INTERNATIONAL JOURNAL OF RESEARCH AND SCIENTIFIC INNOVATION (IJRSI)
ISSN No. 231-2705 | DOI: 10.51244/IJRSI | Volume X Issue III March 2023

Effect of Aqueous Extract of Soya Bean (*Glycine Max*) on the Male Reproductive Hormones and the Testes of Male Albino Wistar Rats

Darlington-Peter Chibuzor UGOJI[1,3*], Emmanuel Chijioke Uwakwe[1,3], Ugochukwu Sunday Julius Ezenyirioha[2], Kelvin Emeka Ortuanya[4], Sunday Emmanuel Ucha[3], Grace Umahi[1], Elizabeth Besong[1], John Chukwuka Igwe[1]

[1]Department of Physiology, Ebonyi State University, Nigeria
[2]Julius Ezenyirioha Memorial Hospital and Maternity, Abakpa Nike, Enugu State, Nigeria
[3]Department of Obstetrics and Gynaecology, Alex Ekwueme Federal University Teaching Hospital Abakaliki, Ebonyi State, Nigeria
[4]Department of Obstetrics and Gynaecology, Enugu State University Teaching Hospital, Parklane, Enugu State, Nigeria

**Corresponding Author*

Received: 14 February 2023; Accepted: 04 March 2023; Published: 05 April 2023

Abstract: - Soya bean is a widely used plant seed that possess phytosteriods components like isoflavones, coumestans and ligans, though rich in isoflavones. This has been shown to act on the steroid receptors thereby either exiting or inhibiting the receptor site and subsequently the function of the receptor. The aim of this study was to determine the effect of soya bean aqueous extract on the testis and male reproductive hormones using adult male albino Wistar rats. Fifteen adult male rats were assigned into three groups of 5 animals each. Group 1 was negative control. Both groups 2 and 3 were treated with 100mg/kg and 200mg/kg of soya bean aqueous extract respectively. Treatment of the animals lasted for 4weeks and was done orally. Blood sample was collected and assayed for FSH, LH and testosterone hormones. The testes were also processed for histological studies. The result showed general increases in hormonal levels. However, FSH was marginally significant only in group 3 when compared to group 1. Histological examination of the testis showed more testicular tubular development in groups 3 and 2 when compared to group 1. We concluded that soya beans have positive effect on the testes and male reproductive hormones. However, a further study in human is recommended especially in the aspect of management of male infertility.

Keywords: soya bean, glycine max, reproductive hormones, FSH, LH, testosterone, testis.

I. Introduction

Soya beans is a plant seed and biological named as *Glycinemax* (Singh, 2014). It has health benefits (Sugano, 2006). It is commonly used as food additive because it is cheap, cholesterol-free, vegetable protein rich in complex carbohydrates and unsaturated fats, high in fiber, and free of lactose (Patisaul and Jefferson, 2010). It is considered to be the richest source of phytosteriods which can metabolize to testosterone (Dadon and Reifen, 2010). Testosterone is the major male hormone that induces spermatogenesis under the influence of FSH and LH (Zhao, 2011). Once the functionality of the hypothalamic-pituitary-testicular pathway is good, fertility is preserved (Zhao, 2011).

Phytosteriods are compound derived from plants which are presents in foods like leafy greens, soy, whole grains, garlic and beans (Wong, 2017). It is defined to be plant-derived xenosteriods that are produced within the endocrine system rather gotten by consumption of phytosteriods plants (Yildiz, 2005). They are also called "dietary steroids". They are natural Nonsteroidal plant compound of diverse origin but due to their similarity in structure with estradiol (17-β-estradiol), they have the strength of excitation or inhibiting the testosterone receptors (Yildiz, 2005). This is achieved due to its ability to sit in and block receptor sites (Crenshaw and Goldberg, 1996). Isoflavones is the commonest compound of phytosteriods found in soya bean and exerts its hormone-like activities as they contain a diphenolic ring that allows them to bind to the testosterone receptor exerting weak activity (Dinsdale and Ward, 2010; Patisaul and Jefferson, 2010).

