Dijlah Journal of Medical Sciences (DJMS) Vol. 3, No. 2, May, 2026, pp. 34-42
P-ISSN: 3078-3178, E-ISSN: 3078-8625, paper ID: 04

ORIGINAL ARTICLE

Estrogenic Performance of High Doses of Lignans in Immature Female Mice

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Article Info

Article history:

Received February, 11, 2026
Revised March, 05, 2026
Accepted March, 15, 2026

Keywords:

Lignans,
Immature Female Mice,
Estrogenic Activity,
Reproductive Organs

ABSTRACT

This study was conducted to evaluate the responses of immature female mice to high doses of Lignans intubated orally for four weeks period. Diethyl ether and 80% ethanol was used for extraction of Lignans from flaxseeds. Thirty immature female mice, 21 one day old, were divided randomly into three equal groups were administered 50 and 100 mg/kg body of Lignans, respectively while the third group served as a control. The results showed that no significant differences in body weight gain among Lignans treated groups as compared to the control was recorded. On the other hand, Lignans had induced a significant increment in uterine and ovarian weight compared with control groups. However, reproductive organs, weight increments were coincided with the significant increments of gonadotropic hormones; follicle-stimulating hormone (FSH), Luteinizing hormone (LH) and estrogen levels. Moreover, thyroid hormones showed a significant alteration manifested by a significant decrease in the levels of triiodothyronine (T3) and thyroxine (T4) and a significant increase in thyroid stimulating hormone (TSH). Interestingly, these results confirmed the estrogenic activity of high doses of Lignans extracted from flaxseed.

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1- INTRODUCTION

Lignans are low molecular weight diphenolic plant-derived secondary metabolites, widely distributed in plants especially Flaxseeds. The considerable role of Lignans as phytoestrogens, may be due to their structural similarity to naturally produced estrogen (endogenous) which facilitates, its binding to estrogen receptors ERα and ERβ, and their capability in gene expression modulation with a consequent alteration in reproductive performance [4, 5, 6]. Assessment of estrogenic activity of phytoestrogens, (non- steroidal compounds similar to 17 β-estradiol), can be carried out in vitro through receptor binding assays [7], cell proliferation and gene expression analysis, while in vivo using immature mice and rats and oral or subcutaneous administration of the test compounds were established [8]. In addition, responses observed in experimental model rodents for evaluating exogenous estrogenic phytoestrogen activity was confirmed by their effects on hypothalamic- pituitary - gonadal axis, changes in uterine and ovarian weight as well as their effects in endocrine homeostasis [9, 10].

In experimental animal models, a particular organ changes and overall significant physiological responses to certain exogenous estrogens may vary with type, does, age and duration of exposure. In This context and according to the accumulative evidence's low doses of exogenous estrogenic compounds do not elicit classical uterotropic responses and may exhibits an antagonistic impacts [11, 12]. However, to avoid potential antagonistic behavior of phytoestrogens at low doses such as uterine growth stimulation failures, inhibition of gonadotropin liberation,

promotion negative feedback at the hypothalamus/pituitary axis, two high doses of Lignans 50, 100 mg/kg body weight was intubated to immature female mice for four weeks and changes in; body, ovarian and uterine weights was recorded as well as reproductive and thyroid hormones concentrations.

2- MATERIALS AND METHODS

1. Lignans Extraction

Flaxseed was purchased from Baghdad local market. Seeds were ground using household grinder and subjected to soxhlet`s extraction using diethyl ether as recommended by [13]. The residual cake in the thimbles were collected and dried in an oven at 40°C, and used a raw material for the extraction of Lignans from flaxseed using 80% methanol for 1h at 40°C according to the method of [14]. Purified Lignans was kept in a tide-sealed container.

