HISTOPATHOLOGICAL EFFECTS OF PHYTOESTROGRN (GENISTEIN) ON THYMUS GLAND OF ADULT AND POST-NATAL FEMALE ALBINO MICE

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Received: 26 Jan., 2021 / Accepted: 03 Apr., 2022 / Published: 18 Apr., 2022 https://doi.org/10.25271/sjouz.2022.10.2.889

ABSTRACT:
This study aimed to evaluate how genistein affected the weight and histological structure of the thymus gland in adult and postnatal (P22) female albino mice. Fifteen adult female albino mice and fifteen postnatal (P22) female albino mice were used and divided between two experiments. The animals were divided into three groups (n=5) in each experiment: Group (I), the control group, Group (II), and Group (III), which received 10mg and 50mg genistein/ kg B. W. respectively. In comparison to the control, treatment with 50mg genistein resulted in a significant increase in the weight of the thymus gland in both adult and postnatal mice. When compared to control, treatment with 10mg genistein resulted in a significant increase in this weight in adult females and a significant decrease in the weight of this gland in postnatal mice. Both genistein concentrations had a negative impact on the gland’s histological features. The formation of a “Starry sky” in cortical and medullary regions, an increase in the thickness of regions due to an increase or decrease in the number of T cells depending on genistein concentration, as well as histocyte hyperplasia and blood vessel congestion, are among these consequences. In conclusion, because genistein affects thymic tissue negatively, it has the potential to create thymic and immunological diseases.

KEYWORDS: Adult female mice, Histopathological changes, Genistein, Post-natal female Mice, Thymus gland.

1. INTRODUCTION

Plant steroids are divided into two categories: isoflavones (genistein and daidzein) and lignans (secoisolariciresinol and matairesinol), (Moutsatsou, 2007; Kim and Park, 2012). Soybean products, which contain high levels of isoflavones, and are structurally comparable to the female sexual hormone estradiol, are the most prevalent sources of phytoestrogens (Sleenisma, 2006). For humans, cattle, and rodents, genistein is the primary source of phytoestrogens (Cederroth and Nef, 2009). Furthermore, soy-based infant formula can be a good source of soy-isoflavones in the early stages of life (Dimdale et al., 2011).

Genistein, like other phytoestrogens, can serve as an agonist or antagonist in the production of estrogen. This occurs when genistein attaches to estrogen receptors, which are specific places on cells called estrogen receptors (ERs). ERs are divided into two types: ER-alpha (α) and ER-beta (β). Many systems and organs include these receptors, including immune system cells, the brain, the pituitary, and reproductive tissues such as the uterus and ovary. They are thought to be the primary mediators of estrogen activity in these organs. Genistein can act as an exogenous ER ligand and compete with estrogen for the binding of ER (Pelletier and El-Alfy, 2000; Taylor and Al-Azzawi, 2000; Patisaal and Adewale, 2009). Thus, genistein stimulates these receptors, though not as effectively as genuine estrogen. At the same time, it prevents estrogen from adhering to the cells. As a result, genistein may partially block estrogen’s effects when there is a lot of it in the body, such as before menopause. Because estrogen appears to increase the risk of certain types of cancer, premenopausal women who take genistein on a regular basis may be able to minimize this risk. On the other side, genistein can partially compensate for a lack of human estrogen, such as after menopause. One of the reasons for utilizing genistein to alleviate menopausal symptoms and prevent osteoporosis is because of this. Genistein may also assist to lower the risk of heart disease (Setchell and Lydeking-Olsen, 2003; Michel et al., 2007). The thymus gland is a central lymphoid organ in the body, however adult mice treated with genistein had their thymus weight and number of thymocytes reduced in a dose-dependent manner (Eick and Thornton, 2011). Thymic atrophy was reported in human soy-fed newborns. Genistein activates NK cells and modulates cytokine synthesis and secretion (Yellayi et al., 2002). The phenolic ring in genistein’s chemical structure is linked to its estrogenic and anti-estrogenic properties. So, Genistein can bind to ERs because of this property (Le- Maire et al., 2010). Genistein has been demonstrated to have a good impact on the prevention of metabolic disturbances linked to cardiovascular disease (CVD), obesity, and diabetes (Yousefi et al., 2017). In the kidneys of ovariectomized rats, genistein is an effective treatment for reducing oxidative stress and inflammation (Javani et al., 2019). The findings also point to genistein’s potential impacts, particularly its antioxidant properties in the face of morphine’s harmful effects. Genistein could be a promising new therapeutic option (Salahshoo et al., 2018).