Controversially, some studies showed harmful effect of soya phytoestrogens - Isoflavones. Overall, there are some indications that phytoestrogens alone or in combination with other endocrine disruptors, may alter reproductive hormones, spermatogenesis, sperm capacitation and fertility (Chavarro *et al.*, 2008; Cederroth*et al.*, 2010). However, review of available research shows that the biological effects of soy Isoflavones exposure are controversial and inconclusive (Dinsdale and Jefferson, 2010). This necessitated the important of this research as soya bean products are widely used in Nigeria and other African Countries. To this we aimed to

ISSN No. 231-2705 | DOI: 10.51244/IJRSI | Volume X Issue III March 2023

study the effect of aqueous extract of soya bean on the reproductive hormones (testosterone, FSH and LH) and the gonads (histological features) of male albino wistar rats.

II. Methodology

Healthy 15 adult Male Albino Wister rats weighing 140-180g were purchased from animal house, Department of Anatomy, Faculty of Medicine, Ebonyi State University, Abakaliki. The rats were housed in a well-ventilated cage (aluminum cage) and allowed to acclimatize for one week under regulated environmental conditions of temperature (25± 5ºC), relative humidity (50± 5%) and 12 hours light /dark cycle. The animals were fed on a standard commercial rat feed twice daily with water at all time. All animals were housed in accordance with international acceptable guideline for laboratory animal use and care as found in European Community guidelines (ECC, 1986).

The fresh seeds of Soya bean sufficient for the study were obtained from the Akpakpa market in Abakaliki, Ebonyi State, Nigeria. The seed of the plant was identified and authenticated with the numbers, *Glycine max (leguminaceae)* (soya bean seed) (UNH No 902) by a Taxonomist in the department of Plant Science and Biotechnology Department (Botany), University of Nigeria Nsukka, Enugu State where a herbarium specimen exist.

Aqueous Extraction of Soya Bean was by the method described by Tazieb*ou et al.,* (2007). Fresh seeds of soya beans was cleaned, washed and boiled for over 30minutes using an ovum at 60^{oC} to cause detoxification of some poisonous constituents and aid removal of the hustle. It was air dried and grinded with a blender. This soya bean powder was mixed in a proportion of 100g to 500ml of distilled water. The mixture was allowed to stand for 72 hours with 12hrs interval shaking of the mixture so that the Isoflavones will settle. It was filtered with NO 1 Whatman filter paper. The filtrate was placed in a rotatory evaporator at a temperature of 40^{0}C until it forms a semisolid. This was stored in a refrigerator. The extract was collected from the refrigerator, weighed and reconstituted at the concentration of 1g/10ml of distilled water and administered to the rat according to their respective group dosage.

Weight matched rats were randomly divided into 3 groups consisting of 5rats per group

Group 1: Served as control group and was given rat feed and distilled water

Group 2: Received100mg/kg daily of the soya bean extract, rat feed and distilled water

Group 3: Received200mg/kg daily of the soya bean extract, rat feed and distilled water.

The treatment of the animals lasted for 28 days. The extract was administered orally to the animals. After the 28^{th} day of the experiment, the animals were sacrificed following the standard procedures for handling experimental animals. Before the sacrificed, the animal were anasthesised with ketamine, blood was collected through the orbital sinus using glass capillary tube into a plain tube free of anticoagulants for hormonal assay test using automated method. Their gonads were harvested, weighed and fixed in 10% formalin solution and processed for histology.

Data obtained from this study was analysed using SPSS Ver. 20.0 statistical package and values were expressed as mean ± SEM. Groups were compared using one-way analysis of variance (ANOVA) followed by a post hoc Newmann keul`s multiple comparison tests. Statistical significance was considered at $P<0.05$.

