Effect of soybean on fertility of male and female albino rats

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Abstract: This study aimed to investigate whether consumption of soybean is useful or harmful on reproductive hormones; ovary; uterus; mammary gland; testis and subsequent fertility. In the former experiment, male and female Wister albino rats were used in the present study. Each sex was randomly divided into 4 groups, control group fed on the basal diet (AIN93 G), three treated groups given 30, 60 and 90 gm cooked soybeans/70 kg human body weight (b.w.) for three months. Female rats showed that soybean significantly decreased free estradiol hormone (E2); progesterone hormone; follicle stimulating hormone (FSH); luteinizing hormone (LH); ovary weight and number of ovarian follicles. On the other hand soybean significantly increased total E2; sex hormone binding proteins (SHBP); uterus weight and caused uterus proliferation and cystic hyperplasia. The mammary gland showed gradual hyperplasia and mammary ducts showed proliferation. In male rats soybean significantly decreased free testosterone hormone; LH and FSH, meanwhile total testosterone hormone, SHBP, testes weight and testes diameter were significantly increased accompanied with spermatogenesis arrest.
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1. Introduction:

Soybean (Glycine max) is considered a valuable crop among legumes as it's the world's largest oilseed and protein and is the primary source of protein for livestock (Shoemaker *et al.,* 2006 and Shao *et al.,* 2007). It's also considered the most important economic oil and cash crops in Egypt (El-Sherif and Ismail, 2009).

In addition soy is the richest dietary source of bioactive phytoestrogens (plant estrogens) called isoflavones (Setchell, 2001 and Caldwell *et al.,* 2005).Many studies have focused on the role of soy isoflavones on fertility but results remain controversial, the question of whether phytoestrogens are beneficial or harmful to fertility remains unresolved. Indeed considerable interest has been focused on the role of soy in females fertility this interest has stemmed from numerous studies which showed that consumption of soy components significantly increased menstrual cycle length; Duncan *et al.,* 1999 and Messina *et al.,* 2006); decreased sex hormone binding globulin (SHBG) level (Kumar *et al.,* 2002); lowered gonadotropin levels (Duncan *et al.,* 1999 and Hooper *et al.,* 2009); decreased progesterone hormone (Lu *et al.,* 2000) and decreased estrogen level (Watanabe *et al.,* 2001; Kumar *et al.,* 2002 and Tamaya, 2005). In contrast consumption of soy increased the level of E2 increased SHBG and progesterone hormone (Duncan *et al.,* 2000). However other studies found no difference in menstrual cycle length or hormone levels with soy supplementation (Wu *et al.,* 2000; Maskarinec *et al.,* 2002 and Nicholls *et al.,* 2002).

Studies focused on male fertility showed that soy lowered testosterone level (Lund *et al.,* 2004; Goodin *et al.,* 2004; Dillingham *et al.,* 2005; and Goodin *et al.,* 2007); lowered sperm concentration (West *et al.,* 2005 and Chavarro *et al.,* 2008) and serum E2 .

However others found that soy increased serum testosterone (Dalu *et al.,* 2002), increased LH level in male rats (Ohno *et al.,* 2003); increased serum E2 and estrone (E1) in men (Dillingham *et al.,* 2005) and influence spermatogenesis (Song *et al.,* 2006).

In contrast, others found that soy protein or isoflavones intake had no effects on serum total and free testosterone and SHBG in men (Teede *et al.,* 2001 and Hamilton-Reeves *et al.,* 2009) and in rats (Fritz *et al.,* 2002 and Assinder *et al.,* 2007); LH or FSH in men (Teede *et al.,* 2001 and Dillingham *et al.,* 2005) and E2 or E1 (Gardner-Thorp *et al.,* 2003).

2. Materials and Methods:

2. 1. Soybean Diet:

Commercial soybean seeds sample (Giza 22) obtained from the Agriculture Research Center; Giza, Egypt was used in the present study because it's the common soybean used in the manufacture of most soy foods present in local markets. Also it's used as a dietary source of proteins for poultry and livestock.

It contains 40 % protein, 20 % fat, 5 % ash and 35 % carbohydrates (soluble sugars and insoluble sugars) Food Technology Research Institute Agriculture Research Center, Giza, 2008.

Oligosaccharides are soluble sugars but are not broken down by the enzymes of the digestive tract and are fermented by the micro-organisms present in

the intestine, with the formation of the intestinal gas flatulence. That's why raw soybean was soaked for 12 hours at room temperature to get rid of these oligosaccharides. Also soybean was cooked at 120°C for 18 minutes in attempt to decrease the amount of the anti-nutrients present such as trypsin inhibitors, phytin, lectins, saponins, and hemaggluttinins (Sat and Keles, 2002)

2.2 .Experimental Animals:-

Male and female Wister albino rats with average body weight 120 gm obtained from the private market Abou-Rawash, Giza, Egypt, were used in the present study. They were kept on vegetables and water *ad libitum* for one week under condition with a 12h light/ dark cycle and temperature of 25-27 ^{0}C prior to the experiment to remove any traces of previous soybean. Following this brief adjustment period, each sex was divided into four groups (n = 12 per group).

