Effect of Phytoestrogen (Genistein) on Histology of Uterine Lining of Adult and Post-Natal Female Albino Mice

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***Abstract*— The goal of this study was to determine the effects of genistein on the weight and histological structure of the uterus in adult and postnatal (P22) female albino mice. Adult female mice given 50 mg genistein had a considerable rise in uterine weight and a highly significant increase in endometrial layer thickness if compared with the other groups. While the thickness of the myometrial layer in both genistein groups was significantly increased in comparison with the control, however, treatment with 10mg genistein caused no significant increase in the weight of the uterus as compared to the control group, but caused a significant increase in the endometrial layer thickness compared with the control group. In postnatal females, the thickness of the endometrium layer was significantly increased in the group of 50mg genistein as compared to the other groups. But the thickness of the myometrium layer was significantly decreased due to the effect of 10 mg genistein when compared to the other groups. Histopathological examination of uteri of genistein groups in both experiments demonstrated variable degrees of degenerative tissues ranging from affected uterine wall thicknesses and luminal (circumference and its branches) and caused hyperplasia in both the lining and glandular epithelium, which is associated with connective tissue edema. In conclusion, genistein has a detrimental effect on the anatomy of the uterus in adult and post-natal female mice, interfering with its functions.**

***Keywords*- Adult and post- natal albino female mice, Genistein, Histopathology, Phytoestrogen, Uterus.**

I. Introduction

Phytoestrogens are plant-derived xenoestrogens that are consumed by eating phytoestrogenic plants, which include beans, sprouts, cabbage, spinach, soya beans, grains, and hops. As a result, it is referred to as "dietary estrogens," a diverse group of naturally occurring non-steroidal plant compounds that share structure and/or function with the endogenous steroidal hormone (17-estradiol) [1,2]. They have either estrogenic and/or anti-estrogenic effects on humans or animals, by sitting in and blocking receptor sites against estrogen [3,4]. Phytoestrogens are much less powerful than estradiol, but they work through similar mechanisms [5].

Phytoestrogens are a type of substituted natural phenolic compounds. Phytoestrogens can be classified into two types based on their chemical structure: flavonoids and non-flavonoids [2]. The phytoestrogen genistein belongs to the best-known category of isoflavone glycones and is the most estrogenically active chemical. Although there are 234 distinct glycones, only a few contain estrogenic properties similar to genistein.

Genistein is an estrogen-like chemical molecule found in plants that is produced by human metabolism from certain plant precursors. These are chemical substances found in nature that may interact with estrogen receptors to create estrogenic or anti-estrogenic effects. They are made up of a diverse range of non-steroidal chemicals that are structurally and functionally identical to human estrogens [2, 6].

Estrogen operates primarily through two subtypes of its receptors located in the target tissues. The alpha (ER) and beta (ER) receptors are the two types of receptors [7]. Although these receptors are produced differently in different mammalian tissues, there are some discrepancies in their location. The interaction of phytoestrogen with these classical receptors, can mediate many of their downstream actions [8]. Divergences in the affinity of distinct estrogen compounds to the individual ER are closely connected with phytoestrogen's ability to bind to these receptors. However, as compared to estradiol, these affinities for ER and ER are weak [8,9], and ER has a much higher affinity than ER [10]. Therefore, depending on whether estradiol is present too, the phytoestrogens, as indicated by Shanel and Xu, [8], depending on whether or not estradiol is present, the activity is either agonist or antagonistic. That means phytoestrogens have estrogenic and or anti-estrogenic effects on humans and animals. These chemicals can either increase or inhibit estrogen responses by interacting with ERs [1]. These effects are caused by interactions with the membranes of transport proteins and ERs, as well as additional modes of action. Biological function at the cellular and molecular level reveals that each of the target tissues' proliferation, differentiation, and protein synthesis are all regulated in this way [1, 9].