III. Results

TABLE I: Comparism of the Effect of Aqueous Extract of Soya Bean on Sex Hormones of Male Albino Wister Rats

Groups	FSH(miμ/ml)	LH(miμ/ml)	TES(ng/ml)
1 (Control)	$2.50\pm0.28^{da,db}$	$2.30\pm0.42^{da,db}$	$1.95\pm0.07^{da,db}$
2 (100mg/kg)	$3.65\pm0.92^{da,dc}$	$2.55\pm0.64^{da,dc}$	$4.20\pm0.99^{da,dc}$
3 (200mg/kg)	$5.35\pm0.64^{db,dc}$	$5.25\pm1.20^{db,dc}$	$6.200\pm1.56^{db,dc}$

Source: Field Work, 2018

ISSN No. 231-2705 | DOI: 10.51244/IJRSI | Volume X Issue III March 2023

a - significant difference between group 1 & 2, b - significant difference between group 1 & 3, c - significant difference between group 2 & 3, da - no significant difference between group 1 & 2, db - no significant difference between group 1 & 3, dc - no significant difference between group 2 & 3.

The result showed that the male Albino Wister rat had increases in the concentration of all the assayed hormones with increase in the quantity of the aqueous extract of soya bean administered: group 1 (FSH 2.50±0.28), (LH 2.30±0.42) and (Testosterone 1.95±0.07); group 2 (FSH 3.65±0.92), (LH 2.55±0.64) and (Testosterone 4.20±0.99); group 3 (FSH 5.35±0.64), (LH 5.25±1.20) and (Testosterone 6.200±1.56) but none was statistically significant ($P<0.05$) when groups were compared. However comparism of group 1 and group 3 were marginally significant: FSH ($P = 0.05$), LH ($P = 0.07$) and Testosterone ($P = 0.06$)

PLATES: Histological Effect of Aqueous Extract of Soya Bean on the testes

Group 1 (Plate 1) that was not treated with soya bean extract showed healthy testicular tubules with spermatozoa in comparism to group 2 (Plate 2) that was treated with 100 mg/kg of soya beans extracts that showed enhanced testicular tubular proliferation and increased perfusion and group 3 (Plate 3) which showed better enhancement of testicular tubular cells proliferation resulting in a more number of testicular tubular cells closer to each other.

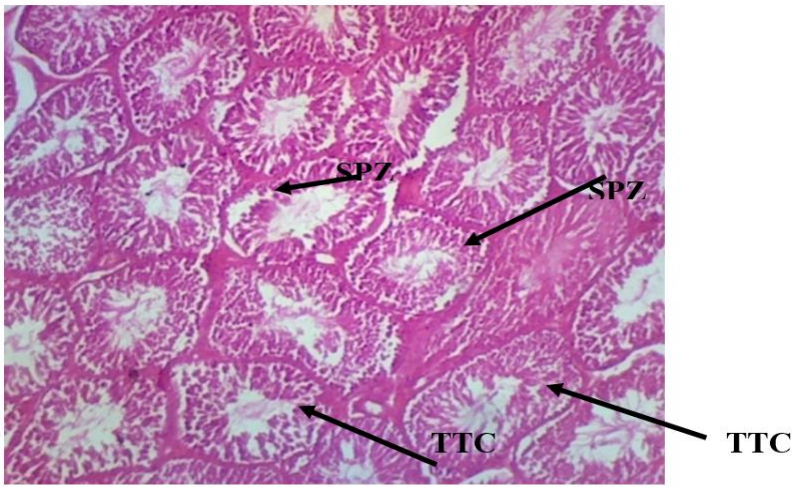

Plate 1: Testicular section of rat not treated with soya bean extract (negative control) showing normal healthy testicular tubular cells (TTC) with spermatozoa maturation (SPZ). H & E; X60.