2. Experimental Animals

The estrogenic activity of Lignans was performed on a total number of thirty immature female Swiss white mice, 21 days old and 14-15 gm of weight. Animals were housed in plastic cages in an air-conditioned room (23-25°C) with an automatically controlled photoperiod of 14 hours light and 10 hours darkness. Mice were fed pelleted diet (Wheat grits 22.5%, Barley grits, 20 %, corn grits 20%, meal 17.5%. and powdered whole milk 20 % fortified with vitamins and minerals and food salt 5 gm per 10 kg of provender and drinking tap water [15, 16].

3. Experimental design

Animals were divided randomly into three equal groups (10 mice/ group) and treated daily for four weeks as follows:

Group C: Immature female mice were received normal saline solution, serving as control.
Group I- Mice of this group were received 50 mg of Lignans/ kg body weight suspended in saline solution by oral intubations with gavage`s needle.
Group II- Mice of this group were received 100 mg of Lignans/ kg body weight suspended in saline solution by oral intubations with gavage needle.

After the completion of the experimental procedure, animals were anesthetized by ethyl ether under glass beaker in order to facilitate blood collection, following body weight estimation; blood was collected from heart directly by heart puncture. Serum was collected and stored in deep freezer (-20°c) until used for hormonal determination.
Animals were then scarified by cervical dislocation, laparotomy was performed, and the whole reproductive system was quickly removed and immersed in a petri dish filled with in vitro normal saline, kept at 37°C. Both ovaries were quickly dissected out using fine surgical scissors, cleaned from surrounding non ovarian tissue, dried by a filter paper, weight by sensitive electronic balance and recorded. The uterus cleaned from surrounded tissue, dried by a filter paper and weight was recorded by a sensitive electric balance [17].

4. Studied parameters were involved:

1. Body weight gain (g), was estimated according to the following equation
   Weight Gain (g) = Final weight- Initial weight (g)
2. Ovarian weight (Ow, w%)
   Weight of ovaries were recorded, and then normalized per 100 gm Body weight as follows
   (Ow, w%) = Ow/ B.W X100
3. Uterine weight (UW, w%.)
   Weigh of uterus were recorded, and then normalized per 100 gm B.W. as follows
   (Uw, w%) = UW/B.W.X100
4. Hormones Assay
   Estrogen (E2), Follicular stimulating hormone (FSH), Luteinizing hormones (LH), thyroid stimulating hormone (TSH), triiodothyronine (T3) and thyroxin (T4) concentrations in the serum of the control and experimental animals were measured by Radio Immuno Assay method [18].

5. Statistical Analysis

A statistical analysis of data was executed on the basis of either one way analysis of variance (ANOVA) using significant level of (P<0-05). The least significant differences (LSD) test was also used to compare the significance between the means [19].

3- RESULTS AND DISCUSSION

1. Body weight gain (g)

Treatments of immature female mice with two high doses of Lignans (50 and 100 mg/kg B.W.) caused 3.18 and 4.57 % increments in body weight gain in both treatments T1 and T2 respectively (Fig 1). These differences were below the calculated LSD value indicating that Lignans have no significant role in body weight gain. The minor increase in body weight gain may attributed to normal physiological growth during the studying period [20, 21], and this may confirm the fact that body weight is a secondary and a nonspecific endpoint in estrogenic testing. Moreover, estrogenic substances often influence water retention, fat distribution and regulations of metabolism in experimental mice by estrogen receptors alpha rather than causing marked changes in overall body weight [22, 23].