There has been concern that phytoestrogens may alter normal hormonal and disease processes that are influenced by hormones in the body because of their hormonal (estrogenic) effects. Phytoestrogens may interfere with hormonal function when the body's natural estrogens are high, as they are before menopause. When the body's own estrogen levels are low, such as after menopause, phytoestrogens can serve as estrogens and boost hormonal activity. Phytoestrogens can play positive role especially in human health care, and these favorable outcomes are for example on the metabolism of bones and prevalence of

DOI: http://doi.org/10.24086/biohs2022/paper.593

occurrence of osteoporosis [11], bone health improvement [12] in addition to their anticancerogenic effects [11,13].

Other researchers found that the organ systems of young animals are more vulnerable to chemical exposures than those of adults. As is well documented, the broad tissue morphogenesis and differentiation of organs necessary for reproductive function occurs both prenatally and postnatally. The majority of these differentiation processes are at least somewhat reliant on steroid hormone signaling. As a result, any endocrine disrupting compounds, such as phytoestrogens, could have a profound impact on development, potentially affecting reproductive health later on. These impacts have the potential to have long-term ramifications for the affected individuals' progeny [14, 15].

Phytoestrogens can affect the female genital system, causing developmental and anatomical defects as well as changes in the estrous cycle and sexual behavior. [16]. Phytoestrogens can induce a variety of morphological and developmental abnormalities. These substances also affect animal sexual development by altering pubertal timing, impaired (estrus cycling and ovarian) function, and altered hypothalamic and pituitary gland functioning in the female genital tract, as well as changes in the estrous cycle and sexual behavior [2], cause variable degrees of degeneration and destruction of ovarian follicles (ovum and granulosa cells) and the formation of apoptotic bodies [17], and have a negative impact on thymic tissue. It has the potential to produce thymic and immunological disorders [18].

In 2007, Michel and his colleagues [19] reported that due to chemo-preventive proprieties, soy and its components are used extensively in the treatment of several cancers like breast, prostate, and colon, in addition to the treatment of other diseases such as osteoporosis, hypercholesteremia, menopausal symptoms, atherosclerosis, and heart disease. This molecule may also have an effect on the volume and content of uterine fluid, resulting in an unfavorable uterine environment for numerous reproductive processes [20]. Salleh *et al.* [21] discovered that large doses of genistein can have negative effects on the uterus organ by promoting uterine fluid secretion and accumulation as well as hyperplasia. For these reasons, the focus of this research was to study the harmful effects of two genistein concentrations on the histological structure of the uterus in adult and post-natal female albino mice.

II. . MATERIALS AND METHODS

Genistein

Genistein (Sigma) synthetic, ≥ 98% (HPLC), powder with a molecular weight of 270.24g/mol was used in this study. According to earlier studies carried out by [22,23], two concentrations of genistein (10 and 50 mg/kg B.W.) were used. The low concentration was 10 mg/kg B.W. genistein. This concentration, as reported by [22], is similar to the total amount of soy phytoestrogens taken daily by children fed soy infant formula, while the 50 mg/kg B.W. genistein was utilized as a high dose.

Tween 80 (Merck-Schuchardt, Germany) was used as a negative control [24]. Distilled water was used to dilute Tween to a concentration of 1:9 v/v [25]. Genistein was dissolved in Tween 80 and orally given once per day for two weeks.

Animals and experimental design

This study includes fifteen adult (8–10 weeks old) and fifteen postnatal (P22) female albino mice of strain Balb/C. These animals were obtained from the Animal Breeding House, Faculty of Science, University of Zakho. These animals were maintained under a regime of 12 hours of light and 12 hours of dark at 22–24 °C and they were given a standard diet of pellets and water all the time. The research was carried out in the Laboratory of Zoology, Biology Department, Faculty of Science, University of Zakho.

Experiment (I): adult female mice: All fifteen female mice were weighed using an electric balance and then randomly divided into three groups (n = 5) for each group, as follows: Group (I): the control group The females of this group were orally given an equivalent volume of Tween 80. Group (II) orally received 10mg of genistein/kg B.W. Group (III): orally received 50mg of genistein/kg B.W.