Phytoestrogens have major effects on sexual development, including delayed pubertal timing, reduced estrous cycle and estradiol, are the most prevalent sources of phytoestrogens (Steensma, 2006). For humans, cattle, and rodents, genistein is one of the reasons for utilizing genistein to minimize this risk. On the other side, genistein can partially block estrogen’s effects when there is a lot of it in the number of T cells depending on genistein concentration, as well as histocyte hyperplasia and blood vessel congestion, are among these consequences. In conclusion, because genistein affects thymic tissue negatively, it has the potential to create thymic and immunological diseases.

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Genistein is typically introduced during the newborn era (Nagao, 2001). Weaning is the process of transitioning from breastfeeding to other nutritional sources in humans. As a result, the chances of being exposed to foods containing genistein are increased during the weaning and post-weaning periods (Jonas, 2005). Gaffler et al., reported in 2018 that at prenatal period the dietary soy isoflavones may have an immunosuppressive effect on cell mediated immunity in male offspring after PHA activation. The goal of this study was to see how genistein affected the weight of the thymus gland, as well as histology and histopathological alterations in the gland.

2. MATERIAL AND METHODS

2.1 Phytoestrogen (Genistein):
Genistein (Sigma) synthetic, ≥ 98% (HPLC), powder with molecular weight of 270.24g/mol was used in this study. In this investigation, two concentrations of genistein (10 and 50 mg genistein / Kg B.W.) were utilized, according to earlier studies such as Setchell et al., (1997) and Li et al., (2012). The low dose was ten mg/kg B.W. genistein (this dose, according to Setchell et al., 1997), is similar to the total quantity of soy phytoestrogens taken daily by children fed soy infant formula, and the high dose was 50 mg/kg B.W. genistein. 

Twee 80 (Merck-Schuchardt, Germany), was used as the vehicle and as negative control (Montani et al., 2008). Twee 80 was diluted in distilled water (1:9 v/v) (Md- Zin et al., 2013). The genistein was dissolved in Twee 80 and given orally once a day for two weeks, as is its usual mode of administration.

2.2 Animals and Experimental Design
Fifteen adult albino female mice (8-10 weeks old) and fifteen postnatal (P22) albino female mice of strain Balb/C Mus musculus were used in this study. These animals were obtained from the Animal Breeding House \ Faculty of Science University of Zakho and maintained under a regime of 12-hour light and 12 hours dark at 22-24 °C. They were given standard diet as pellets and water all time (ad libitum). The experimental work of this study was carried in the Laboratory of Zoology, Department of Biology, Faculty of Science, University of Zakho.

2.2.1 Experiment I: Adult female mice: This experiment includes fifteen adult female albino mice of strain Balb/C (8-10 weeks old), with average weight (18-18.5 gm), and randomly divided into three groups (n=5) for each group, as follow; Group (I): the control group. The adult females of this group were orally received an equivalent volume of the vehicle only (Twee 80). Group (II) orally received 10mg genistein / kg B.W. Group (III): orally received 50mg genistein / kg B.W.

2.2.2 Experiment II: Postnatal (P22) female mice: In this study, fifteen postnatal (P22) albino female mice of the strain Balb/C were used with average weight (4.8-5.2 gm) and then randomly separated into three groups (n=5) as follows: The control group is group (I). This group's postnatal females were simply given an equivalent volume of the vehicle orally (Twee 80). Group (II) got 10mg genistein / kg B.W. orally. Group (III): 50mg genistein / kg B.W. was given orally. Animals were given tap water and a diet free of phytoestrogens. Genistein was administered once daily for two weeks via oral gavage tube in order to mimic the common route of human exposure.

2.3 Dissection and Histological Preparation
After two weeks of treatment, all animals were weighted with an electrical balance, slaughtered by cervical dislocation, dissected, and the thymus gland was removed and weighted with a sensitive electrical balance, then fixed in 10% formalin for 24 hours, dehydrated, cleared, and embedded in paraffin. Then by using a rotary microtome (KEDEE: Korea), 6 μm thick sections were cut from paraffin block. Then stained by Harris haematoxylin and eosin (H and E) for histological studies (Montani et al., 2008; Md-Zin et al., 2013). Twenty-five representative sections were inspected microscopically in both experiments and for each group to determine the histopathological alterations of the thymus gland in and photographed using a digital camera (Sony: China).