Control group: rats were kept on the basal diet (AIN 93 G) according to (Reeves, 1997) and water *ad libitum* for three months.

Three treated groups: each rat was fed individually on 30, 60 & 90 gm cooked soybean /70 kg human b.w. daily for three months. Doses used in our study are according to Messina, 1999 and Chang *et al.*, 2008.

All three treated groups were then given the basal diet (AIN 93G) and water *ad libitum* throughout the experimental period

2.2.1 . Organs Weight Analyses:

Weight of some organs as ovary; uterus and testes of control and treated rats were recorded at the end of the experimental period.

2.2.2. Analysis of hormones:

Determination of Free E2, Total E2, Progesterone, Free Testosterone Hormone, Total Testosterone Hormone, FSH and LH in serum samples were measured by enzyme-linked immunosorbent assay (ELISA) according to the method of Tietz (1995a) for Free E2, Maxey *et al*.(1992) for Total E2, Tietz (1995b) for progesterone Hormone, Chen *et al.* (1991) for Free Testosterone Hormone ; Griffen and Wilson(1992) for Total Testosterone Hormone; Rose (1998) for FSH; Rebar *et al.* (1982) for LH.

SHBP calculated according to De Ronde *et al*. (2006) equation.

2.2.3. Histopathological Analysis:-

Uterus, ovary, testis and mammary gland were dissected and carefully cleaned from adhering tissues then immediately fixed in bouin`s solution for uterus, ovary and testis only while mammary gland was fixed in 10% neutral buffered formalin for 24 hours. Routine steps of dehydrating and embedding were applied, then transverse or longitudinal sections of 3-5µ were prepared then stained with haematoxylin and eosin (H&E).

2.3 Statistical Analysis:

Data were analyzed using the SPSS for windows (version 12.0). Analysis of variance (one-way ANOVA) was performed to test for any significant differences among groups and independent sample t-test was used to calculate statistical significant between the control group and each treated group. The level of significance was set as $P< 0.05$ for all statistical tests (Tello *et al.*, 2003)

3. Results:

3.1.Analysis Studies

Table I and Figs.(1-8) shows that serum level of free E2, LH, FSH in rats fed on 30mg soybean/70 kg human b.w. decreased significantly($p<0.05$ & $p<0.001$ for 60 & 90mg/kg b.w.,respectively) compared with control group. Likewise serum level of progesterone significantly decreased in the three treated groups with ($p<0.05$) However serum levels of total E2 and SHBP increased significantly in the three treated groups ($p<0.001$) compared with control . Left ovary weight significantly decreased ($p<0.001$) in all three treated groups . Meanwhile 60 and 90 gm soybean/ 70 kg human b.w. induced significant increase in uterus weight($p<0.01$ and $p<0.001$, respectively) compared to control.

Table II and Figs.(9-14) reveals that male serum level of free testosterone, LH and FSH in rats fed on the three doses significantly decreased for groups feed on 60, 90mg/70kgm($p<0.001$) and for 30mg/kg($p<0.05$) . Meanwhile serum levels significantly increased of total testosterone($p<0.05$) and SHBP($p<0.01$) in male rats fed only on 60 and 90 gm soybean/70 kg human b.w. In addition testis weight significantly increased ($p<0.01$)for 30mg/kg and for 60 ,90 mg/kg($p<0.001$) , respectively, compared with control groups .

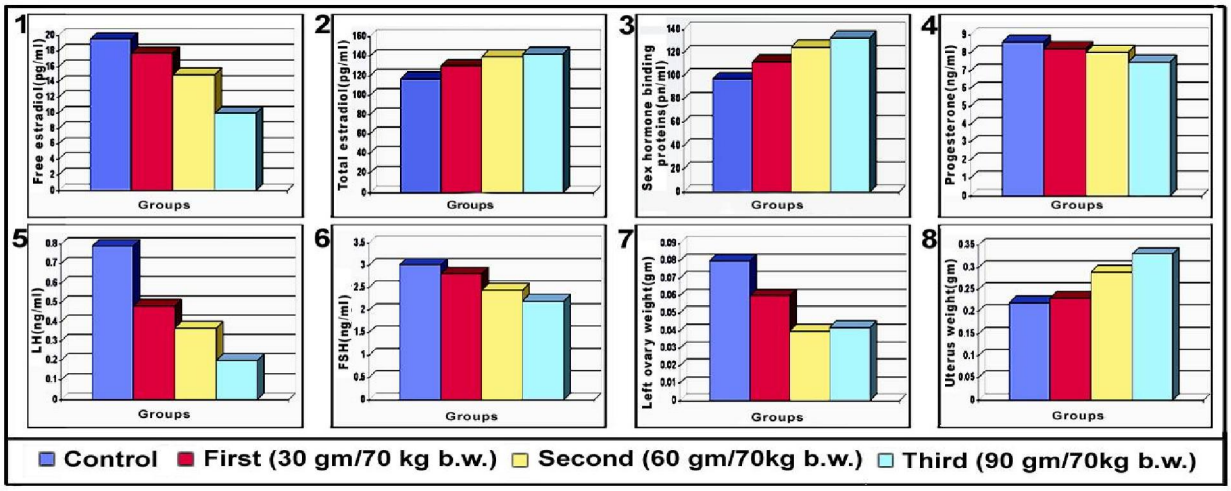
3.2. Histopathological Studies

The histopathological studies (Figs. 15,16)showed that soybean decreased the number of ovarian follicles and caused endometrial proliferation (uterus proliferation) and cystic hyperplasia . Soybean also caused mammary gland gradual hyperplasia in a dose dependent manner as well as mammary ducts proliferation. Soybean increased testes diameter and caused spermatogenesis arrest at spermatid stage. Also Leydig cells showed decreased in number.