Experiment (II): Postnatal (P22) female mice: This experiment involves using fifteen postnatal (P22) albino female mice. The animals were also weighed on an electric balance and then randomly divided into three groups (n = 5) as follows: Group (I): the control group This group's postnatal females were simply orally given an equivalent volume of the (Tween 80). Group (II) got 10mg of genistein/kg B.W. orally. Group (III): 50mg genistein/kg B.W. was given orally. In both experiments, the animals were given tap water and a diet free of phytoestrogens. The genistein was given once a day for two weeks through an oral gavage tube to imitate the prevalent route of human exposure.

Dissection and histological preparation and studies

After two weeks of treatment, all these animals were weighted, sacrificed by cervical dislocation, dissected, and the uterus was taken, then weighted with a sensitive electrical balance. Then they were fixed for 24 hours in 10% formalin, dehydrated, cleared, embedded in paraffin and sectioned using a rotary microtome. Then they were stained with Harris hematoxylin and eosin (H and E) for histological studies [24,25]. Twenty-five typical left and right uterus sections (for each animal) were chosen for microscopic analysis to measure the thickness of the endometrium and myometrium (125 uterus sections per group). Using ocular and stage micrometers in postnatal and adult female albino mice, then these sections were examined microscopically to find out the histopathological changes of the uterus in mice delivered genistein and Tween 80 and photographed using a digital camera.

Analytical statistics

The experiment was set up in a completely randomized design (CRD). The SAS program was used to analyze the data from this study, and Duncan's multiple range tests were used to compare the means [26].

III. RESULTS

The effect of genistein on uterine weight and uterine layer thicknesses (endometrium and myometrium) in adult and post-natal female albino mice

DOI: http://doi.org/10.24086/biohs2022/paper.593

Treated adult female mice with 50mg genistein /kg B.W. caused a significant increase (P≤0.05) in the uterus weight in comparison with 10mg genistein and with the control group. However, when compared to the control group, treatment with 10mg genistein generated no significant increase in uterine weight (Table 1).

These two genistein concentrations had no significant (P>0.05) variations in their impact on the weight of the uterus in the treated post-natal females. When compared to the control group, the effect of these concentrations resulted in a significant decrease (P≤0.05) in uterine weight (Table 2).

The results also showed a highly significant increase (P≤0.01) in the thickness of the adult female endometrial layer was observed in 50mg genistein as compared to 10mg genistein and the control group table (1). Similarly, a significant difference was observed between the 10mg genistein treated group and the control group at (P≤0.05). The result also indicated that both concentrations caused an increase in the thickness of the myometrium layer as compared with control. Statistical analysis revealed no significant differences in their effects on this layer between the 10 and 50 mg groups (P>0.05), but these results were significantly different from those of control group.

Significant increases (P≤0.05) in the thickness of the postnatal female endometrium layer were recorded in the group of 50mg genistein as compared to the 10mg genistein and control groups. When compared to the control group, those treated with 10mg genistein had no significant effect (P>0.05) on the thickness of this layer. The thickness of the myometrium layer significantly decreased (P≤0.05) due to the effect of 10mg genistein (31.36±2.463) compared to 50mg genistein and control groups. No significant differences (p>0.05) were observed between the thicknesses of myometrium in the 50mg genistein and control groups (Table 2).

Table (1): Mean ± S.E for the effect of genistein on the uterus weight (grams), and thicknesses of uterine layers (endometrium and myometrium) of the adult female albino mice.

Groups	Uterus weight	Endometrial layer thickness (μm)	Myometrial layer thickness (μm)
Group (I) Control	0.028±0.0054 [b]	204.32±3.289 [c]	1333.5±49.859 [a]
Group (II) 10mg genistein/kg (B.W.)	0.039±0.003 [b]	226.96±2.003 [b]	280.24±11.213 [a]
Group (III) 50mg genistein/kg (B.W.)	0.088±0.003 [a]	88.4±4.133 [a]	93.94±6.859 [a]

- At (P > 0.05), the same letters indicate no significant differences. The different letters denote statistically significant differences (P≤ 0.05), or highly significant differences at (P≤ 0.01) between them according to Duncan's multiple range tests.

