Asia to enhance the flavor of a wide range of prepared dishes and aid in digestion. Bacteria called *Aspergillus oryzae* are used to ferment the paste of roasted soybeans and brine **[1]**. Soybean is one of the main sources of bioactive phytoestrogenic compounds (plant estrogens) called isoflavones **[2,3]**. The major isoflavones in soybean are genistein, daidzein, and glycitein. Isoflavones mimic the structure and/or function of the mammalian steroidal estrogens **[4]**. Their structural and functional similarity to estrogens allows the isoflavones to elicit estrogenic or anti-estrogenic effects and affect a number of the estrogen-regulated systems including the reproductive system **[5,6]**. Soy sauce and isoflavones improved the antioxidants properties and decreased the LPO **[7–9]**. Isoflavones were reported to have no significant changes in hematological parameters **[7,10]**. However, others reported a significant increase in lymphocytes, a slight increase in RBCs **[11]**. Genistein showed no significant change in body weight **[12,13]**, but others showed weight loss in numerous studies on animals and humans **[14]**. Soy dietary intake may have a profound physiological impact on the growth and function of male reproductive tissues **[15]**. Seminiferous tubule lumen and testis diameter were significantly larger in the high phytoestrogen-fed adult male rats **[16]**, but in other studies, the tubular diameter and interstitial spaces were reduced in size **[17]**. Soya caused a presence of cellular debris in the seminiferous tubules, sloughing of the germ cells and absence of maturing spermatids in rodents **[18–21]**, and hyperplasia of Leydig cells **[22,23]**. Genistein and soy *in vivo* study decreased sperm counts in testes **[17,22,24]**. However, isoflavones had no effects on histology and sperm numbers or morphology in the postpubertal **[25]**. Soy isoflavones (SIF) promote spermatogenesis in diet-induced obese male rats **[26]** and decreased serum-free testosterone levels in rodents

[17,24,27,28]. Phytoestrogens are also caused hormonal imbalances in men **[29-31]**. However, soy raised serum testosterone levels **[25,33]**. Others reported that consuming soy protein or isoflavones had no impact on men's serum levels of total and free testosterone in men **[29,32,33]** and rats **[16,34]**. So, due to contradictory results obtained in the previous work, the primary goal of this study was to evaluate the effect of soybean sauce supplementation in a low for 4 weeks and the height dose for 2 weeks then on serological, oxidative stress, and histopathological changes in the testis of rats.

MATERIALS AND METHODS

2.1. Animal care conditions

Twenty-four mature male Wistar albino rats, with a body weight (170±20g), were purchased from Assuit University's animal house, in Egypt. The rats were transferred to the animal house, Zoology Department, Faculty of Science, Assiut University where the experiment proceeded. They were kept in cages under controlled conditions of temperature (28±3°C), humidity (55-60%), and normal photoperiod cycle. The animals were acclimated to the laboratory conditions for two weeks. The human care of animals was according to regulations set by the National Institutes of Health guidelines.

2.2. Experimental design

Rats were categorized randomly into three groups (8 rats each). Group I was the control males; Groups II was received 1.3 mg/kg.b.w (orally by gavage) soybean sauce (local market, Assuit, Egypt), for 30 consecutive days. Groups III was received orally 2.6 mg/kg.b.w (orally) soy sauce, for consecutive 15 days then recovery for another 15 days (36,37). Every day the total body weight for every rat in previous groups were measured **[35-37]**. On the 31st day of the experimental onset, all the animals were sacrificed.

2.3. Complete Blood Count (CBC)

The evaluation of CBC was done by Automated Hematology Analyzer (Diff3) Mek-6410/Mek-6420 in the veterinary Exigo Hematology Analyzer at the Clinical Pathology

Laboratory in the Pathology Department, Faculty of Veterinary Medicine, Assiut University.

2. 4. Histological preparations

The testes were taken out and washed in saline solution. One testis from each rat was fixed in neutral buffered formalin (10%) formalin pH 7.2, dried, cleaned in xylene, and embedded in paraffin for histological and histopathological investigations. Sections were cut at 5 µm and stained with hematoxylin and eosin, Picrosirius Red Staining Protocol **[38]**. Photographs of the stained sections were captured by an industrial digital camera (LCMOS05100KPA, China) at Zoology Department Central Lab, Faculty of Science, Assuit University. Image J software (version 1.8) was used for morphometric analysis to measure the collagenous fiber in the testis, Fibrosis percentage % quantification is as follows: =Total positive area / Total section area X 100 **[39,40]**.

2.5 Preparation of testis homogenate

For preparing 10% w/v homogenate of testis; 500 mg of each testis was homogenized using a homogenizer (IKA Yellow line DI 18 Disperser, Germany), in 5 ml (0.1 M) phosphate buffer (pH 7.4). The homogenates were centrifuged at 5000 rpm for 30 min at 4 °C and the supernatant cytosols were kept frozen at -20 °C for the subsequent biochemical assays.

2.6. Biochemical measurement

Hormonal assay:

Testosterone (free and total) in serum samples was assayed by enzyme-linked immunosorbent assay (ELISA) according to the method of **[41]** for free testosterone and **[42]** for total testosterone.

Estimation of some oxidative stress markers:

Malondialdehyde as thiobarbituric acid was used to measure the lipid peroxidation (LPO) in the testis, as described by **[43]**. To prevent additional oxidation, 1 % v/v DMSO was added after homogenization. The reaction buffer was added to 0.2 ml aliquots of tissue homogenates and subjected to spectrophotometric measurement. Nitric oxide was calculated as the concentration of nitrite in the tissue cytosols using the method of **[44]**.

Estimation reduced glutathione

The concentration of GSH was estimated as described by **[45]**. Aliquots of 50 µl of tissue homogenate were added to 14.5 mg EDTA at 4000 rpm for 10 minutes. A mixture of 1 ml PBS at pH 8 and 100 µl DTNB to 100 µl was added to the supernatant, which was kept at room temperature for 5 minutes, before being used for the estimation of reduced glutathione at 412 nm.

2.7. Statistical Analysis:

One-way analysis of variance (ANOVA) was used, followed by the student Newman-Keuls T test, using the software Graph Pad Prism 3 (Graph Pad Software Inc., USA). The results are reported as mean ± SE. Statistical significance was accepted at $p < 0.05$.