Table (I): Effect of soybean on the levels of serum reproductive hormones, ovary and uterus weight of female rats treated for three months with three different doses

Groups \ Parameters		Free estradiol (pg/ml)	Total estradiol (pg/ml)	Sex hormone binding proteins (pg/ml)	Progesterone (ng/ml)	LH (ng/ml)	FSH (ng/ml)	Left ovary weight (gm)	Uterus weight (gm)
Control	Range	17.20 — 21.80	110.12 — 119.78	90.58 — 102.58	7.20 — 9.80	0.40 — 0.95	2.44 — 3.68	0.063 — 0.088	0.15 — 0.30
	Mean ± S.E	19.54±0.49	116.95 ± 1.09	97.41 ± 1.20	8.61 ± 0.23	0.79 ± 0.05	3.01 ± 0.11	0.08 ± 0.002	0.22 ± 0.01
First group (30 g/ 70 kg b.w.)	Range	15.70-21.60	123.00 — 136.25	103.00 — 117.75	7.40 — 9.70	0.40 — 0.57	2.22 — 3.15	0.050 — 0.080	0.18 — 0.30
	Mean ± S.E	17.74±0.58	130.04 ± 1.22	112.30 ± 1.33	8.24 ± 0.17	0.48 ± 0.02	2.81 ± 0.08	0.06 ± 0.002	0.23 ± 0.01
	% of change	-9.21	11.19	15.29	-4.30	-39.24	-6.64	-25.00	4.55
	P value	P < 0.05	P < 0.001	P < 0.001	N.S.	P < 0.001	N.S.	P < 0.001	N.S.
	Range	14.20–15.90	130.25 — 143.18	115.95 — 127.90	7.36 — 8.36	0.27— 0.50	2.10 — 3.00	0.030 — 0.060	0.18 — 0.38
	Mean ± S.E	14.95±0.14	139.81 ± 0.97	124.86 ± 0.94	8.06 ± 0.08	0.37 ± 0.02	2.45 ± 0.07	0.04 ± 0.003	0.29 ± 0.02
Second group (60 g/ 70 kg b.w.)	% of change	-23.49	19.55	28.18	-6.39	-53.16	-18.60	-50.00	31.82
	P value	P < 0.001	P < 0.001	P < 0.001	P < 0.05	P < 0.001	P < 0.001	P < 0.001	P < 0.01
	Range	8.90-12.30	132.25 — 150.36	122.25 — 140.93	6.39 — 9.00	0.10 — 0.27	1.98 — 3.07	0.020 — 0.060	0.29 — 0.38
	Mean ± S.E	9.93±0.32	142.51 ± 1.83	132.58 ± 1.83	7.48 ± 0.26	0.20 ± 0.02	2.20 ± 0.10	0.042 ± 0.004	0.33 ± 0.01
	% of change	-49.18	21.86	36.11	-13.12	-74.68	-49.18	-47.50	50.00
Third group (90 g/ 70 kg b.w.)	P value	P < 0.001	P < 0.001	P < 0.001	P < 0.01	P < 0.001	P < 0.001	P < 0.001	P < 0.001
	ANOVA	F = 90.26 P < 0.001	F= 82.67 P < 0.001	F= 33.16 P < 0.001	F= 5.50 P < 0.05	F = 69.19 P < 0.001	F = 14.74 P < 0.001	F = 43.55 P < 0.001	F = 14.78 P < 0.001

P= probability N.S. = non significant S.E= standard erro



Figs.(1-8) (1) **:** Effect of 30, 60 and 90 g soybean/70 kg b.w. on female free E_2 level.**(2)** Effect of 30, 60 and 90 g soybean/70 kg b.w. on female total E2 level.**(3)** Effect of 30, 60 and 90 gm soybean/70 kg b.w. on female SHBP level..**(4)**)Effect of 30, 60 and 90 gm soybean/70 kg b.w. on female progesterone hormone level.**(5)** Effect of 30, 60 and 90 gm soybean/70 kg b.w. on female LH level.**(6)**Effect of 30, 60 and 90 gm soybean/70 kg b.w. on female FSH level.**(7)** Effect of 30, 60 and 90 gm soybean/70 kg b.w. on female left ovary weight.**(8)** Effect of 30, 60 and 90 gm soybean/70 kg b.w. on female uterus weight.