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Table (2): Mean ± S.E for the effect of genistein on the uterus weight (grams), and thicknesses of uterine layers (endometrium and myometrium) of the postnatal female albino mice.

Groups	Uterus weight	Endometrial layer thickness (μm)	Myometrial layer thickness (μm)
Group (I) Control	0.0720±0.007 [a]	81.24±3.377 [b]	34.94±1.094 [a]
Group (II) 10 mg genistein/kg (B.W.)	0.0480±0.005 [b]	76.1±7.554 [b]	31.36±2.463 [b]
Group (III) 50mg genistein/kg (B.W.)	0.0320±0.003 [b]	90.48±2.278 [a]	36.64±2.140 [a]

- At (P > 0.05), the same letters indicate no significant differences. The different letters denote statistically significant differences (P≤ 0.05), or highly significant differences at (P≤ 0.01) between them according to Duncan's multiple range tests.

Histological and histopathological studies of the effect of genistein on the uterus in both adult and postnatal (P22) female albino mice

The general structure of the uterine wall in the adult (Fig.1) and postnatal (Fig.3) female mice was made up of three main layers: perimetrium on the outside, myometrium in the middle, and endometrium on the inside.

Histological examination of uterine sections treated with genistein revealed a slight increase in the uterine luminal circumference in genistein groups; the uterine lumen became more branched in genistein groups (Fig.1 B; C; D and E) in comparison with the control (Fig.1 A). Treated genistein groups also showed an increase in the uterine gland number, as shown in figures 1D and E) in comparison to a control group (Fig.1 A). As seen in table 1, the thicknesses of the endometrial and myometrial layers in genistein treated groups were increased significantly when compared to control, and this result led to an obvious increase in the thickness of the uterine wall. This increase was attributable to hyperplasia in the lining and the glandular epithelium as seen in [Fig. (2 A; B; 10mg genistein) and (D)], and also treated with genistein resulted in connective tissue edema and caused congestion in the blood vessels (Fig.2. B and C). Figure (2E) showed that treatment with 50mg genistein caused damage to the endometrium layer and uterine glands. However, in postnatal female mice, figures (3B and C) showed an obvious decrease in the uterine luminal circumference following treatment with 10 and 50 mg genistein compared to the size of this lumen in the control group (Fig. 3A). These figures also showed an improvement in the

DOI: http://doi.org/10.24086/biohs2022/paper.593

thickness of the uterine wall compared to control, but the number of uterine glands and uterine lumen branches appeared less than in adult female mice (Fig.1 B; C; D and E).

Treatments with 10mg of genistein caused: destruction of the endometrial layer (Fig.4A); hyperplasia in the lining epithelium (Fig.4B); and a decrease in the number of the uterine glands (Fig.4C) compared to control (Fig.4F). This feature was observed in both concentrations. Figure (4D) showed that 50mg of genistein also caused hyperplasia in the lining epithelium and congestion in the blood vessels.

However, a few histological sections of animals that were treated with 50 mg of genistein show no histological changes (Fig.4E) when compared with the control group (Fig.4F).