RESULTS

3.1. Bodyweight

Supplementation of rats with low and high dose soybean sauce resulted in decreased in body weight by 7.92% and 5.3%, respectively, versus those of control. While rats supplemented with high dose and then left for recovery showed an increased (2.94%) in body weight versus those of the low dose group **(Fig. 1).**

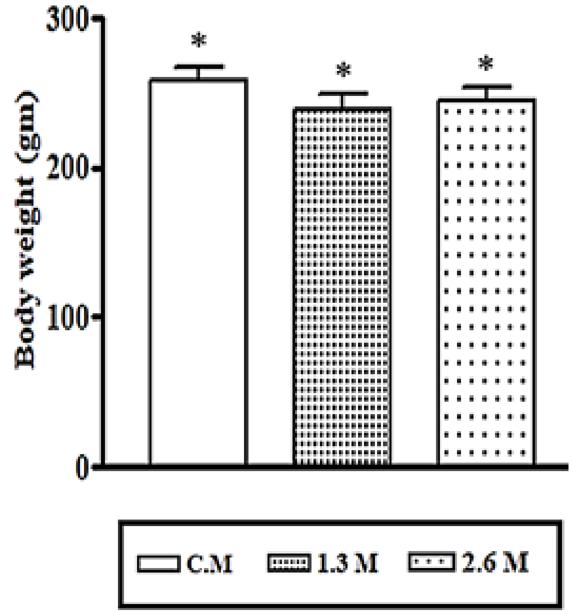


Fig. 1. The effects of low and high dose of soybean supplementation on body weight. Results presented as mean ± SEM. Values with similar superscript signs are non-significantly different at $P > 0.05$.

3.2. Complete Blood Count (CBC)

Supplementation of rats with a low dose of sauce caused a significant decrease in platelets and total WBCs counts ($P < 0.05$), but does not affect RBCs, hematocrit, HB, neutrophils, eosinophils, monocytes, lymphocytes, MCV, MCH and MCHC levels compared to the control rats. However, supplementation of rats with high doses caused non-significants in all the CBC parameters **(Table 1).**

	Control	Low dose vs. Control		High dose vs. Control	
	Mean	**Mean**	**% Change**	**Mean**	**% Change**
RBCs	7.087 *	6.853 ↓*	3.30	6.997 ↓*	1.30
HB	14.13 *	13.37 ↓ *	5.40	13.60 ↓*	3.75
HCT	37.73 *	36.40 ↓*	3.50	37.20 ↓*	1.40
MCV	52.30 *	53.60 ↑*	2.50	53.23 ↓*	1.80
MCH	19.60 *	19.50 ↓*	0.50	20.63 ↑*	5.30
MCHC	36.50 *	36.33 ↓*	0.50	36.67 ↑*	0.50
TWBCs	11.50 *	9.900 ↓**	13.9	10.87 ↓	5.50
Neutrophils	18.10 *	23.00 ↑ **	27.1	18.37 ↑*	1.50
Eosinophils	0.1667 *	0.5000 ↑**	199.9	0.2 ↑ *	20.0
Lymphocytes	75.83 *	71.53 ↓*	5.70	76.73 ↑*	1.20
Monocytes	3.667 *	3.967 ↑ *	8.20	4.733 ↑*	29.1
Platelets	868.0 *	761.3↓ **	12.3	852.7 ↓**	1.80

Table 1. Complete blood count (CBC) values, expressed as mean ± SE, and the % of changes between the treated and control group.

3.3. Histopathological Examination:

Observation of the testis of the control rats showed the normal histological structure of the seminiferous tubules and normal spermatogenesis **Fig.2 (A&B).** The supplementation of rats with a low dose of sauce caused marked irregularity of the seminiferous tubule periphery and atrophy in seminiferous tubules with edema. There was degeneration and detachment of germ cells from basement membrane of seminiferous tubules, loss of regular distribution of spermatogenic cells, primary spermatogonia are vacuolated, spermatogenesis arrest at spermatid stage, degenerated spermatozoa in the center of tubules, widening of the intertubular spaces with decreased number and degeneration of Leydig cells when compared with control **Fig. 2 (C&D).** Supplementation of rats with a high dose of sauce with recovery caused high restoration of the normal architecture of the testis and the progressing spermatogenesis with its regular stages appeared. Another finding in this group was the remarkable abundance of interstitial cells between the seminiferous tubules. Also, mitotic figures were seen in the germ cells which form a continuous layer **Fig. 2 (E&F).**

Collagen staining of picrosirius red of testis sections from control showed normal content and distribution of collagen fibers, which take the red color, the seminiferous tubules lined with a definite layer of connective tissues fibers **Fig2. (G).** Testis of rats supplemented with a low dose of sauce revealed a mild increase in the collagen fibers content and distribution than control and high dose with the recovery of sauce groups **Fig.2 (H).** Also a high dose with the recovery of sauce group showed a slight increase of connective tissue fibers (collagenous) through the testicular tissue vs. the control group **Fig. 2 (I).**