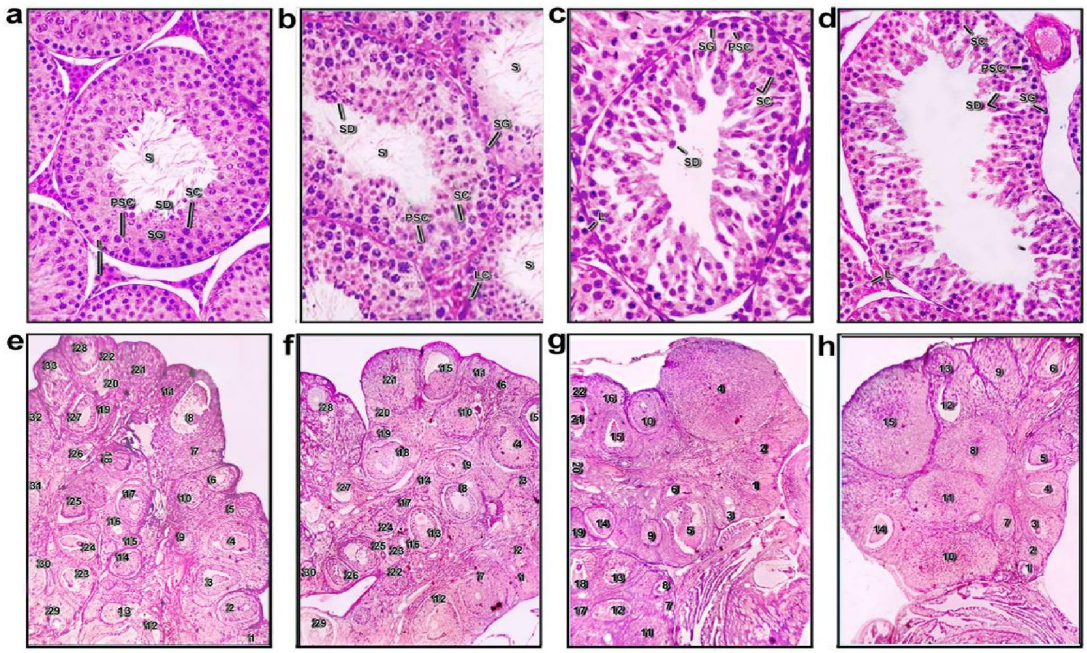


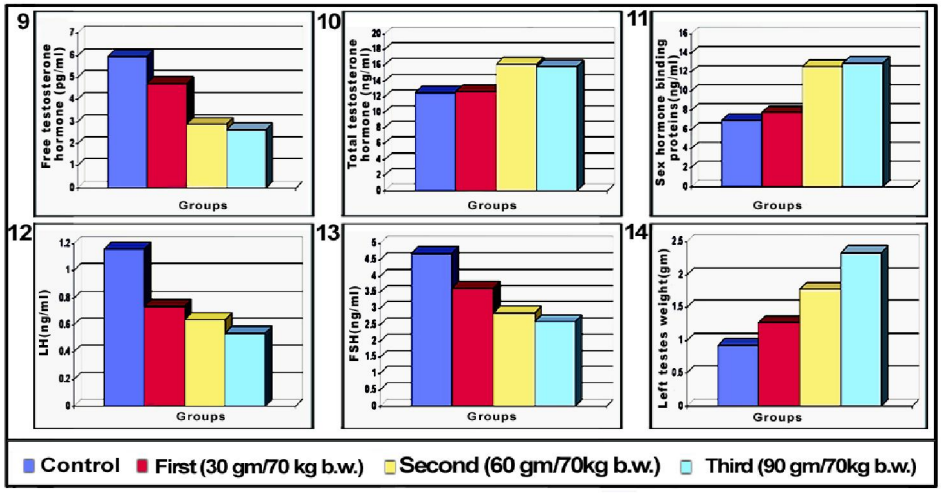
Fig. 15 (a-d) . Effect of soy on testis of treated rats compared with control groups**:(a)** T.S. in testis of control rat showing the normal spermatogenesis and histological structure of the seminiferous tubules,SG.(Spermatogonia), PSC. (Primaryspermatocyte), SC. (Spermatocyte), SD(Spermatid),S.(spermatozoa) and LC.(Leydig cell) (H&E 400x) (b) T.S. in testis of a treated rat with 30gm soy bean/70 kg human b.w. showing normal spermatogenesis while seminiferous tubules diameter slightly increased and leydig cells slightly decreased. (H&E 400x) **(c)** T.S. in testes of a treated rat with 60 gm soy bean/70 kg human b.w. showing spermatogenesis arrest at spermatid stage, increased in seminiferous tubules diameter and decreased in leydig cells (H&E 400x) (d)T.S. in testes of a treated rat with 90 gm soy bean/70 kg human b.w. showing spermatogenesis arrest at spermatid stage, increased in seminiferous tubules diameter and marked decreased in leydig cells (H&E 400x) Effect of soy on ovary of treated rats compared with control groups:

Fig .15(e-h) T.S in ovary of control rat showing the normal appearance and normal number of follicles. Approximately there are (31) follicles. (H&E 100x) (f) T.S in ovary of a treated rat 30 gm soy bean/70 kg human b.w. showing the normal appearance and normal number of follicles. Approximately there are (29) follicles. (H&E 100x) **(g)** T.S in ovary of a treated rat with 60 gm soy bean/70 kg human b.w. showing decrease in number of follicles. Approximately there are (22) follicles. (H&E 100x) **(h)** T.S in ovary of a treated rat with 90 gm soy bean/70 kg human b.w. 100x showing an obvious decrease in number of follicles. Approximately there are (15) follicles. (H&)

Table (II): Effect of soybean on serum reproductive hormones and testis weight of male rats treated for three months with three different doses.