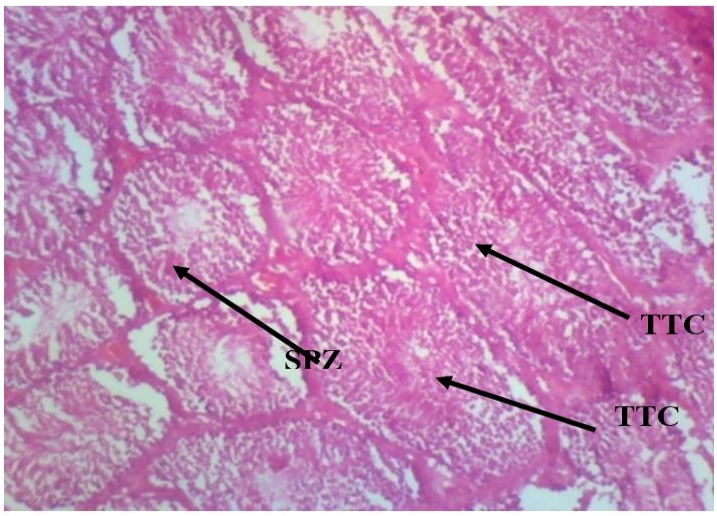

Plate 2: Testicular section of rat treated with soya bean extract (100mg/kg) showing proliferation of testicular tubular cells (TTC) with spermatozoa maturation (SPZ). H & E; X60.

ISSN No. 231-2705 | DOI: 10.51244/IJRSI | Volume X Issue III March 2023

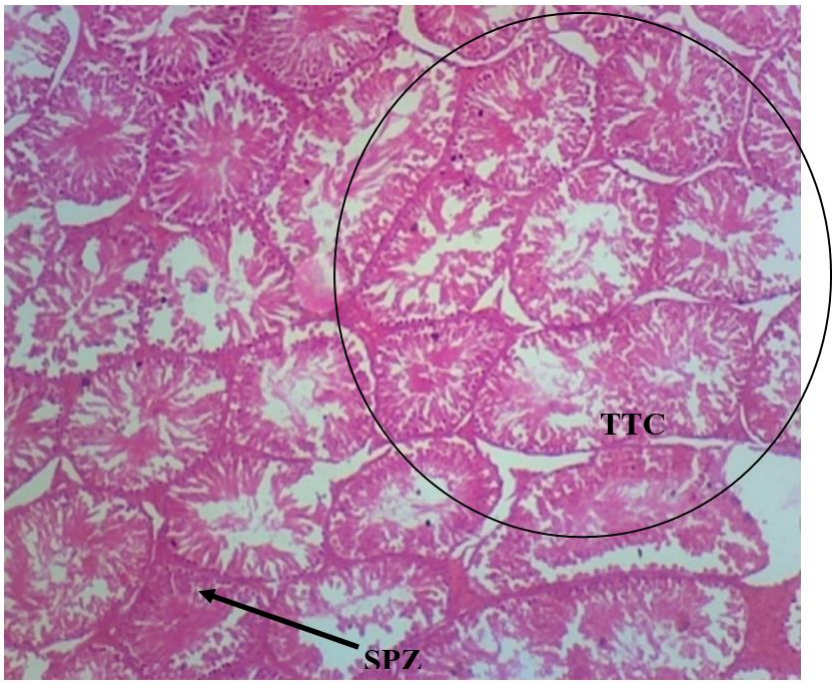

Plate 3: Testicular section of rat treated with soya bean extract (200mg/kg) showing well perfuse testicular tubular cells (TTC) proliferation with spermatozoa maturation (SPZ). Testicular tubular cells lie in a very close contact. H & E; X60.

IV. Discussion

The hypothalamo-pituitary-testicular axis is the principal endocrine system pathway that regulates male reproduction (Zhao, 2011). The Gonaldotropin (FSH and LH) are released into the general circulation to stimulate sex steroid hormones production and to control spermatogenesis in males (Zhao, 2011). Our research brought out the relationship between the soya bean extract and this hypothalamo-pituitary axis through its suspected developmental effect on the hypothalamus. This resulted to testicular function enhancement. Berrino et al. in 2001 reported that phytosteriods lead favourable changes on reproductive hormones. This was noted in this study as most of the assayed hormones were elevated compared to control, though not all were statistically significant. This resulted to increase in amount of LH and FSH that were released leading to sperm maturation and increase in level of testosterone released.