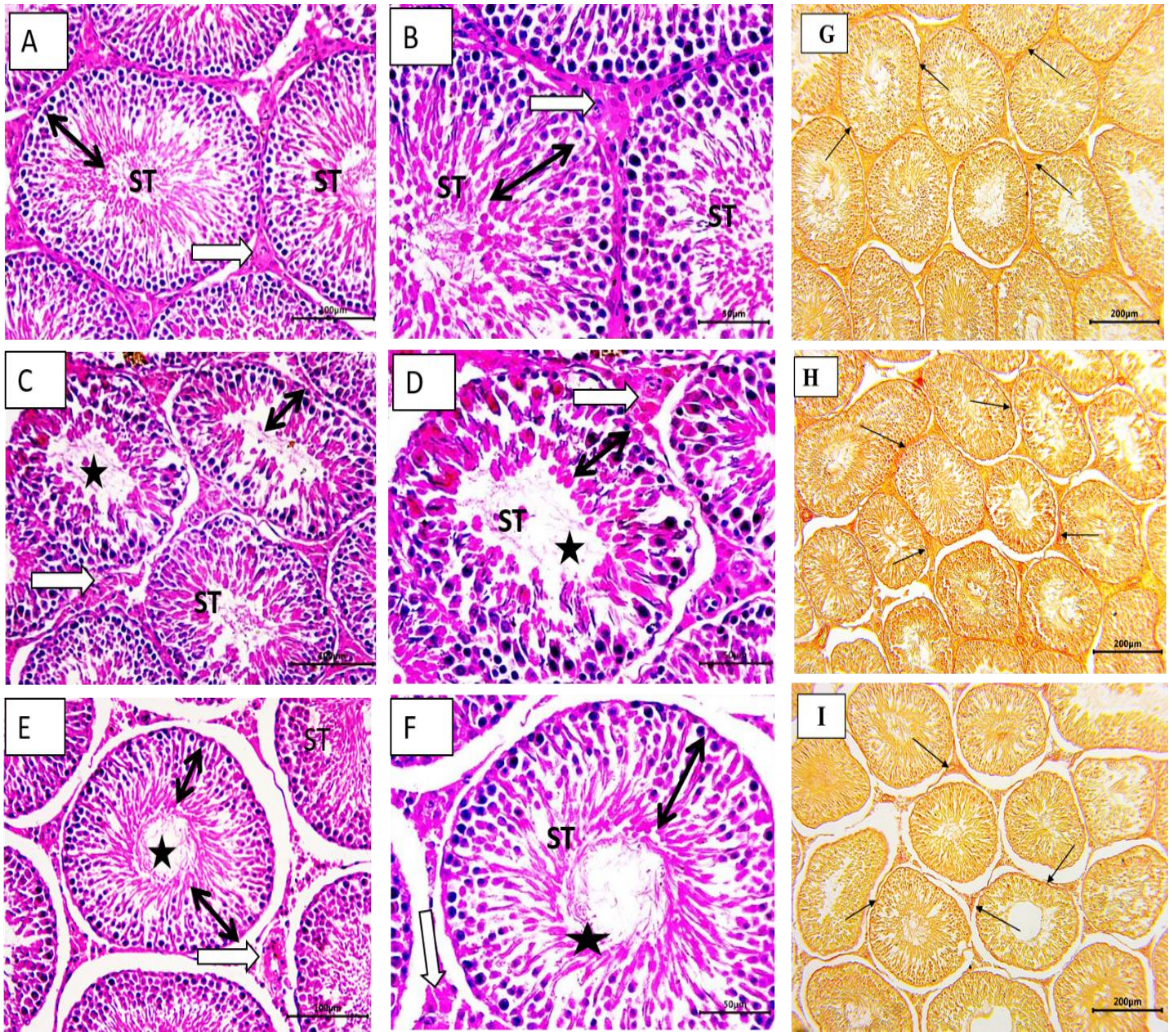


Fig. 2. Photomicrograph of H&E and Picrosirius Red Staining of rat testis sections of the different groups photographed at (HE, Bar =100 um & Bar = 50 um / PSR, Bar = 200 um). **(A&B)** control group, showing seminiferous tubules (ST) with regular thickness germ cell layer (double head arrows). Interstitial cells of normal population (white arrow). (**C&D),** low dose group showing ST larger than control with disorganization of germ cell layer. Gem cell layer is less in thickness (double heads arrow). Mature sperms are absent from most tubules (Black star). Interstitial cells looked decreased compared to control. **(E&F),** of high dose group showing nearly normal ST with full thickness germ cell layers (double head arrow) and mature sperms in the lumen (star). Interstitial cells of normal population (white arrow). **(G),** control showing normal collagenous fibers in the basement membrane of seminiferous tubules (black arrows). **(H)** low dose group showing

collagen was increased (black arrows). **(I)** high dose group showed slightincrease in collagen deposition (black arrows).

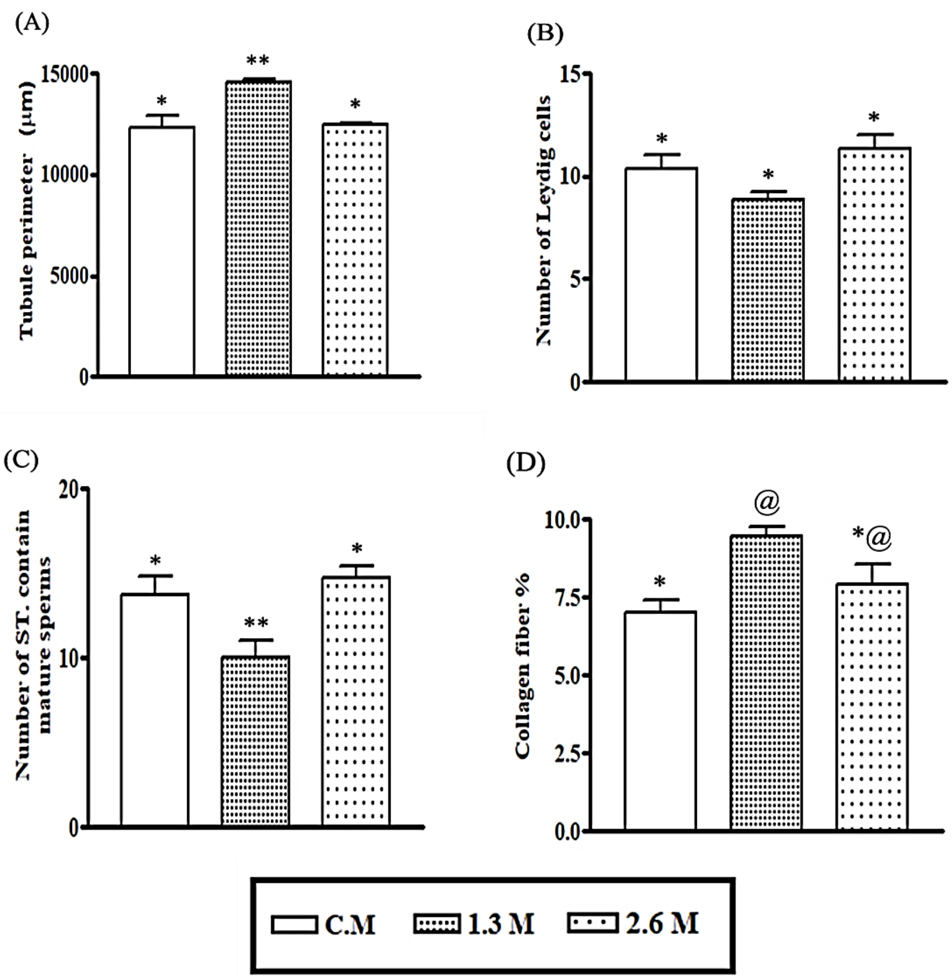
3.4. Morphometric analysis

Tubule perimeter*:* Quantitative results by image analysis of H&E-stained sections of testes showed that the tubule perimeter in the low-dose supplemented group with sauce significantly increased by 18.1%, however, in the high-dose group non- significantly increased by 1.4% vs. control. Statistically, it was a significant decrease (14.2%) in the tubules perimeter of the high-dose group versus these of the low-dose group ($P < 0.01$) **(Fig.3 A)**.

Leydig cells: The number of Leydig cells in the low-dose group non-significantly decreased by 14.5% and the high-dose group non-significantly increased 9.7%, respectively vs. the control group (C). Also, in high-dose group revealed non -a significant increase of 28.3% vs. L dose group **(Fig. 3B)**.

Tubules contained mature sperms*:* The number of tubules containing mature sperms in the low-dose group significantly decreased by 26.8% vs. the control group, ($P < 0.05$). While this number was in the high dose group, non-significantly increased by 7.3% vs. the control group (C). When comparing this number in both high dose group and low dose group, it showed a significant increase of 46.7%, ($P < 0.05$) **(Fig. 3C)**.