Parameters / Groups		Testosterone hormone (ng/ml)	Total testosterone (ng/ml)	Sex hormone binding proteins (ng/ml)	LH (ng/ml)	FSH (ng/ml)	Left testes weight (gm)
Control	Range	4.90 — 7.00	12.05 — 13.00	6.54 — 7.5	0.76 — 1.60	3.36 — 5.76	0.85 — 1.07
	Mean ± S.E	5.96 ± 0.16	12.53 ± 0.27	6.94 ± 0.20	1.16 ± 0.08	4.69 ± 0.23	0.93 ± 0.02
First group (30 gm / 70 kg b.w.)	Range	3.54 -— 7.00	12.25 — 12.95	6.82 — 8.95	0.40 — 2.08	2.00 — 4.80	1.05 — 2.10
	Mean ± S.E	4.73 ± 0.32	12.61 ± 0.20	7.78 ± 0.62	0.74 ± 0.15	3.63 ± 0.23	1.27 ± 0.09
	% of change	-20.64	0.64	12.10	-36.21	-22.60	36.56 %
	P value	P < 0.01	N.S.	N.S.	P < 0.05	P < 0.01	P < 0.01
Second group (60 gm / 70 kg b.w.)	Range	2.00 — 3.54	13.90 — 18.47	10.36 — 14.47	0.40 — 0.92	2.08 — 4.00	1.22 — 2.30
	Mean ± S.E	2.89 ± 0.13	16.12 ± 1.32	12.53 ± 1.19	0.64 ± 0.05	2.86 ± 0.17	1.78 ± 0.10
	% of change	-51.51	28.65	80.55	-44.83	-42.86	91.40%
	P value	P < 0.001	P < 0.05	P < 0.01	P < 0.001	P < 0.001	P < 0.001
Third group (90 gm / 70 kg b.w.)	Range	2.27— 2.98	14.25 — 17.84	11.27 — 15.09	0.20 — 0.72	1.28 — 4.00	1.96 — 3.12
	Mean ± S.E	2.64 ± 0.07	15.78 ± 1.07	12.90 ± 1.13	0.54 ± 0.06	2.60 ± 0.38	2.34 ± 0.15
	% of change	-55.70	25.94	85.88	-53.45	-44.56	151.61%
	P value	P < 0.001	P < 0.05	P < 0.01	P < 0.001	P < 0.001	P < 0.001
	ANOVA	F = 61.02 P < 0.001	F = 5.10 P < 0.05	F = 12.52 P < 0.05	F = 8.29 P < 0.001	F = 15.31 P < 0.001	F = 39.79 P < 0.001

P= probability N.S. = non significant S.E= standard error



Figs.(9-14) (9) Effect of 30, 60 and 90 gm soybean/70 kg b.w. on free testosterone hormone level of male rats **(10)** Effect of 30, 60 and 90 gm soybean/70 kg b.w. on total testosterone hormone level of male rats **(11)** Effect of 30, 60 and 90 gm soybean/70 kg b.w. on sex hormone binding proteins level of male rats **(12)** Effect of 30, 60 and 90 gm soybean/70 kg b.w. on LH level of male rats **(13)** Effect of 30, 60 and 90 gm soybean/70 kg b.w. on FSH level of male rats **(14)**Effect of 30,60and 90gm soybean/70kgb.w. on left tests weights of male rats.

