

## Effect of alcoholic extract of soy bean consumption by pregnant mice on the testis and epididymis of their male offspring's

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### Abstract:

Soy is flavones are phytoestrogens with potential hormonal activity due to their similar chemical structure to 17-B-estradiol. The increasing availability of Soy is flavones throughout the food supply and through use of supplements has prompted extensive research on biological benefits to humans in chronic disease prevention and health maintenance. The objective of this study was to assess the effect of consumption of soy bean by pregnant mice on histomorphology of testis, epididymis in the male offspring's. Twenty pregnant female mice at age of six weeks were used as a model for this study.

After 40 days (around the puberty age) the animals were sacrificed to get their testes and epididymis each testis and epididymis was weighted, then prepared using routine histological technique mouse born from mother treated with Soy bean extract (1.5mg daily), Note the absence of mature sperms and the appearance of immature sperms in the lumen seminiferous tubules and the appearance immature sperms connected to the germinal epithelium, and also we note in the epididymis of young male mouse from mother treated with soy bean extract absence of mature sperms in the lumen of the epididymis.

Diameters of seminiferous tubules and epididymis together with the thickness of their germinal wall were calculated, these observation indicate that the perinatal exposure of male mice to is flavones affected in testies and epididymis, and they imply that Soy bean has potential implication for testis function.

### الخلاصة:

تعتبر الصويا ايزوفلافونيد من الفايثو ايستروجين الذي يتميز بانه يمتلك فعالية هرمونية كامنة تعزى لتركيبها الكيميائي المشابه لـ - 17 -  $\beta$  - estro - diol. ان الزيادة المتاحة او الموجودة في الصويا من خلال الغذاء المجهز كان لها اثر في عمل بحوث واسعة وشاملة لمعرفة الفوائد البايولوجية للانسان في منع الامراض المزمنة والمحافظة على الصحة. الهدف من هذه الدراسة هو تحديد او تقييم التأثيرات الناجمة عن استهلاك فول الصويا من قبل الفئران الحوامل والمتمثلة بمعرفة التغيرات الحاصلة من خلال المقاطع النسيجية التي اخذت للخصا والبربخ في الذكور الاسلاف. استخدمت في هذه الدراسة (20) فأر, حامل ناضجة بعمر ستة اسابيع والتي اعتمد عليها كنموذج في هذه الدراسة, وبعد (40) يوم (خلال عمر النضج) تم تشريح الحيوانات لاختذ الخصى والبربخ لها وتم قياس وزن كل واحد, ثم تم تحضير المقاطع النسيجية لها باتباع الطريقة العملية المعروفة في تحضير المقاطع النسيجية للفئران المولودة من امهات تم معاملتها بمستخلص فول الصويا وكانت الجرعه (1.5 mg) يوميا وقد لوحظ غياب الحيامن الناضجة وظهور حيامن غير ناضجة مرتبطة في التجويف الحيوانات المنوية مع ظهور حيامن غير ناضجة مرتبطة بالطبقة الطلائية لخلايا جينية, وايضا لوحظ في بربخ ذكور الفئران المولودة من امهات تم معاملتها بمستخلص فول الصويا غياب الحيامن الناضجة في البربخ. وقد تم قياس قطر انعدام الحيوانات للانابيب والبربخ معا وقياس تتخن الجدار لكلاهما, وهذه الملاحظات تدل على ان تعرض الفئران الذكور المسبق لـ isoflavones ايزوفلافونيد يؤثر في الخصى والبربخ, وهذا يشير الى ان فول الصويا يمتلك تأثير كامن على وظائف الخصى.

**Introduction:**

Soy protein based infant formula (SBIF) has been used throughout the world for over 100 years<sup>[1]</sup>. SBIFS were initially developed as an alternative to cow's milk based formula for infants with immuneoglobulin E-mediated milk allergies, post-infections diarrhea due to lactose intolerance, galactosemia, or for infants who required a vegetable substitute<sup>[1]</sup>. Genistein and daidzein are present at high concentration (0.2-1.2mg/g dry wt) in soy beans<sup>[2]</sup>. Genistein and daidzein may reach micromolar concentration in infants fed soy protein formulas<sup>[3]</sup>.

Evidence indicates that genistein and daidzein act as estrogen receptors alpha (ESR1) and beta (ESR2) agonists and/or antagonists and have the capacity to regulate cell proliferation, growth and function<sup>[4]</sup>. Because reproductive tract tissues express high levels of steroid hormone receptors, including ESRs, consumption of high levels of Soy proteins for prolonged periods of time may affect reproductive tract development and function. In addition to exerting hormone like effects, isoflavones may also act through a non-hormonal mechanism by inhibiting tyrosine kinases and inducing some growth arrest and apoptosis<sup>[5]</sup>. According to the infant formula Act of 1980, amended in 1986, SBIFs meet all nutritional requirements for term infants<sup>[6]</sup>.

Data from North America suggest that approximately 37.2% to 43.8% of infants are formula fed three to six months postpartum<sup>[4]</sup>. Recent data suggests the prevalence of feeding SBIF is 20-25% in Canada<sup>[7]</sup>, and the United States<sup>[8]</sup>, and markedly lower (2-3%) in the United Kingdom<sup>[9]</sup> and Australia<sup>[10]</sup>.