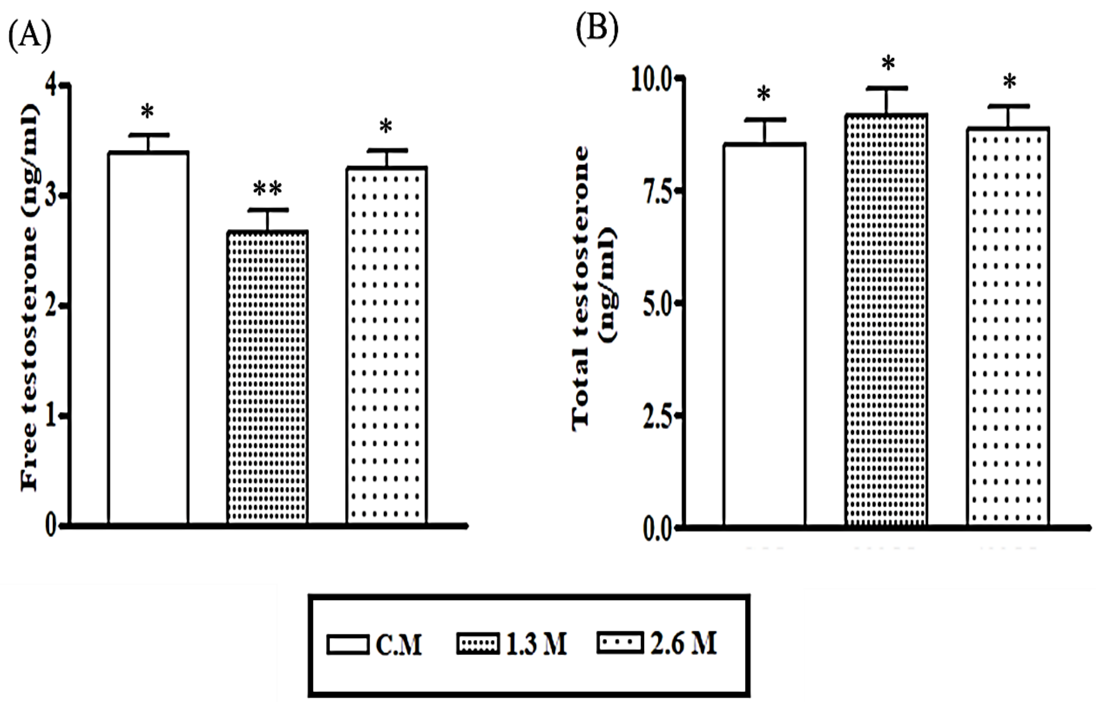
Collagen fibers content: Quantitative results by image analysis of collagen-stained sections of testes showed that collagen content in the testis of low dose group showed a significant increase by 34.46 % vs. control. Statistically, the collagen fiber content in the high-dose group revealed a non-significant increase 12.7% vs. the control group. At the same time, high dose group was decreased by 16.2% vs. the low dose group **(Fig. 3D)**.



Figs. 3 (A-D). Effect of low and high dose supplementation with sauce on seminiferous tubule perimeter, Leydig cells number, number of seminiferous tubules contained mature sperms, and the % of testis fibrosis. The data are expressed as Mean ± S.E.M. Means with different superscripts are significantly different.

3.5. Analysis of hormones:

The level of serum-free testosterone was significantly decreased (21.65%) for the rats group that received a low dose of sauce ($P < 0.05$), and non-significantly decreased (4.62%) for the high dose group compared with the control group. While free testosterone level in high dose group was significantly increased (21.82%) compared with low dose group (P < 0.05) **Fig. 4A**. Serum levels of total testosterone non-significantly increased (7.55% and 3.7%) in rats who received a low and high dose of sauce, respectively, compared with the control group. While in high dose group, total testosterone non-significantly decreased (3.57%) compared with low dose group **(Fig. 4B)**.



Figs. 4. Effect of low and high dose supplementation with sauce on free and total testosterone in blood serum of male rats. The data are expressed as Mean ± S.E.M. Means with different superscripts are significantly different at P < 0.05.

3.6. Oxidative stress biomarkers:

Lipid peroxidation (LPO) in a low dose of sauce supplemented group significantly declined (46.4%), while in rats supplemented with high dose group; it was non-significantly decline (19.9%) vs. control group. Also, there was a significant increase (49.4%) in high dose (P < 0.05), when compared with the low dose group **(Fig. 5A)**. Nitric oxide as nitrite levels significantly decreased (45.1%) (P < 0.001), while the decrement of NO non-significantly (15%) in the high dose group vs. control. Also, there is a significant increase (54.8%) in high dose group vs. low dose group (P < 0.01) **(Fig. 5B)**. Glutathione content significantly was increased (48.78% and 30.5%) in low and high-dose supplemented groups vs. control (P < 0.01 and P < 0.05), respectively. Also, the changes non-significantly decreased in high dose group (12.28%) vs. low dose group **(Fig. 5C)**.

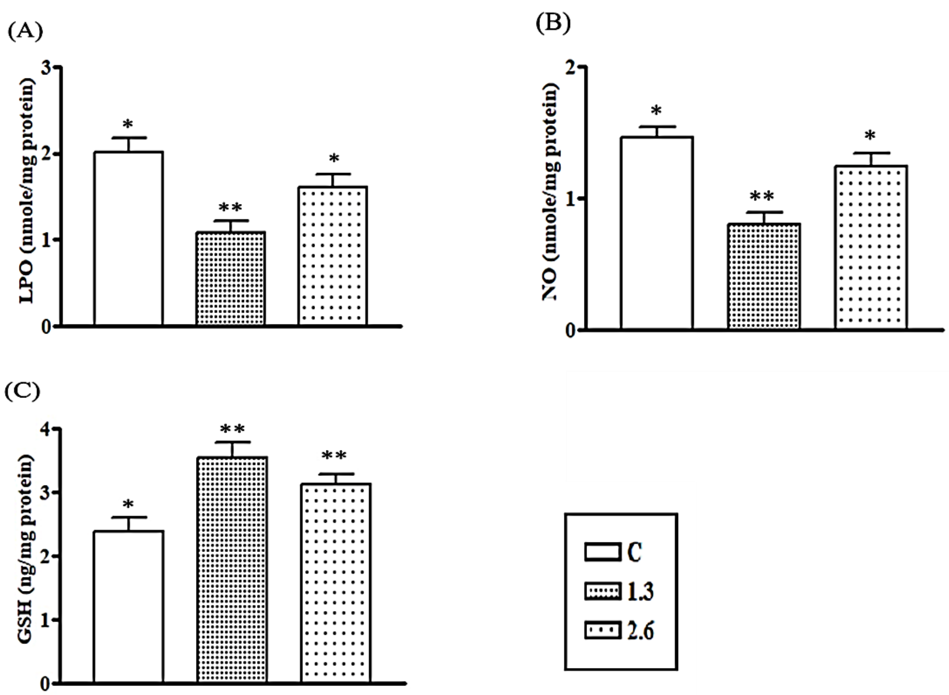


Fig. 5. Effect of L and H dose of SBS (1.3 and 2.6 mg/kg B.W.) in testicular activities of LPO, NO, and GSH level in male albino rats. The data are expressed as Mean ± S.E.M. Means with different superscripts are significantly different.