There were increases in the concentration of all the assayed hormones with increase in the concentration of the aqueous extract of soya bean administered but they were not statistically significant when groups were compared. This is in keeping with some available studies. Hamilton-Reeves (2010) noted that soy foods supplements did not alter bioavailable testosterone concentrations in men, also Allen et al, (2001) showed that soya intake was not associated with changes in serum concentrations of testosterone, free testosterone or luteinizing hormone. More so, Mitchell et al (2001) reported that daily supplementation with a soy extract did not alter sex hormone and gonadotrophin levels. A randomized dietary intervention study by Nagata et al, (2001b) documented that there were no statistical differences in testosterone or SHBG levels between the treatment and control groups after administration of soya food.

Comparism of group 1 and group 3 were marginally significant for all the assayed hormones. This is in line with Van Putte et al., (2016) and Ezilo, (2009) that noted elevation of LH level causes the interstitial cells to secrete large amount of testosterone. Also, Van Putte et al., (2016) that reported increase in FSH and LH after the administration of the soya bean extract as a result of developmental changes that occurred at the hypothalamus while the administration was on. However, this study contradicts studies done by Svechnikov et al., (2010) and Weber et al., (2001) that noted that long term dietary administration of genistein in rats, suppresses the steroidogenic response of Leydig cells thereby reducing serum levels of testosterone and androstenedione. Also, Srilatha and Adaikan (2004) showed that long term administration of daidzein a component of soya bean to male rats causes a decrease in plasma levels of testosterone. These outcomes were because their study used only selected chemical compounds (genistein and daidzein) from soya bean that has been established in literatures as an endocrine disruptor for their studies.

ISSN No. 231-2705 | DOI: 10.51244/IJRSI | Volume X Issue III March 2023

Histology of the testes showed enhanced testicular tubular proliferation and increased perfusion but with a higher concentration results in a more number of testicular tubular cells closer to each other. This is in line with study by Van Putte et al., (2016) that reported increase in testicular cell proliferation in the testes after the administration of the soya bean extract as a result of developmental changes that occurred at the hypothalamus while the administration was on but against the study by Dabrowski (2004) and Mitchell et al., (2001) that noted that Soya isoflavones supplementation has no effect on sperm concentration, count or motility, and it leads to no observable changes in testicular or ejaculate volume. This however may be that their study was on the isoflavones component not soya bean as a whole.

V. Conclusion

Soya bean is widely used as food addictive and this study assessed the effect of phytosteriods in it on the male wistar rat at different concentrations. The male reproductive hormones were assayed and the histology of the testis studied. The conclusion of the study is as follows:

- That soya bean increased testosterone hormone, follicle stimulating hormones (FSH) and luteinizing hormones (LH).
- The histological outcome of the testes showed enhancement of spermatogenesis
- These effects were dose dependent.

We therefore recommend more research on its benefit in the management of male infertility.

Ethical Approval

Ethical approval for animal studies was obtained from the Faculty of Medicine Animal Ethical Committee, Ebonyi State University, Abakaliki.