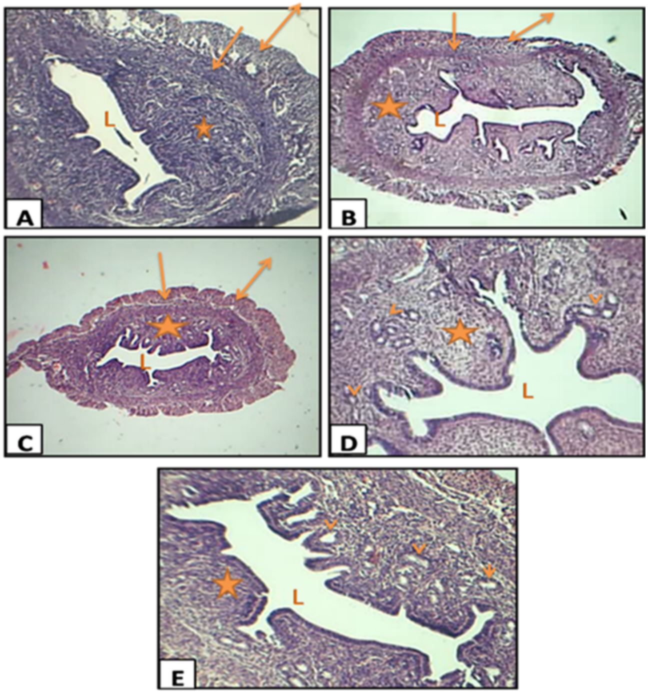

Fig. (1): Photomicrographs of uterine sections tissue of adult female albino mice (exp.1) (A: control; B and D: 10mg; C and E: 50mg genistein) showing: A slightly increase in the uterine luminal circumference in the (D: 10mg and E: 50mg genistein) compared to the control group (A). In addition to an obvious increase in both the number of uterine glands and uterine lumen branches in genistein groups compared with control. Uterine lumen (L); Endometrium (star); myometrium (arrow); perimetrium (double head arrow). (A; D and E:100X; B and C:40X).

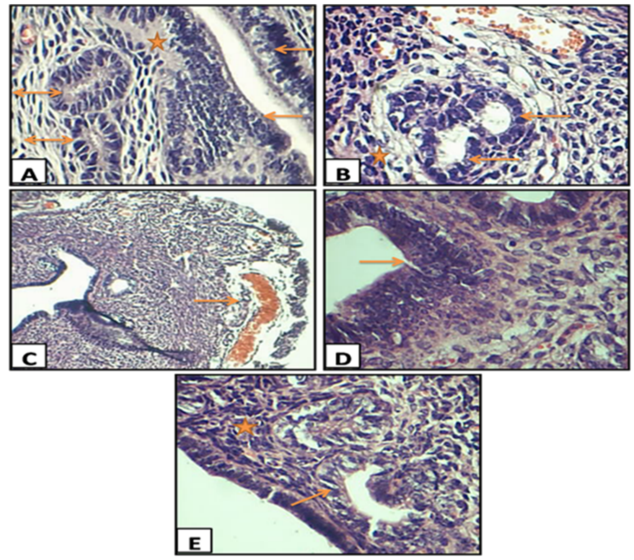

Fig. (2): Photomicrographs of uterine sections tissue of adult female albino mice (exp.1) showing: A (10mg genistein) hyperplasia in the lining (arrow) and the glandular epithelium (double head arrow) and connective tissue edema (star). B (10mg genistein): hyperplasia in glandular epithelium (arrow), connective tissue edema (star) and RBC infiltration (double head arrow). C (10mg genistein): congested blood vessels (arrow). D (50mg genistein): hyperplasia in the lining epithelium (arrow). E (50mg genistein): damaged to the endometrium layer (star) and uterine glands (arrow). (A; B; D and E: 400X; C: 100X).