DISCUSSION

Despite the various stated pharmacological benefits soy sauce has been linked to high blood pressure and an increased risk of cardiovascular illnesses and stroke when ingested at levels beyond the daily recommended dose (46). In the present experimental groups, no significant change in body weight occurred.

Akhlaghi et al. (2017) confirmed numerous animal and human studies have shown the beneficial effect of soy on weight reduction (12). The anti-obesity effect of soy is partly attributed to its high protein content. High-protein diets have been effective in ad libitum food consumption in weight maintenance and reduction (13). Changes in leucocytes composition of low dose group may be due to the proliferation of some leukocytes that are indicative of immune response to foreign antigens. In this aspect, RBC, WBC, and HB decreased after xenobiotic oral administration that induce oxidative stress and the interaction between the xenobiotic and erythrocytes membrane proteins (47,48).

The present histological study of the testis showed, supplementation of rats with a low dose of sauce for 30 days caused an increase in testis perimeter. Also, it decreased in tubules containing mature sperms, a mild decrease in Leydig cells number, and degeneration in seminiferous tubules. These findings supported previous observations on rodents (16,18–22). It is well established that testosterone is an important for germ cells and spermatid development. The decrease of this hormone increase germ cells and spermatids apoptosis and thereby sperms apoptosis (49–51), which attributed to the disruption of the hypogonadal–pituitary–testicular axis (18). Also, it decreased tubules containing mature sperm may be associated with the anti-estrogenic effect of soy isoflavones. Earlier studies revealed that estrogen is responsible for the proliferation and differentiation of Leydig cells, therefore the decrease in the number of Leydig cells may be due to the anti-estrogenic and or weak estrogenic effect of isoflavones s (18,20,52). Moreover, increased testis perimeter is due to the anti-estrogenic properties isoflavones due to estrogen receptors involved in the regulation of fluid reabsorption in efferent ductless (53,54).

Consequently, any disruption in estrogen action, by the administration of an anti-estrogen to adult rats, reduced fluid absorption in the ex-current duct system, thus increasing water retention and its accumulation in the lumen and the flattening of epithelial cells (18,55). Also, the present work observed, no toxic effect of sauce oral administration with a high dose for 15 days and then left for recovery but the result was near to control. a high restoration of the normal architecture of the testis and the progressing spermatogenesis with its regular stages appeared. Our morphometric analysis of collagen had a significant increase in testis fibrosis in both two treated groups, and this support an earlier study that reported that soy increased collagen type I (56,57). The antioxidant properties of soya sauce polyphenols may be reflected in the current histopathological data. Phenols, a compound found in soybeans, help to significantly offset the negative effects of xenobiotic that cause the generation of free radicals in the liver (58).

In male rats who received a low dose of sauce, a reduction in serum-free testosterone levels occurred which matched with earlier studies on rodents (17, 59–66). While rats received a high dose of sauce showed, a non-remarkable difference in serum testosterone level occurred. Total testosterone had not to be affected in all experimental groups, and this finding agrees with the study (34). Reduction in serum testosterone level related to estrogenic activity of isoflavones and an inhibition of the steroidogenic enzymes (27). Our results supported that isoflavones suppressed steroidogenic capacity in the Leydig cells as suggested previously (28). Isoflavones may also interfere with the metabolism of steroid hormones, thus inhibited the enzyme activity involved in the steroidogenic pathway (67). Isoflavones were reported to elevate sex hormone-binding globulin production in the liver which binds to biologically active testosterone and thus declines the levels of free testosterone and its bioavailability to the target cells, consequently elevating total testosterone (29,68).

In the present study, soy sauce supplementation reduced LPO, NO and increased GSH in rats which confirmed previous studies (7–9). Isoflavones may repress free radical generation by enhancing endogenous antioxidants, such as superoxide dismutase and GSH (69). These effects are associated with hormonal and physiological activity (70). Isoflavones and genistein were reported to reduce concentrations of free radicals and activate antioxidant enzymes in various organs (48,71). The antioxidant potencies of isoflavones are structurally associated with the presence of hydroxyl groups at positions 4′ and 5′ and the position of the aromatic ring (13,72, 73). Soybeans compounds, such as isoflavones, play a significant role in neutralizing the harmful effects of xenobiotics in the liver (74) due to physiological changes induced by multiple constituents (75) and enhancement of antioxidants isoflavones (76).

CONCLUSION

The present histopathological and biochemical observations in rats supplemented with a high dose of soya sauce; may reflect the estrogenic properties of soy isoflavones caused by hormonal imbalances.

REFERENCES

[1] Wang Y, Jiang C, Bazinet L, Xu T. Electrodialysis-based separation technologies in the food industry [Internet]. Separation of Functional Molecules in Food by Membrane Technology. Elsevier Inc.; 2018. 349–381 p. Available from: http://dx.doi.org/10.1016/B978-0-12-815056-6.00010-3.

[2] Mizushina Y, Shiomi K, Kuriyama I, Takahashi Y, Yoshida H. Inhibitory effects of a major soy isoflavone, genistein, on human DNA topoisomerase II activity and cancer cell proliferation. Int J Oncol [Internet]. 2013 Oct;43(4):1117–24. Available from: https://pubmed.ncbi.nlm.nih.gov/23900272.

[3] Chen L-R, Ko N-Y, Chen K-H. Isoflavone supplements for menopausal women: A systematic review. Nutrients. 2019 Nov 1;11(11).

[4] Křížová L, Dadáková K, Kašparovská J, Kašparovský T. Isoflavones. Molecules. 2019;24(6).

[5] Ososki AL, Kennelly EJ. Phytoestrogens: a review of the present state of research. Phytother Res [Internet]. 2003 Sep;17(8):845–69. Available from: https://pubmed.ncbi.nlm.nih.gov/13680814.

[6] Rietjens IMCM, Louisse J, Beekmann K. The potential health effects of dietary phytoestrogens. Br J Pharmacol [Internet]. 2017;174(11):1263–80. Available from: https://pubmed.ncbi.nlm.nih.gov/27723080.

[7] Dixit AK, Bhatnagar D, Kumar V, Chawla D, Fakhruddin K, Bhatnagar D. Antioxidant potential and radioprotective effect of soy isoflavone against gamma irradiation induced oxidative stress. J Funct Foods. 2012 Jan;4(1):197–206.

[8] Teixeira CP, Florencio-Silva R, Sasso GRS, Carbonel AAF, Simões RS, Simões MJ. Soy isoflavones protect against oxidative stress and diminish apoptosis in ovary of middle-aged female rats. Gynecol Endocrinol. 2019 Jul 3;35(7):586–90.