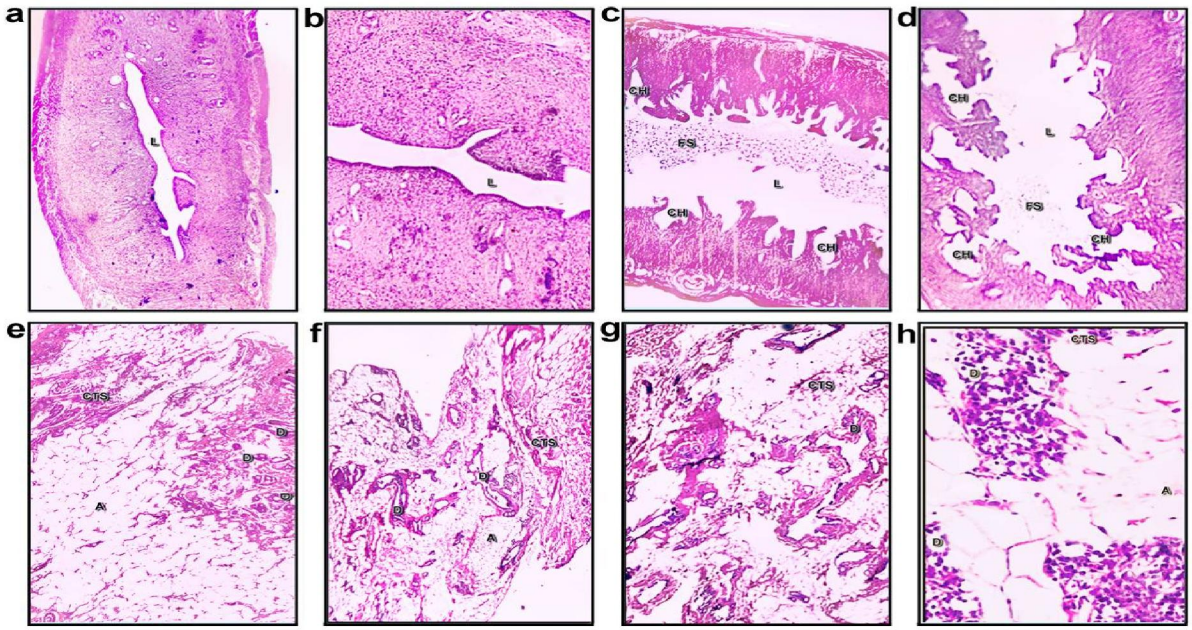


Fig.16 (a-d) Effect of soy on uterus of treated rats compared with control groupsFig.16 **(a)** L.S. in uterus of control rat showing normal luminal epithelia and normal storma. (H&E 200x) **(b)** L.S. in uterus of a treated rat with 30 gm soy bean/70 kg human b.w. showing normal luminal epithelia and normal storma.(H&E 200x) **(c)** L.S. in uterus of a treated rat with 60 gm soy bean/70 kg human b.w. showing obvious proliferative changes in the luminal epithelia and uterine cystic hyperplasia. (H&E 200x) **(d)** L.S. in uterus of rat a treated with 90 gm soy bean/70 kg human b.w. showing obvious proliferative changes in the luminal epithelia and cystic hyperplasia. (H&E 200x)

Fig.16 (e-h) Effect of soy on mammary gland of treated rats compared with control groups**:(e)**T.S. in mammary gland of control rat showing normal histological structure: adipose tissue; connective tissue septa running through the adipose tissue; ducts growing up through the connective tissue septa. (H&E 200x) **(f)** Testes T.S. in mammary gland of a treated rat with 30gm/70 kg human b.w. showing slight proliferation of ducts. (H&E 200x) **(g)** T.S. in mammary gland of a treated rat with 60gm/70 kg human b.w. showing proliferation of ducts and mild hyperplasia. (H&O 200x) (h) T.S. in mammary gland of a treated rat with 90gm/ 70 kg human b.w. showing proliferation of ducts and marked hyperplasia. (H&E 200x).

4. Discussion:

This study showed that serum levels of free E_2 and LH significantly decreased in female rats fed 30, 60 and 90 g soybean/70 kg b.w. Meanwhile serum levels of progesterone and FSH significantly decreased only in rats fed with 60 and 90 g/70kg human b.w.

These results correlate with previous studies showing that administration of isoflavones resulted in significant reduction of progesterone and free E_2 concentrations in rodents (Lamartiniere *et al.*, 2002). They also agree with studies on premenopausal women which showed that soy isoflavones significantly reduced serum free E_2 (Tamaya, 2005) and progesterone hormone concentration (Lu *et al.*, 2000 and 2001).

The possible explanation for the reduction of serum free E_2 by soy may be due to the weak estrogenic and / or anti-estrogenic action of isoflavones. Indeed, Ososki and Kennelly (2003) and Dixon (2004) cited that the biological effects of isoflavonoids vary according to the female biological phase. In premenopause, when the concentration of circulating hormones are high, the estrogen receptors are active and phytoestrogen exerts anti-estrogenic effect which competes with estrogen to bind with ER and displace it from its binding sites, but once bound, have a far weaker estrogenic potency than endogenous estrogens; the result is only due to a weak estrogenic action. Also Hwang *et al.* (2006) observed that isoflavones may exert their effects as estrogen antagonists in a high estrogen environment, or they may act as estrogen agonists in a low estrogen environment.

Previous studies by Tamaya (2005) interpreted that the weak estrogenic action of soy isoflavones may be due to their ability to stimulate or up regulate SHBG which will bind with free E_2, or down regulate enzymes involved in estrogen biosynthesis, such as aromatase (CYP 19), which converts androgens into estrogens (Rice *et al.*, 2006) or inhibit 17β-hydroxysteroid dehydrogenase type I (17β-HSD) that converts E_1 to the more potent E_2 (Lacey *et al.*, 2005 and Brooks and Thompson, 2005); isoflavones also may inhibit the reductive/ oxidative activity of 17β-HSD type 5 which inhibits the conversion of androsterone to testosterone and androstenediol to androstenedione. Testosterone and androstenedione are substrates for the action of aromatase which converts testosterone to E_2 and androstenedione to E_1, so isoflavones may inhibit the conversion of these androgenic precursors to E_2 or E_1 in the estrogen dependent tissues. Also our results agree with Tamaya (2005) who found that soy suppressed the gonadotropins (FSH and LH) which stimulates E_2 production.