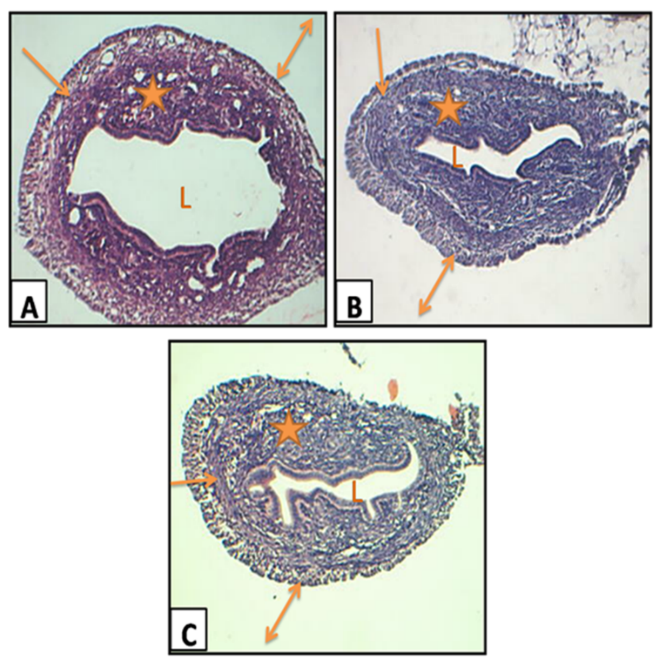

Fig. (3): Photomicrographs of uterine sections tissue of postnatal female albino mice (exp.2) showing: an obvious decrease in the size of the uterine lumen which is associated with increasing the uterine wall thicknesses in the [B: 10mg genistein and C: 50mg genistein] groups compared to the control group (A). Note the uterine lumen become less branches in genistein groups compared to the adult genistein groups (Fig. 4-19). Uterine lumen (L); Endometrium (star); myometrium (arrow); perimetrium (double head arrow). (A; B; and C: 100X).

DOI: http://doi.org/10.24086/biohs2022/paper.593

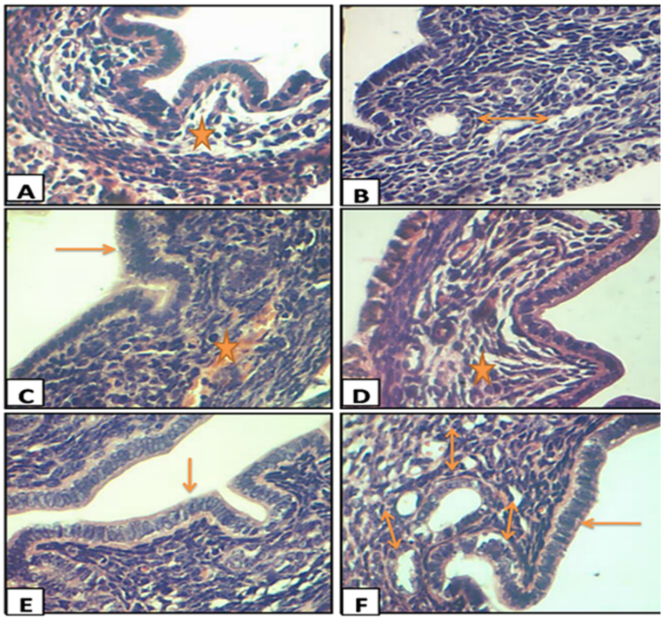

Fig. (4): Photomicrographs of uterine sections tissue of postnatal female albino mice (exp.2) [(A and B: (10mg genistein) (C; D and E: (50mg genistein) (F: control)]: showing: A destruction of endometrial layer (star). B: A decrease in the number of the uterine gland when compared to control (F) (double head arrow). C: hyperplasia in the lining epithelium (arrow) and congested blood vessel (star). D: destruction of endometrial layer (star). E: normal epithelial lining (arrow) compared to the control (F). (A; B; C; D; E and F: 400X).

IV. DISCUSION

In the current study, the treated animal model was an adult and postnatal (P22) female albino mouse. Age at P22 was dependent because this age, as reported by Rasier et al. [27], was a week before the body's natural estrogen levels began to rise and two weeks before puberty began (around P35). Nago et al. [28] and Jefferson et al. [29] show that genistein exposure occurs often during the neonatal era. Genistein was given orally via an oral gavage tube according to Zin et al. [30] to imitate the most prevalent human exposure route.

Growth and development of the male and female reproductive systems are accurately regulated by gonadal steroids. Therefore, these systems are very sensitive to exogenous hormonal stimuli [31]. In 2009, Evanthia et al. [32] reported that the endocrine disruptor was an exogenous chemical or mixture of chemicals. These compounds prevent natural hormones from being synthesized, secreted, transported, bound, or eliminated. As a result, they have a negative impact on hormone-mediated processes such as homeostasis, reproduction, development, and behavior. One of these endocrine disruptors is phytoestrogen genistein, which shows estrogen-like activity. Therefore, in the present study, when postnatal female mice were exposed to the (10 or 50mg) genistein, both concentrations caused a significant decrease in the weight of the uterus compared to the control group. But this result did not agree with Lewis and his colleague [33], who showed that when female pups were exposed to high dose genistein (40gm/kg/day) at day 22, the uterine weight was two times that of the control pups, while a low dose (4 mg genistein) had no effect on the weight of these pups' uterus. In 2010, Cimafranca et al. also showed that neonatal genistein (25mg/ml) exposure caused a 41% increase in uterine weight to body weight ratio. That means mean genistein resulted in hypertrophic effects on the uterus. Singh and Lata in 2014 [31] also reported that the uterine weight showed a significant increase at 8 and 12 weeks of age in genistein (0.5 mg/kg B.W.) exposed groups. Their finding suggested that low concentrations of genistein have an estrogenic effect.