[9] Alipour MR, Karimi-Sales E. Molecular mechanisms of protective roles of isoflavones against chemicals-induced liver injuries. Chem Biol Interact. 2020 Sep 25;329:109213.

[10] Laddha AP, Murugesan S, Kulkarni YA. In-vivo and in-silico toxicity studies of daidzein: an isoflavone from soy. Drug Chem Toxicol [Internet]. 2022;45(3):1408–16. Available from: https://pubmed.ncbi.nlm.nih.gov/33059469.

[11] CM I, IH I, CM I, IH I. Hematological indices and sensory quality of fermented soybean condiments. https://wjarr.com/sites/default/files/WJARR-2022-0411.pdf [Internet]. 2022 May 30;14(2):435–42. Available from: https://wjarr.com/content/hematological-indices-and-sensory-quality-fermented-soybean-condiments.

[12] Akhlaghi M, Zare M, Nouripour F. Effect of Soy and Soy Isoflavones on Obesity-Related Anthropometric Measures: A Systematic Review and Meta-analysis of Randomized Controlled Clinical Trials. Adv Nutr [Internet]. 2017 Sep 1;8(5):705–17. Available from: https://pubmed.ncbi.nlm.nih.gov/28916571.

[13] Rajan RK, Kumar M. SS, Balaji B. Soy isoflavones exert beneficial effects on letrozole-induced rat polycystic ovary syndrome (PCOS) model through anti-androgenic mechanism. Pharm Biol [Internet]. 2017;55(1):242–51. Available from: http://dx.doi.org/10.1080/13880209.2016.1258425.

[14] Velasquez MT, Bhathena SJ. Role of dietary soy protein in obesity. Int J Med Sci [Internet]. 2007 Feb 26;4(2):72–82. Available from: https://pubmed.ncbi.nlm.nih.gov/17396158.

[15] Patisaul HB. Endocrine disruption by dietary phyto-oestrogens: impact on dimorphic sexual systems and behaviours. Proc Nutr Soc [Internet]. 2017 May 1;76(2):130–44. Available from: https://www.cambridge.org/core/journals/proceedings-of-the-nutrition-society/article/endocrine-disruption-by-dietary-phytooestrogens-impact-on-dimorphic-sexual-systems-and behaviours/870318B231ED5818E44BD2F01FFFD011.

[16] Assinder S, Davis R, Fenwick M, Glover A. Adult-only exposure of male rats to a diet of high phytoestrogen content increases apoptosis of meiotic and post-meiotic germ cells. Reproduction. 2007;133(1):11–9.

[17] Adesanya OA, Oyesola OA, Adesanya RA, Bamitale KDS, Odubela OO. Effects of Soy-flour Enriched Diet on the Testis and Gonadal Hormone Status of Male Wistar Rats Control Low dose soya diet High dose soya diet. 2016;4:1657–61.

[18] Serag El Din OS, Batta, H. AEA, Abd El Fattah N. Effect of soybean on fertility of male and female albino rats. J Am Sci. 2011;7(6):872–83.

[19] Norazit A, Mohamad J, Razak SA, Abdulla MA, Azmil A, Mohd MA. Effects of Soya Bean Extract, Bisphenol A and 17β-Estradiol on the Testis and Circulating Levels of Testosterone and Estradiol Among Peripubertal Juvenile Male Sprague-Dawley Rats. Sains Malaysiana. 2012;41(1):63–9.

[20] Hancock KD, Coleman ES, Tao YX, Morrison EE, Braden TD, Kemppainen BW, et al. Genistein decreases androgen biosynthesis in rat Leydig cells by interference with luteinizing hormone-dependent signaling. Toxicol Lett [Internet]. 2009 Feb 10;184(3):169–75. Available from: https://pubmed.ncbi.nlm.nih.gov/19059320.

[21] Cederroth CR, Zimmermann C, Beny JL, Schaad O, Combepine C, Descombes P, et al. Potential detrimental effects of a phytoestrogen-rich diet on male fertility in mice. Mol Cell Endocrinol [Internet]. 2010;321(2):152–60. Available from: https://pubmed.ncbi.nlm.nih.gov/20171261.

[22] Zhu Y, Xu H, Li M, Gao Z, Huang J, Liu L, et al. Daidzein impairs Leydig cell

testosterone production and Sertoli cell function in neonatal mouse testes: An in vitro study. Mol Med Rep. 2016 Dec 1;14(6):5325–33.

[23] Jung EY, Lee BJ, Yun YW, Kang JK, Baek IJ, Yon JM, et al. Effects of exposure to genistein and estradiol on reproductive development in immature male mice weaned from dams adapted to a soy-based commercial diet. J Vet Med Sci [Internet]. 2004 Nov;66(11):1347–54. Available from: https://pubmed.ncbi.nlm.nih.gov/15585947.

[24] Ekaluo UB, Udoh PB, Ikpeme E V., Udensi O. Effect of soybean (Glycine max L) on the hormonal milieu of male rats. Pakistan J Biol Sci. 2011;14(14):752–4.

[25] Modaresi M, Messripour M, Khorami H. Effect of Soybean on Levels of LH, FSH and Testosterone Hormones and Testis in Adult Male Mice. Nature, Environ Pollut Technol [Internet]. 2011;10(3):337–42. Available from: https://www.researchgate.net/publication/236680939_Effect_of_Soybean_on_Levels_of_LH_FSH_and_Testosterone_Hormones_and_Testis_in_Adult_Male_Mice.

[26] Faqi AS, Johnson WD, Morrissey RL, McCormick DL. Reproductive toxicity assessment of chronic dietary exposure to soy isoflavones in male rats. Reprod Toxicol [Internet]. 2004 Jun;18(4):605–11. Available from: https://pubmed.ncbi.nlm.nih.gov/15135855.

[27] Luo Q, Li Y, Huang C, Cheng D, Ma W, Xia Y. Soy Isoflavones Improve the Spermatogenic Defects in Diet-Induced Obesity Rats Through Nrf2 / HO-1 Pathway. 2019;1–19.

[28] Napier ID, Simon L, Perry D, Cooke PS, Stocco DM, Sepehr E, et al. Testicular development in male rats is sensitive to a soy-based diet in the neonatal period. Biol Reprod. 2014;90(2).

[29] Tanaka M, Fujimoto K, Chihara Y, Torimoto K, Yoneda T, Tanaka N, et al. Isoflavone supplements stimulated the production of serum equol and decreased the serum dihydrotestosterone levels in healthy male volunteers. Prostate Cancer Prostatic Dis [Internet]. 2009;12(3):247–52. Available from:

https://pubmed.ncbi.nlm.nih.gov/19597532.

[30] Hamilton-Reeves JM, Vazquez G, Duval SJ, Phipps WR, Kurzer MS, Messina MJ. Clinical studies show no effects of soy protein or isoflavones on reproductive hormones in men: results of a meta-analysis. Fertil Steril [Internet]. 2010 Aug;94(3):997–1007. Available from: https://pubmed.ncbi.nlm.nih.gov/19524224.