**Materials and methods:**

Animals and treatment 20% female albino mice at age of six weeks were used as model for this study obtained from the animal house at the Al-Nahrain University. The animals were kept in the laboratory under constant temperature (25<sup>±</sup>0c) for at least one week before and along the period of the experimental work. The mice were distributed randomly into two groups: the control group (C) (10) and the experimental group (G) (10) received (1.5 mg/kg) alcohol extract of Soy bean dissolved in (1ml) distilled water orally for the whole pregnancy while the (C) was given distilled water only in the same amount and for same period of time. Histological and morphometrical examination: After 40 days (around the puberty age), the animals were sacrificed to get their testes and epididymis. Each testis and epididymis was weighted, then fixed in (10%) normal saline and histological sections with thickness of (5micrometer) were prepared using the routine histological technique and stained with Haematoxylin-eosin<sup>[11]</sup>. Diameters of seminiferous tubules and epididymis together with the thickness of their germinal wall were calculated by ocular micrometer about a total of 25 cross sections of round shape seminiferous tubules and epididymis randomly selected from each mouse for measuring their diameter a calibrated eye (X400) ocular microscope in order to draw their mean value for individual mice<sup>[12]</sup>.

**Statistical analysis:**

The goal of the analysis was to test the effects of Soy bean or isoflavonoid treatment on weight, diameter, thickness for testes and epididymis. Descriptive statistics were calculated to assess normality and equality of variances. Analyses were performed using SAS (2004) and all result was expressed as means  $\pm$  standard deviation. Differences were considered significant at P<0.05.

**Table-1: Pregnancy outcome (e.g., weight, diameter and thickness for both testis and epididymis), anogenital sex organ and testes weights in adult mice.**

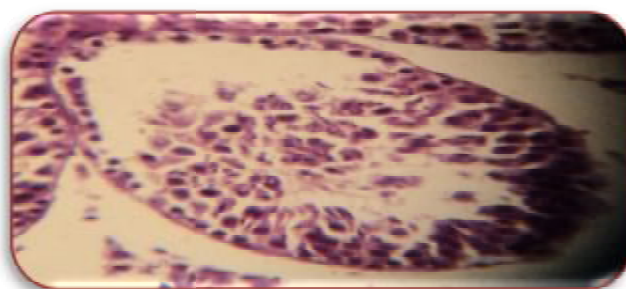
	Weight of testis (g)	Weight of epididymis (g)	Diameter of seminiferous tubules (µm)	Diameter of epididymis (µm)	Thickness of seminiferous tubules(µm)	Thickness of epididymis (µm)
<b>Treated</b>	0.28* ±0.004	0.54* ±0.005	180.2* ±0.4	152.3* ±0.57	87.3* ±0.67	24.7* ±0.12
<b>control</b>	0.8 ±0.005	0.73 ±0.008	199.7 ±0.19	167.2 ±0.62	95.4 ±0.78	27.8 ±0.26

**Results:**

The result showed that treatment with extract of Soy bean caused significant ( $P < 0.05$ ) decrease in epididymis, and significant increase in testis ( $P < 0.05$ ). Whereas the findings of histology in the testis of young male

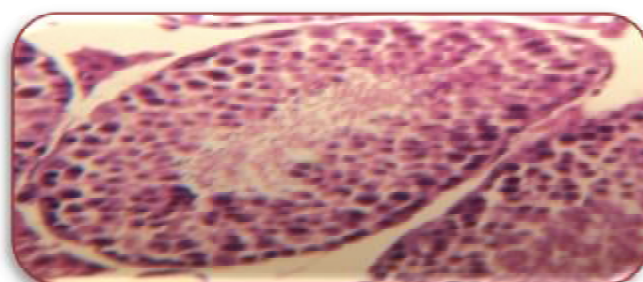
(40 days) mouse born from treated with Soy bean extract (mg daily),

Note the absence of mature sperms in the lumen of the seminiferous tubules and the appearance of clumps of immature sperms connected to the germinal epithelium figure-1.



**Figure-1: Cross section in the testis of young male(40 days) mouse born from mother treated with soy bean extract (mg daily), Note the absence of mature sperms in the lumen of the seminiferous tubules and the appearance of clumps of immature sperms connected to the germinal epithelium (arrows). 400X (H&E).**

Compared with control figure-2



**Figure-2: Cross section in the testis of young male mouse born from control group mother. Showing the wide lumen of seminiferous tubules filled with mature sperm (arrows) and complete development of the lining epithelium 400 X(H&E).**

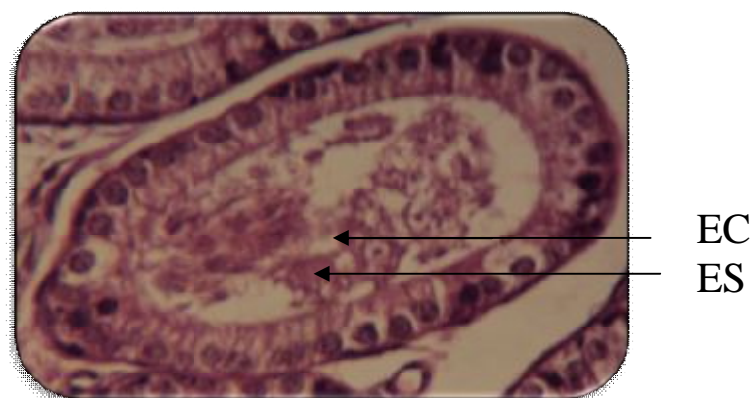
And the results of epididymis of young male (40 days) mouse from mother treated with Soy bean extract (mg daily)

.Note the absence of mature sperms in the lumen of the epididymis fig(3).