From these results, and as indicated by Bern et al. [35], the uterine tissues are highly responsive to estrogen hormone analogues and function as a biomarker for estrogenic exposure. But the result of the present study was similar to that shown by Zin et al. [30], who reported the reduction in the uterine weight of post-weaning female rats after three weeks of treatment with 10mg genistein, while these animals showed a slight increase in the weight of the uterus after three weeks of treatment with 100mg genistein. As a result of its impact on the hypothalamic-pituitary-gonadal (HPG) axis through modulating hormones and estrogen receptors, the author cited above concluded that post-weaning exposure to genistein could affect the development of the reproductive system of a variant intact experimental rat. The results of the present study showed that when the adult female animal was treated with the 50mg genistein group, it resulted in a considerable increase in the uterus's weight, but the 10mg genistein showed no significant increase in this organ.

Histopathological studies of the effect of genistein in adult and postnatal female mice on the uterus

The results of the present study demonstrated that treatment with genistein (10 and 50 mg) caused some adverse effects on the uterus layers, such as in adult female mice. Both concentrations caused a slight increase in the uterine luminal circumference in addition to the increase in the numbers of uterine glands, and the lumen of the uterus became more branched. Alternatively, both concentrations in postnatal female mice showed an obvious decrease in the uterine luminal circumference, and the decrease in the number of uterine (glands and lumen branches) appeared less than in adult and control female mice. In both adult and postnatal female mice, treatment with genistein caused the thickness of the uterine wall to grow, and hyperplasia in the lining and glandular epithelium caused this rise. But these results are not consistent with other findings such as Zin et al.'s [30]. In that study, postnatal (P22) female rats treated with (10 mg genistein/kg B.W.) showed a decrease in endometrium and myometrium thickness, which led to a decrease in uterine wall thickness. However, the findings of this study are consistent with those of Nago et al. [28], who discovered that treating neonate mice with 100mg/kg/day genistein caused the formation of histological abnormalities in the uterus later in adulthood. Following a high dose of genistein treatment, Diel et al. [36,37] showed that there was an increase in the uterus' weight and thickness, as well as uterine epithelial cell proliferation. Another study showed that treating Arabi

DOI: http://doi.org/10.24086/biohs2022/paper.593

female lambs with different levels of genistein caused a significant increase in the weights of the uterus, right ovary, and ovary duct length [38]. Salleh et al. [21] also discovered that at doses of more than 10 mg/kg/day, the increase in lumen circumference was larger than in the control group. When compared to control, therapy with 50 and 100 mg of genistein/kg/day resulted in a 4.65 and 5.82-fold increase in luminal circumferences, respectively. In their histological investigation of uteri, Helmy et al. [39] discovered that in the high dietary phytoestrogen group, the uterine wall thickens and the uterine lumen becomes more branching. They also discovered that hyperplasia in the uterine lining and glandular epithelium, as well as connective tissue edema and increased blood supply, increased the thickness of the uterine wall. According to all these results, the authors mentioned above suggested that soy phytoestrogens acted in the uterus in a manner similar to that of estradiol. This is because estrogen-responsive genes are induced by binding to the ER and the ligand receptor complex, which leads to an increase in uterine mass.

Generally, when uterine morphology changes, the endometrium's transformation into a receptive condition is delayed or inhibited, resulting in the embryo's failure to implant. [40]. In conclusion, genistein has a detrimental effect on the anatomy of the uterus in adult and post-natal female mice, interfering with its functions.

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