[31] Goodin S, Shen F, Shih WJ, Dave N, Kane MP, Medina P, et al. Clinical and biological activity of soy protein powder supplementation in healthy male volunteers. Cancer Epidemiol Biomarkers Prev [Internet]. 2007 Apr;16(4):829–33. Available from: https://pubmed.ncbi.nlm.nih.gov/17416779.

[32] Chavarro JE, Toth TL, Sadio SM, Hauser R. Soy food and isoflavone intake in relation to semen quality parameters among men from an infertility clinic. Hum Reprod [Internet]. 2008;23(11):2584–90. Available from: https://pubmed.ncbi.nlm.nih.gov/18650557.

[33] Messina M. Soybean isoflavone exposure does not have feminizing effects on men: a critical examination of the clinical evidence. Fertil Steril [Internet]. 2010 May 1;93(7):2095–104. Available from: https://pubmed.ncbi.nlm.nih.gov/20378106.

[34] Sathyapalan T, Rigby AS, Bhasin S, Thatcher NJ, Kilpatrick ES, Atkin SL. Effect of soy in men with type 2 diabetes mellitus and subclinical hypogonadism: A randomized controlled study. J Clin Endocrinol Metab. 2017 Feb 1;102(2):425–33.

[35] Fritz WA, Wang J, Eltoum IE, Lamartiniere CA. Dietary genistein down-regulates androgen and estrogen receptor expression in the rat prostate. Mol Cell Endocrinol [Internet]. 2002 Jan 15;186(1):89–99. Available from: https://pubmed.ncbi.nlm.nih.gov/11850125.

[36] Song J. Anti-colitic effects of kanjangs (fermented soy sauce and sesame sauce) in dextran sulfate sodium-induced colitis in mice 1.2014;17(9):1027-35.

[37] Nagahara A, Ohshita K, Nasuno S. Investigation of soy sauce treated with nitrite in the chromosomal aberration test *in vitro* and the micronucleus test *in vivo*

Summory. 1991;262:171–6.

[38] Courtoy GE, Leclercq I, Froidure A, Schiano G, Morelle J, Devuyst O, et al. Digital image analysis of picrosirius red staining: A robust method for multi-organ fibrosis quantification and characterization. Biomolecules.2020;10(11):1–23.

[39] Bataller R, Schwabe RF, Choi YH, Yang L, Paik YH, Lindquist J, et al. NADPH oxidase signal transduces angiotensin II in hepatic stellate cells and is critical in hepatic fibrosis. J Clin Invest [Internet]. 2003;112(9):1383–94. Available from: https://pubmed.ncbi.nlm.nih.gov/14597764.

[40] Wang H, Zhang Y, Heuckeroth RO. PAI-1 deficiency reduces liver fibrosis after bile duct ligation in mice through activation of tPA. FEBS Lett. 2007 Jun 26;581(16):3098–104.

[41] Chen A, Bookstein JJ, Meldrum DR. Diagnosis of a testosterone-secreting adrenal adenoma by selective venous catheterization. Fertil Steril. 1991;55(6):1202–3.

[42] Kovacs WJ, Orth DN. The Adrenal Cortex BT - Williams Textbook of Endocrinology. Williams Textb Endocrinol [Internet]. 1998;(12):517–664. Available from: http://www.worldcat.org/title/williams-textbook-of-endocrinology/oclc/22184127.

[43] Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem [Internet]. 1979 ;95(2):351–8. Available from: https://pubmed.ncbi.nlm.nih.gov/36810.

[44] Ding AH, Nathan CF, Stuehr DJ. Release of Reactive Nitrogen Intermediates and Reactive Oxygen Intermediates from Mouse Peritoneal Macrophages: Comparison of Activating Cytokines and Evidence for Independent Production. . Immunol [Internet]. 1988 141(7):2407–12. Available from: https://www.scirp.org/(S(lz5mqp453edsnp55rrgjct55))/reference/referencespapers.aspx?referenceid=2810152.

[45] Ravi K, Ramachandran B, Subramanian S. Protective effect of Eugenia jambolana seed kernel on tissue antioxidants in streptozotocin-induced diabetic rats. Biol

Pharm Bull. 2004 Aug;27(8):1212–7.

[46] Jeong H, Noh M, Choi J, Lee H, and Kim S. Neuroprotective and antioxidant activities of bamboo salt soy sauce against H_2O_2-induced oxidative stress in rat cortical neurons, Experimental and Theraputic Medicine. 2016 11: 1201-1210.

[47] Enodien EO, Akpan OU, Umoh and IO. Comparative Hematological Effects of Cimetidine , Ascorbic Acid , Citrus Aurantifolia and Tetracarpidium Conophorium in Adult. Eur J Pharm Med Res. 2018;5(5):619–27.

[48] Atia MM, Abdel- F. Soya milk alleviates toxicity caused by citric acid in male mice : Histopathological and hematological studies. 2021;(April):1–10.

[49] Asadi A, Ghahremani R, Abdolmaleki A, Rajaei F. Role of sperm apoptosis and oxidative stress in male infertility: A narrative review. Int J Reprod Biomed [Internet]. 2021 Jun 1 ;19(6):493–504. Available from: https://pubmed.ncbi.nlm.nih.gov/34401644.

[50] Smith LB, Walker WH. The regulation of spermatogenesis by androgens. Semin Cell Dev Biol [Internet]. 2014 ;30:2–13. Available from: https://pubmed.ncbi.nlm.nih.gov/24598768.

[51] McLachlan RI, O'Donnell L, Meachem SJ, Stanton PG, De Kretser DM, Pratis K, et al. Identification of specific sites of hormonal regulation in spermatogenesis in rats, monkeys, and man. Recent Prog Horm Res [Internet]. 2002 ;57:149–79. Available from: https://pubmed.ncbi.nlm.nih.gov/12017541/

[52] Yu J, Bi X, Yu B, Chen D. Isoflavones: Anti-Inflammatory Benefit and Possible Caveats. Nutrients [Internet]. 2016 Jun 10 ;8(6). Available from: /pmc/articles/PMC4924202.

[53] Hess RA, Fernandes SAF, Gomes GRO, Oliveira CA, Lazari MFM, Porto CS. Estrogen and its receptors in efferent ductules and epididymis. J Androl [Internet]. 2011 Nov ;32(6):600–13. Available from: https://pubmed.ncbi.nlm.nih.gov/21441425.

[54] Hess RA, Cooke PS. Estrogen in the male: a historical perspective. Biol Reprod

[Internet]. 2018 Jul 1 ;99(1):27–44. Available from: https://academic.oup.com/biolreprod/article/99/1/27/4847876.