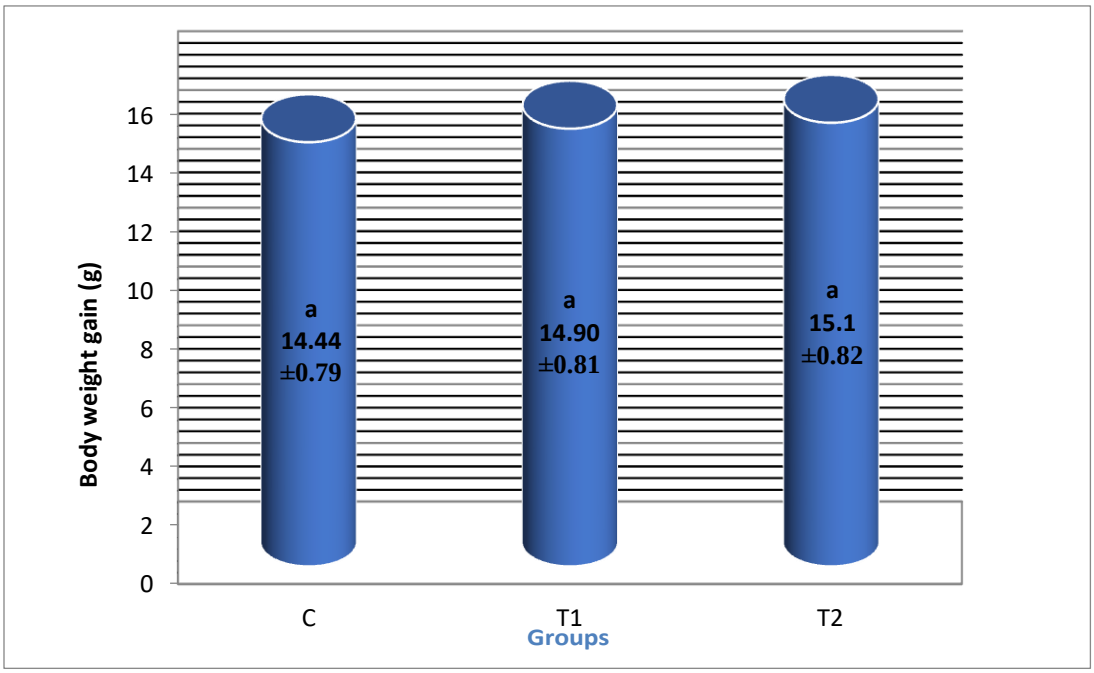

Fig (1): Effect of Flax seed Lignans at a level 50mg/kg B.wt (T1), and 100mg/kg B.wt (T2) on body weight gain in immature female mice. C is the control

LSD= 1.69

Small letter represents the presences of significant (P<0.05) differences between groups

The Effect of Lignans in uterine weight of immature female mice

Immature female mice treated with 50 and 100 mg Lignans/ kg B.W. for four weeks were showed a significant increment (P<0.05) in the weight of the uterus for both doses (figure 2) comparing to the control. A direct positive relationship was recorded among increased Lignans concentration and the significant increments in uterine weight in both groups. The estrogenic activity of a large number of phytoestrogen was confirmed by many workers, gavage`s administration of naringenin to immature female mice caused a significant increase in the uterine weight [24]. Similar results were recorded in genistein and flaxseeds extract treated female mice [25, 26]. However, the significant increases in the weight of uterus in this study may attributed to the histological changes and increments in the thickness of uterine wall layers; epithelia, stroma and myometrium in immature and mature female mice as mentioned by some workers [11].

Furthermore, in immature female mice and during maturation Lignans as an exogenous estrogenic component may binds estrogen receptors alpha (ERα) the most plentiful one in the uterus and induces DNA transcription pathway, with a consequent alteration in tissue proliferation, structure and early uterine growth stimulation. These changes ultimately lead to a significant uterine weight changes [27, 28].