References

1. Allen, N.E., Appleby, P.N., Davey, G.K. and Key, T.J. (2001). Soy Milk Intake in Relation to Serum Sex Hormone Levels in British Men. *Nutritionand Cancer*, **41**:41-46.
2. Axelson, M., Sjovall, J. and Gustafsson, B.E. (1984). Soya – A Dietary Source of the Nonsteroidal Estrogen Equol in Man and Animals. *Journal of Endrinology*,**102**:49-56.
3. Berrino, F., Bellati, C., Secreto, G., Camerini, E., Pala, V., Panico, S., Allegro, G. and Kaaks R. (2001). Reducing Bioavailable Sex Hormones Through a Comprehensive Change in Diet: The Diet and Androgens (DIANA) Randomized Trial. *Cancer Epidemiology, Biomarkers and Prevention*,**10**(1): 25-33.
4. Cederroth, C.R., Auger, J., Zimmermann, C., Eustache, F. and Nef, S. (2010). Soy, Phyto-oestrogens and Male Reproductive Function: A Review. *International journal of Andrology*,**33**: 304–316
5. Chavarro, J.E., Toth, T.L., Sadio, S.M. and Hauser, R. (2008). Soy Food and Isoflavone Intake in Relation to Semen Quality Parameters Among Men from an Infertility Clinic.*Human Reproduction*,**23**(11): 2584–2590.
6. Chen, A. and Rogan, W.J. (2004). Isoflavones in Soy Infant Formula: A Review of Evidence for Endocrine and Other Activity in Infants. *Annual Revision of Nutrition*,**24**:33–54.
7. Crenshaw, T.L and Goldberg, J.P. (1994). Sexual Pharmacology: Drugs that Affect Sexual Function. Norton, W.W. and Company, Inc. **pp**. 420-451.
8. Dabrowski, W.M. (2004). Toxins in Food. *CRC Pres*, **376**: 50.
9. Dadon, S.B. and Reifen, R. (2010). Soy as an Endocrine Disruptor: Cause for Caution? *Journal of Pediatrics Endocrinology and Metabolism*,**23**(9): 855-861.
10. Dinsdale, E.C. and Ward, W.E. (2010). Early Exposure to Soy Isoflavones and Effects on Reproductive Health: A Review of Human and Animal Studies. *Nutrition*,**2**: 1156-1187.
11. Duncan, A.M., Merz, B.E., Xu, X., Nagel, T.C., Phipps, W.R, and Kurzer, M.S. (1999). Soy Isoflavones Exert Modest Hormonal Effects in Premenopausal Women. *JournalofClinicalEndocrinolMetabolism*,**84**:192-197.
12. Ezeilo, G.C. (2009). Textbook of Physiology: Reproduction.4th Edition. Oxford University Press, New Delhi. **Pp** 351-380.
13. Hamilton-Reeves, J.M., Vazquez, G., Duval, S.J., Phipps, W.R., Kurzer, M.S. and Messina, M.J. (2010). Clinical Studies show no Effects of Soy Protein or Isoflavones on Reproductive Hormones in Men: Results of a Meta-Analysis. *Fertility and Sterility;* **94** (3): 997–1007.
14. Jefferson, W.N., Newbold, R., Padilla-Banks, E. and Pepling, M. (2006). Neonatal Genistein Treatment Alters Ovarian Differentiation in the Mouse: Inhibition of Oocyte Nest Breakdown and Increased Oocyte Survival. *BiologyofReproduction*,**74**:161–168.
15. Kelly, G.E., Joannou, G.E., Reader, A.Y., Nelson, X.C. and Waring, M.A. (1995). The Variable Metabolic Response to Dietary Isoflavones in Humans. *Proceedings of the Society for Experimental Biology and Medicine*,**208**(1):40-43.