[55] Oliveira CA, Carnes K, França LR, Hess RA. Infertility and testicular atrophy in the antiestrogen-treated adult male rat. Biol Reprod. 2001;65(3):913–20.

[56] Tokudome Y, Nakamura K, Kage M, Todo H, Sugibayashi K, Hashimoto F. Effects of soybean peptide and collagen peptide on collagen synthesis in normal human dermal fibroblasts. Int J Food Sci Nutr [Internet]. 2012 Sep ;63(6):689–95. Available from: https://pubmed.ncbi.nlm.nih.gov/22264122.

[57] Accorsi-Neto A, Haidar M, Simões R, Simões M, Soares-Jr J, Baracat E. Effects of isoflavones on the skin of postmenopausal women: a pilot study. Clinics (Sao Paulo) [Internet]. 2009 Jun ;64(6):505–10. Available from: https://pubmed.ncbi.nlm.nih.gov/19578653.

[58] Jiang CX, Pan LJ, Feng Y, Xia XY, Huang YF. High-dose daidzein affects growth and development of reproductive organs in male rats [Internet]. Vol. 14, Zhonghua nan ke xue = National journal of andrology. 2008. p. 351–5. Available from: https://pubmed.ncbi.nlm.nih.gov/18481431.

[59] Zhang WZ, Cui WM, Zhang X, Wang W, Jia XD, Zhang XP, et al. Subchronic Toxicity Study on Soy Isoflavones in Rats. Biomed Environ Sci. 2009 Jun 1;22(3):259–64.

[60] Musameh NI, Zin SR, Kassim NM. Effects of genistein on male sprague dawley rats reproductive development . 2014;25(3):391–400.

[61] Weber KS, Setchell KDR, Stocco DM, Lephart ED. Dietary soy-phytoestrogens decrease testosterone levels and prostate weight without altering LH, prostate 5alpha-reductase or testicular steroidogenic acute regulatory peptide levels in adult male Sprague-Dawley rats. J Endocrinol [Internet]. 2001 ;170(3):591–9. Available from: https://pubmed.ncbi.nlm.nih.gov/11524239.

[62] Ohno S, Nakajima Y, Inoue K, Nakazawa H, Nakajin S. Genistein administration decreases serum corticosterone and testosterone levels in rats. Life Sci [Internet].

2003 Dec 26 ;74(6):733–42. Available from: https://pubmed.ncbi.nlm.nih.gov/14654166.

[63] Svechnikov K, Supornsilchai V, Strand ML, Wahlgren A, Seidlova-Wuttke D, Wuttke W, et al. Influence of long-term dietary administration of procymidone, a fungicide with anti-androgenic effects, or the phytoestrogen genistein to rats on the pituitary-gonadal axis and Leydig cell steroidogenesis. J Endocrinol. 2005;187(1):117–24.

[64] Pan L, Xia X, Feng Y, Jiang C, Huang Y. Exposure to the Phytoestrogen Daidzein Attenuates Apomorphine-Induced Penile Erection Concomitant with Plasma Testosterone Level Reduction in Dose and Time-Related Manner in Adult Rats. Urology. 2007 Sep;70(3):613–7.

[65] Akingbemi BT, Braden TD, Kemppainen BW, Hancock KD, Sherrill JD, Cook SJ, et al. Exposure to phytoestrogens in the perinatal period affects androgen secretion by testicular Leydig cells in the adult rat. Endocrinology [Internet]. 2007 Sep ;148(9):4475–88. Available from: https://pubmed.ncbi.nlm.nih.gov/17569756.

[66] Bae M, Woo M, Kusuma IW, Arung ET, Yang CH, Kim YU. Inhibitory Effects of Isoflavonoids on Rat Prostate Testosterone 5α-Reductase. JAMS J Acupunct Meridian Stud. 2012;5(6):319–22.

[67] Hu GX, Zhao BH, Chu YH, Zhou HY, Akingbemi BT, Zheng ZQ, et al. Effects of genistein and equol on human and rat testicular 3beta-hydroxysteroid dehydrogenase and 17beta-hydroxysteroid dehydrogenase 3 activities. Asian J Androl [Internet]. 2010 ;12(4):519–26. Available from: https://pubmed.ncbi.nlm.nih.gov/20453869.

[68] Sivoňová MK, Kaplán P, Tatarková Z, Lichardusová L, Dušenka R, Jurečeková J. Androgen receptor and soy isoflavones in prostate cancer (Review). Mol Clin Oncol. 2019;10(2):191–204.

[69] Huang QH, Xu LQ, Liu YH, Wu JZ, Wu X, Lai XP, et al. Polydatin Protects Rat Liver against Ethanol-Induced Injury: Involvement of CYP2E1/ROS/Nrf2 and

TLR4/NF- B p65 Pathway. Evidence-based Complement Altern Med. 2017;2017.

[70] Zaheer K, Humayoun Akhtar M. An updated review of dietary isoflavones: Nutrition, processing, bioavailability and impacts on human health. Crit Rev Food Sci Nutr [Internet]. 2017;57(6):1280–93. Available from: http://dx.doi.org/10.1080/10408398.2014.989958.

[71] Jourdehi YA. Effects of dietary isoflavone-genistein on hematological and immunological parameters in pre-brood stock beluga, Huso huso. Iran J Fish Sci. 2016;15(1):390–401.

[72] Coxam V. Phyto-oestrogens and bone health: Symposium on 'Diet and bone health.' Proc Nutr Soc [Internet]. 2008 May ;67(2):184–95. Available from: https://www.cambridge.org/core/journals/proceedings-of-the-nutrition-society/article/phytooestrogens-and-bone-health/25BCD5B25D08EED725E6815325C37025.

[73] Setchell KDR, Brown NM, Lydeking-Olsen E. The clinical importance of the metabolite equol-a clue to the effectiveness of soy and its isoflavones. J Nutr [Internet]. 2002 Dec 1 ;132(12):3577–84. Available from: https://pubmed.ncbi.nlm.nih.gov/12468591.

[74] Yakubu N, Mohammed UA. International Journal of Pharmaceutical Sciences and Drug Research CODEN (USA): IJPSPP Protective Properties of Flavonoid Extract of Coagulated Tofu (Curdled Soy Milk) Against Acetaminophen-Induced Liver Injury in Rats. 2016;8(1):21–9.

[75] Fernandez-Orozco R, Frias J, Zielinski H, Piskula MK, Kozlowska H, Vidal-Valverde C. Kinetic study of the antioxidant compounds and antioxidant capacity during germination of Vigna radiata cv. emmerald, Glycine max cv. jutro and Glycine max cv. merit. Food Chem. 2008;111(3):622–30.

[76] Thi D, Lien P, Thi C, Hoang K, Hanh NT, Chu DX, et al. Hepatoprotective effect of tofu processed from germinated soybean on carbon tetrachloride induced chronic liver injury in mice. Food Heal Sci. 2017;3(1):1–11.