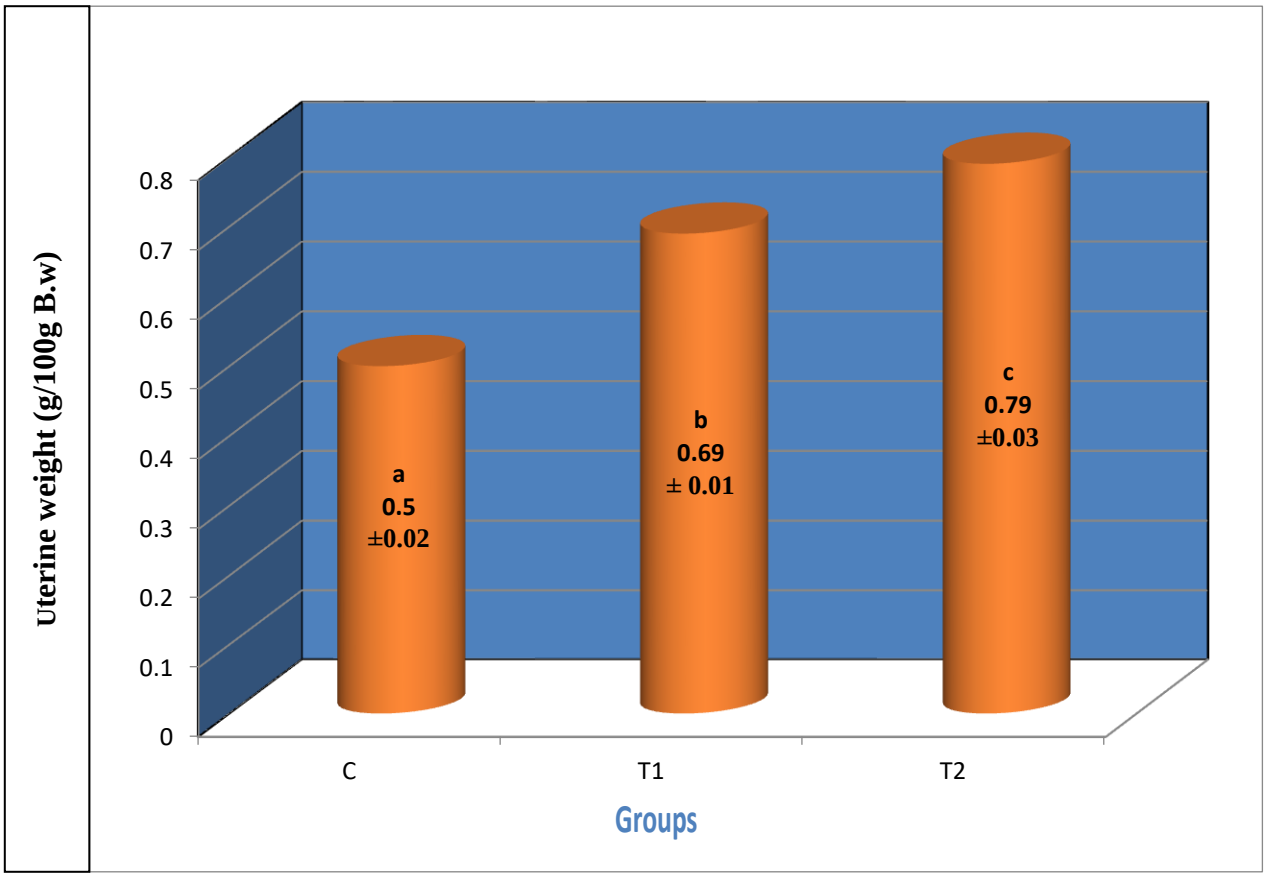

Fig (2): Effect of Flax seed Lignans at a level of 50mg/kg B.W. (T1) and 100mg/kg B.W. (T2) on uterine weight (g/100g B.W.) in immature female mice. C, is the control

LSD= 0.055

Small letter represents the presences of significant (P<0.05) differences between groups

Effect of Lignans in the ovarian weight of immature female mice

A significant (P<0.05) increments in the ovarian weight was observed in both immature female mice groups (T1 and T2) intubated orally 50 and 100 mg Lignans/ kg body weights (Fig 3). This ovarian weight gain may be attributed to structural [29] similarities of Lignans as a phytoestrogen to the endogenous 17β-estradiol which makes their binding to estrogen receptors easier particularly to estrogen receptors beta (the dominant receptors the ovaries [30], with a consequent elevation in follicular growth and developments during folliculogenesis, in addition to the increments of granulosa cell proliferation [31,32, 33]. On the other hand, excessive follicular growths may be caused by an indirect effect of Lignans on hypothalamic- pituitary-gonadal axis leading to an increase in gonadotropin secretion from pituitary gland (LH and FSH).