ISSN No. 231-2705 | DOI: 10.51244/IJRSI | Volume X Issue III March 2023

16. Martini, M.C., Dancisak, B.B., Haggans, C.J., Thomas, W. and Slavin, J.L. (1999). Effects of Soy Intake on Sex Hormone Metabolism in Premenopausal Women. *Journal of Nutrition and Cancer*,**34**:133-139.
17. Mitchell, J.H., Cawood, E., Kinniburgh, D., Provan, A., Collins, A.R. and Irvine, D.S. (2001). Effect of a Phytoestrogen Food Supplement on Reproductive Health in Normal Males. *ClinicalScience*; **100**(6): 613-618.
18. Murkies, A.L., Wilcox, G and Davis, S.R. (1998). Clinical Review 92: Phytoestrogens. *Journal of Clinical Endocrinology and metabolism*,**8**(2): 297-303.
19. Nagata, C., Shimizu, H., Takami, R., Hayashi, M., Takeda, N. and Yasuda, K. (2000b). Serum Concentrations of Estradiol and Dehydroepiandrosterone Sulfate and Soy Product Intake in Relation to Psychologic Well-Being in Peri- and Postmenopausal Japanese Women. *Metabolism*,**49**:1561-1564.
20. Nicholls, J., Lasley, B.L., Nakajima, S.T., Setchell, K.D. and Schneeman, B.O. (2002). Effects of Soy Consumption on Gonadotropin Secretion and Acute Pituitary Responses to Gonadotropin-Releasing Hormone in Women. *JournalofNutrition*,**132**:708-714.
21. Patisaul, H.B. and Jefferson, W. (2010).The Pros and Cons of Phytoestrogens.. *Front Neuroendocrinology*,**31**(4):400–419.
22. Price, K.R. and Fenwick, G.R. (1985). Naturally Occurring Estrogens in Foods: A Review. *FoodAdditiveContamination*, **2**:73-106.
23. Setchell, K.D.R. and Adlercreutz, H. (1988). Mammalian Lignans and Phytoestrogens. Rescew Studies on their Formation, Metabolism, and Biological Role in Health and Disease. In:Rowland L., edition. Role of the gut flora in toxicity and cancer. London: *AcademicPress*; 315-345.
24. Singh, J. (2014). Benefits and Side Effects of Soybeans. Ayurvedic Consultations. AYUR TIMES.
25. Srilatha, B. and Adaikan, P.G. (2004). Estrogen and Phytoestrogen Predispose to Erectile Dysfunction: Do Era and ERß in the Cavernosum Play a Role? *Urology*,**63**:382-286.
26. Svechnikov, K., Izzo, G., Landreh, L., Weisser, J. and Söder, O. (2010). Endocrine Disruptors and Leydig Cell Function. *Journal of Biomedicine and Biotechnology*,**2010**:1-10.
27. Van Putte, C.L., Regan, J.L. and Russo, A.F. (2016). Seeley's Essentials of Anatomy and Physiology; Reproductive System. 9th edition. Mc Graw Hill Education, 2 Pann Plaza New Yolk. **Pp** 529-559.
28. Watanabe, S., Terashima, K., Sato, Y., Arai, S. and Eboshida, A. (2000). Effects of Isoflavone Supplement on Healthy Women. *BioFactors*,**12**:233-241.
29. Weber, K., Setchell, K., Stocco, D. and Lephart, E. (2001)**.** Dietary Soy Phytoestrogens Decrease Testosterone Levels and Prostate Weight without Altering LH, Prostate 5 Alpha Reductase or Testicular Steroidogenic Acute Regulatory Peptide Levels in Adult Male Sprague Dawley Rats.*Journal of Endocrinology*, **170***:*591- 599.
30. Wendy, N., Padilla-Banks, J.E. and Newbold, R.R. (2007). Disruption of the Developing Female Reproductive System by Phytoestrogens: Genistein as an Example. *Molecular Nutrition of food Research*,**51**(7):832-844.
31. Wong, C. (2017). Are Phytoestrogens Good for Your Health? *TaiwanHealthCareExposure*,
32. Wu, A.H., Stanczyk, F.Z., Hendrich, S., Murphy, P.A., Zhang, C., Wan, P. and Pike, M.C. (2000). Effects of Soy Foods on Ovarian Function in Premenopausal Women. *BritishJournalofCancer*; **82**:1879-1886.
33. Yildiz, F. (2005). Phytoestrogens in Functional Foods. *TaylorandFrancis* Ltd. pp3-5, 210-211.
34. Zhao, E. and Mu, Q. (2011). Phytoestrogen Biological Actions on Mammalian Reproductive System and Cancer Growth. *ScientiaPharmaCentica*,**79**(1): 1-20