In addition, the decrease in progesterone level may be due to the ability of isoflavones to decrease FSH which stimulates progesterone production in granulosa cells from preovulatory follicles in rats (Whitehead and Lacey, 2000 and Nejaty *et al.*, 2001), also Tiemann *et al.* (2007) found that the isoflavones inhibit 3β-HSD an enzyme involved in progesterone synthesis from granulosa cells.

Soybean also led to the increase of total E_2 in all three treated groups. This is because the total E_2 content is composed of endogenous estrogen plus the weak phytoestrogen from soybean. And due to the increase in level of total E_2, likewise SHBP will increase as shown in the current results in the three treated groups to bind excess total E_2 present.

Moreover Tamaya (2005) cited that isoflavones induce human HepG2 hepatoblastoma cells to increase SHBG synthesis and secretion.

Tollefsen *et al.* (2002) showed that isoflavones potentially disrupt the endocrine function of SHBP through interacting with it and inducing changes in its internal hydrophobic binding sites and decreasing the number of it's binding sites to endothelial glycocalyx and megalin cells and thus decreasing the dissociation rate of sex steroids from its binding proteins.

Hammes *et al.* (2005) explained the role of endothelial glycocalyx and megalin cells in the dissociation of SHBP from it's bound hormone. They clarified that the interaction of binding proteins with the endothelial glycocalyx leads to a structural modification of the hormonal binding site and thereby change it's affinity. As a result sex hormones are set free and diffuse freely into the target cells. Or sex steroids are bound together with SHBP to megalin cell which mediate endocytosis of proteins bound hormones.

A possible explanation for the reduction of FSH and LH may be due to the increase in the level of total E_2 which produces a negative feedback mechanism leading to unalleviated levels of gonadotrophic releasing hormone (GnRH) of the hypothalamus which intern will stop the secretion of FSH and LH.

The present study showed a significant decrease of ovarian weight in the three treated groups and a remarkable decrease in the number of ovarian follicles in the histological study (Fig. 3 a-p). This may be due to the decrease in serum levels of free E_2 and FSH by soybean which disturb follicles development (Dubey *et al.*, 2000 and Rosselli *et al.*, 2000).

Also the present study showed significant increase in uterine weight in rats fed only 60 and 90 g soybean/70 kg human b.w. , and histological results showed endometrial proliferation and cystic

hyperplasia of uterus after soybean, as seen in Figs (3a-3d) these results are supported by Kayisli *et al.* (2002) who found that soy isoflavones induced endometrial stromal-cell proliferation, yet the proliferative effect occurred at high concentrations of isoflavones and was 8–15% lower than that induced by E2 indicating that isoflavones are weak estrogens. Also Seidlova-Wuttke *et al.* (2003) and Archer (2004) cited that the increase in uterine mass may be caused by liquid imbibitions of the tissue, and subsequently through proliferation of endo- and myometrial cells.

Moreover Moller *et al.* (2010) cited that the possible explanation of increased water impaction in rats treated with soy isoflavones may be due to that isoflavones up regulate AQP3 which in turn increases the permeability of uterus to glycerin and/or urea and water, whereas isoflavones down regulate AQP1 & AQP5 which increase the permeability to water only. This would finally lead to elevated uterus luminal fluid volumes resulting in increased uterine weight. It is likely to note that during dissection huge amount of water was observed coming out of the uterus.

As far the effect of soybean on the histology of mammary gland, the present work showed gradual hyperplasia in a dose dependent manner as well as mammary duct proliferation (Figs. 4 a-d), this agrees with Thomsen *et al.* (2006) who explained the proliferation effect of soy on mammary gland due to the weak estrogenic effect of soy isoflavones.

As far male rats treated with 30, 60 and 90 g soybean/70 kg b.w., a significant decrease in serum testosterone level occurred. Likewise serum levels of FSH and LH significantly decreased compared with control group.

These results correlate with previous studies on rodents (Ohno *et al.,* 2003; Svechnikov *et al.,* 2005; Pan *et al.,* 2007; Akingbemi *et al.,* 2007; Jiang *et al.,* 2008; Zhang *et al.,* 2009 and Hancock *et al.,* 2009) and men (Kurzer, 2002 and Spentzos *et al.,* 2003) for testosterone. Also, Pan *et al.,* 2007 and Jiang *et al.,* 2008 for LH.

Decrease in serum testosterone level may be due to the ability of soy isoflavones to inhibit the enzymes involved in steroid hormones synthesis. In fact various types of cell culture studies have demonstrated the ability of isoflavones to inhibit 3β-HSD an enzyme involved in the conversions of pregnenolone to progesterone; DHEA to androstenedione; and 5-androstenediol to testosterone (Ohno *et al.,* 2002; Whitehead *et al.,* 2002 and Ohno *et al.,* 2004) and also inhibit the enzyme 17β- HSD involved in the conversion of DHEA to 5-androstenediol and androstenedione to testosterone (Krazeisen *et al.,* 2001).