Then, elevated levels of LH and FSH therefore increases ovarian estrogen production and promote follicular developments. In this study, reproductive hormone elevations may (table 1) confirmed the indirect role of this axis in ovarian weight increments.

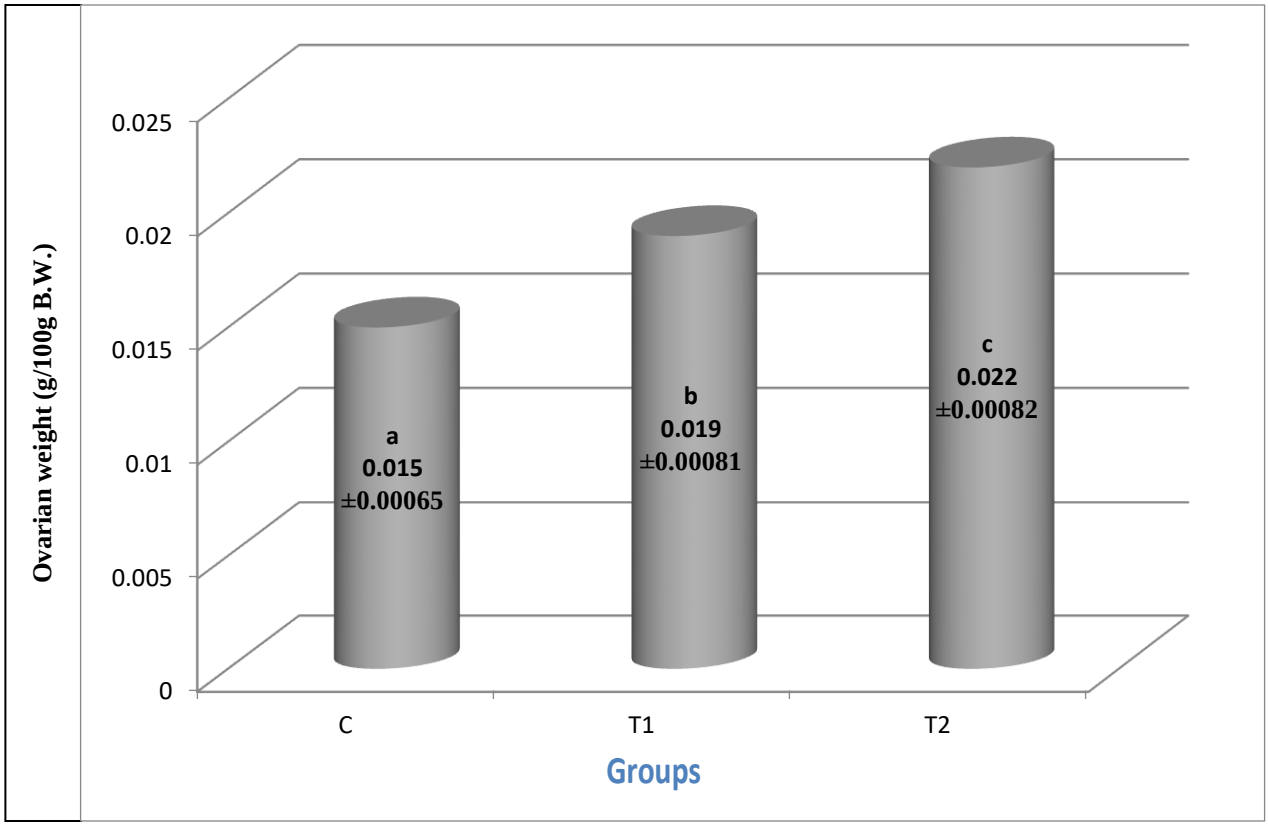

Fig (3): Effect of Flaxseed Lignans at a level of 50mg/kg B.W. (T1) and 100mg/kg B.W. (T2) on ovarian weight (g/100g B.W.) in immature female mice. C, is the control