**Figure-3:** Cross section in the epididymis of young male (40 days) mouse born from mother treated with soy bean extract (mg daily), Note the absence of mature sperms in the lumen of the epididymis (arrows). EC; Epithelial cell. (←) 400X (H&E)

Compared with control figure-4



**Figure-4:** Cross section in the epididymis of young male mouse born from control group mother. Showing the wide lumen of epididymis filled with mature sperm (arrows) and complete development of the lining epithelium. EC; Epithelial cell layer (↔), ES; Epididymal spermatozoa (←).

### Discussion:

The present results demonstrate that alcoholic extract of Soy bean causes a significant decrease in fertility in male mice. This finding has implications for the use of soy bean by pregnant mothers and in infant formulas. Organ weights, testis and epididymis and histological findings for testis and epididymis were evaluated at (1.5mg/kg) alcoholic extract of soy bean causes changes in the organs weight and variables evaluated.

These findings agree with our previous bioflavonoid treatment study demonstrating marked accessory sex gland atrophy by Cline *et al.* [13]. All mice

receiving the isoflavonoid exhibited accessory sex gland atrophy Spearow *et al.* [14]. 750,000 infants fed soy based cereals in the United States each year are exposed to genistein and daidzein which attain blood concentrations in the range between 300 and 600 nm [15, 16], and in other study the testes of the soy infant formula-fed monkeys had larger numbers of both- germ cell (Sertoli cell) and Leydig cells (interstitial cells) with no evidence of a long-term adverse effect of soy isoflavonoids fed during development [17]. Giampietro *et al.* [18]. evaluated the effects of the use of soy-protein-based formula in children ranging from 7 to 96 months of

age and found no evidence of precocious puberty, gynecomastia, or altered bone metabolism.

In other study show that low concentrations of genistein ( $\leq 10\text{mM}$ ) tend to induce cell proliferation in estrogen sensitive tissues<sup>[19, 20]</sup>. Increases in serum testosterone levels were previously associated with decreasing steroidogenic enzyme activity, indicating the possibility of greater Leydig cell numbers, especially as animals were fed soy diets beginning in the prepubertal and pubertal periods<sup>[21, 22]</sup> of intense mitotic activity in Leydig cells<sup>[23]</sup>, and also<sup>[24]</sup> observed that genistein causes direct inhibition of  $3\beta$ -hydroxy steroid dehydrogenase (HSD3 $\beta$ ) activity in human and rat testis. Thus, perinatal exposures to soy isoflavones possibly exert differential effects on steroidogenic enzyme capacity in Leydig cells, moreover, genistein is known to exert other biological effects, including tyrosine kinase inhibition and antioxidant activity<sup>[25, 26]</sup>.

Further studies investigating the effects of soy isoflavonoids on Leydig cell density and function are warranted, although we are unaware of data demonstrating reduced fertility as a result of ingesting soy protein or isoflavonoids by nonhuman primates or humans. Thus exposure to isoflavonase occurring in the fetal and neonatal periods have the potential to alleviate effects associated with deficits in androgen biosynthesis and/or augment testicular and serum testosterone levels<sup>[27]</sup>.

This effect has implications for reproductive health, because high intratesticular testosterone concentrations may adversely affect the process of spermatogenesis and sperm production. On the other hand, a number of studies have shown that isoflavones may act within the seminiferous epithelium to impact spermatogenesis without concomitant endocrine changes<sup>[28]</sup>.

Thus multiple cell types in the testis are likely targets for the action of

isoflavones. Also, the testicular actions of daidzein and equol, which are present in body tissues (along with genistein) after consumption of soy-based meals, have received little or no attention. Additional studies are warranted to examine the outcome of combined action by isoflavones in Leydig cells and germ cells. These studies will provide information relevant to risk assessment of the population regarding the potential for soy-based food products to cause testicular toxicity, especially in neonates, also future studies should address the effects of other soy components commonly consumed by human beings that act alone or in combination with isoflavonoids.

### **Conclusions:**

The biological effects of soy isoflavone exposure as a result of SBIF consumption is controversial and inconclusive. In summary, only one retrospective study has reported effects of feeding SBIF on health outcomes at adulthood and few studies have examined infant health after exposure to SBIF.

While studies using a variety of animal models report negative effects of soy isoflavones exposure during development, it is unclear whether these data can be extrapolated to human infants, these studies do however suggest effects of early exposure to isoflavones is warranted.

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