Moreover, isoflavones interfere with the first and rate-limiting step in steroidogenic pathway and may be due to their ability to decrease the activity of the side-chain cleavage enzyme P450 which catalyses the conversion of cholesterol to pregnenolone (Svechnikov *et al.,* 2005).

Also Hancock *et al.* (2009) stated the reason of decreased testosterone by isoflavones which interfere with coupling of transmembrane LH receptors (LHR) with G proteins. Uncoupling of LHR from G proteins adversely affects adenylate cyclase function and impacts LH-dependent stimulation of Leydig cells these led to decreased testicular steroidogenesis.

We also found significant increase in serum levels of total testosterone and SHBP in male rats fed only on 60 and 90 g soybean/70 kg b.w.

Tanaka *et al.* (2009) cited that isoflavones could increase the production of SHBG in the liver which binds to biologically active testosterone and consequently lowers the free testosterone levels and its bioavailability to the target cells, thus increasing total testosterone.

A possible reason for the reduction of FSH and LH may be due to the increase in the level of total testosterone which produces a negative feedback mechanism leading to unalleviated levels of GnRH of the hypothalamus which intern will lead to a decrease in FSH and LH.

We also found significant increase in rat's testis weight in the three treated groups in a dose dependent manner. Also the histological study showed significant increase in testis diameter and spermatogenesis arrest at spermatid stage as seen in Figs. (3a-p). These results confirmed previous observations on rodents reported by Roberts *et al.,* 2000 and Cederroth *et al.,* 2010 and studies on men (West *et al.,* 2005 and Chavarro *et al.,* 2008).

It is well established that the development of germ cells is dependent on testosterone and FSH. The decrease of both hormones increase germ cells apoptosis (McLachlan *et al.,* 2002). FSH regulates spermatogonial development in the adult rat (Meachem *et al.,* 1999), testosterone is essential for spermatid development, while both FSH and testosterone are required for spermatocyte development (McLachlan *et al.,* 2002). Therefore, increased apoptosis of spermatocytes; round spermatids and thereby sperms is due to the disruption of the hypogonadal–pituitary–testicular axis. Our study found significant decrease in serum testosterone and FSH as cited above. Also, decreased sperm concentration may be due to the anti-estrogenic effect of phytoestrogens, indeed ERα is expressed in Leydig cells (Pelletier *et al.,* 2000); ERß in sertoli cells (Saunders *et al.,* 1998) and spermatogonia (Saunders *et al.,* 1998 and Van Pelt *et*

al., 1999); both receptor types are present in spermatocytes and round spermatids (Pelletier *et al.*, 2000). A direct role for estrogen in the prevention of human germ cell apoptosis has been described by Assinder *et al.* (2007) who demonstrated that feeding a diet of high phytoestrogen content to adult male rats, not previously exposed to elevated dietary phytoestrogens disrupt normal spermatogenesis by increasing apoptosis of developing germ cells.

Also *in vitro* studies showed that incubation of seminiferous tubules in serum and hormone-free media induce apoptosis of spermatocytes and spermatids. This apoptosis is inhibited by 17 ß-E2. The same population of seminiferous tubules exhibited increased apoptosis when a diet of high phytoestrogen content is consumed. This induction of apoptosis suggests, therefore, that phytoestrogens are anti-estrogenic in this respect (Pentikainen *et al.*, 2000).

Also the present histological study showed decrease in the number of Leydig cells or interstitial cells which produces testosterone hormone in a dose dependent manner as seen in Figs. (3.a-p).

It is known that LH and FSH play a major role in leydig cell development, differentiation, maintenance and function by modulating the production of sertoli cell-derived factors (Sriraman *et al.*, 2005), so the decrease in serum levels of LH and FSH may be the reason for the decrease of leydig cells. Also previous studies showed that estrogen play an important role in leydig cell proliferation and differentiation thus the anti-estrogenic and / or weak estrogenic action of isoflavones may cause decrease in number of leydig cells (Hancock *et al.*, 2009).

Moreover, increased testes weight and diameter is due to the anti-estrogenic action of phytoestrogen. Hess *et al.* (1997) stated that ERα has been found to be abundant in the efferent ductules and is responsible for the reabsorption of almost 90% of the luminal testis fluid. Thus, it was logic by Oliveira *et al.* (2001) to hypothesize that estrogen receptors play a role in the regulation of fluid reabsorption in efferent ductules. So, the disruption of estrogen action, by the removal of functional ER in mice or the administration of an anti-estrogen to adult rats causes reduced fluid absorption in the excurrent duct system, leading to the increase in water retention and it's accumulation in the lumen and the flattening of epithelial cell height.

In conclusion, the present study recommend Food and Agriculture Organization (FAO) to reconsider the benefits of soybean in our daily food since it was found to have adverse effects on male and female fertility. Also, care must be taken in the composition of rodent's diet as nearly all rodent's diet contains soybean which may interfere with results reached in any experiment.

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