LSD= 0.002

Small letter represents the presences of significant (P<0.05) differences between groups

Changes in the reproductive and thyroid hormones Levels

A significant (P>0.05) increments in the level of serum LH, FSH and estrogen post four weeks of oral intubation of high doses of Lignans in both treated groups comparing to the control was (table 1). This alteration may be to attribute incomplete maturation of hypothalamic -pituitary- gonadal axis at initial stages of the development [34]. In Immature female mice, the negative feedback sensitivity of the pituitary and hypothalamus to estrogen is reduced, permitting Lignans as an estrogenous compound to stimulate gonadotropin release rather than suppress it [35, 36]. Lignans can act as selective estrogen receptor modulator binding to estrogen receptors alpha and beta, with higher affinity for estrogen receptors- β, which is abundantly expressed in hypothalamus and ovaries [37]. This interaction may enhance gonadotropin- releasing hormone (Primary regulator of the reproductive system), leading to elevated pituitary release of FSH and LH, which in turn stimulate follicular development and ovarian estrogen production [38]. Further, a significant change in thyroid hormone levels was recorded in this study (table 1) manifested by a significant decrease in the level of triiodothyronine (T3), Thyroxin (T4) and a significant increase in thyroid hormone stimulating hormone (TSH). The observed reduction in T3 and T4 levels in immature female mice post four weeks of treatments with high doses of Lignans may be caused by the interaction of Lignans with hypothalamic pituitary thyroid axis, thyroid hormone synthesis and metabolism. The mode of action of Lignans in this context was similar to genistein in inhibiting thyroid peroxidase [39].

Table (1): Effect of flaxseed Lignans 50 mg/kg B.W. and 100 mg/kg B.W. for four weeks in reproductive and thyroid hormones concentration in immature female mice

Hormones	Groups			
	C	**T1**	**T2**	**LSD**
Estrogen (Pg/ml)	10.35 c ± 0.12	16.22 b ± 0.22	19.89 a 0.10	0.444
FSH (mIu/ml)	11.02 c ± 0.09	19.49 b ± 0.37	20.92 a 0.11	0.655
LH (mIU/ml)	11.60 c 1± 0.19	21.74 b + 0.19	23.95 a 0.18	0.525
T3 ng/ml	0.906 c ±0.06	0.832 b ±0.05	0.776 a ±0.08	0.046
T4 ng/ml	48.70 c ±0.06	44.65b ±0.19	41.70a 0.18	1.95
TSH ng/ml	0.201 ± 0.004	0.227 ±0.006	0.239 ±0.009	0.010

Values are expressed as mean ± SE, n= 10/group

C: considered as a control group

T1: Immature female mice received (50 mg/kg B.W./day) of flaxseed Lignans for four weeks

T2: Immature female mice received (100 mg/kg B.W./day) of flaxseed Lignans for four weeks

Small letter represents the presence of a significant (P<0.05) difference between groups

4- CONCLUSION

Treatments of in immature female mice stages with high doses of Lignans (50 and 100 mg/ kg B.W.) caused a marked estrogenic alterations manifested by a significant increments in uterine and ovarian weight gain, elevation in FSH, LH and estrogen levels, as well as alteration in thyroid hormone. the results of this study was confirmed the biological efficacy of phytoestrogens in direct activation of estrogenic receptors in reproductive organs at early stages of the developments, as well as, modifications of the hypothalamic - pituitary- gonadal axis.

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