2.4 Statistical Analysis
The experiment was set up in a Complete Randomized Design format (CRD). The SAS program was used to analyse the trial's data, and Duncan's multiple range tests were used to compare the means (SAS, 1999).

3. RESULTS

3.1 The effect of genistein on the weight of the adult and post-natal female albino mice thymus gland

In comparison to 10mg genistein and the control group, 50mg genistein caused a highly significant increase in thymus gland weight (P≤0.01). In comparison to the control group, treated with 10 mg genistein resulted in a significant (P≤0.05) rise in this weight. While the post-natal data showed that treatment with 50mg genistein reduced the weight of this gland significantly when compared to the weight in the 10mg genistein and control groups. When compared to the weight of the control group, treatment with 10mg genistein resulted in a significant reduction in the weight of this gland (Table 1).

Table (1): Mean ± S.E for the effect of genistein on the thymus gland weight (grams) of the adult and post-natal female albino mice.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Groups: Thymus gland weight (gram). Mean ± S. E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group(I) Control</td>
</tr>
<tr>
<td>(1) Adult female albino mice</td>
<td>0.023±0.002 a</td>
</tr>
<tr>
<td>(2) Postnatal female albino mice</td>
<td>0.065±0.001 a</td>
</tr>
</tbody>
</table>

The same letters mean no significant differences at (P > 0.05). The different letters mean significant differences at (P≤0.05) or highly significant differences at (P ≤ 0.01) between them according to Duncan's multiple range tests.

3.2 Histological and Histopathological Studies

3.2.1 Histological and histopathological studies of the effect of genistein on the thymus gland of adult female albino mice
The thymus gland in control group of adult female albino mice was surrounded by a connective tissue capsule, and connective tissue partitions extending into the gland from the inner surface of this capsule partially split it into interconnected lobules of varying size and orientation.

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(Fig.1A). As seen in the same figure, the thymus gland was divided into a morphologically distinct dark portion cortex and a white medulla separated by a vascular corticomedullary zone. The cortex was mostly composed of mature lymphocytes (thymocytes: T cells), which accounted for around 90% of thymus cells, with B cells, natural killer cells, epithelial reticular cells, and macrophages (histiocytes) accounting for the remaining 10%. (Figures 1B and C). The medulla portion of the thymus gland had the same structure as the cortex and had epithelial reticular cells, but the medulla had fewer lymphocytes and more macrophages than the cortex. There are also spherical, acidophilic structures (Hassall's corpuscles) in this region (Fig.1D).

Treated adult female albino mice with 10mg genistein caused simple atrophic changes in the cortex (which contain less T cells) and widening of medulla. Therefore, the thickening of the cortex observed was thinner than of the medulla and the usual control (Fig.2A). This concentration also resulted in reverse of follicles that mean mature lymphocyte (T cells) was observed in the center and the faint area was observed at the periphery (Fig. 2B and C), and in dense increased congestion (Fig.2 D). While in the group of 50mg genistein, this concentration resulted in the increase the thickness of the cortex more than medulla (Fig.3 A and B). The cortex layer consists of mature lymphocytes (Fig.3 A and 4A). Figure (3 A; B; C and D) showed the appearance of "Starry sky" in the cortex and medulla which, in turn, consists of an increase number of the histocyte cells (macrophages) (histiocyte hyperplasia) (Fig.3B) This concentration also caused an increase in germinal center (which contain a large number of large cells and few number of small cells) and caused an increase in follicular hyperplasia (Fig.4B; C and D).

Figure (1): Photomicrographs of sections in the thymus of adult female mice (control group) showing: (A) Thymus lobules, these lobules showed the dark cortical (arrow) and the light medullary (star) zones, between them the blood vessels (arrow head). (B) Thymus cortical zone showing dark stained T lymphocytes. (C) Thymus cortical zone showing epithelial reticular cells (arrows) surrounded by dark stained T lymphocytes. (D) Portion of medulla; identified by its lighter staining and a Hassall corpuscle (arrow) (A: 40X; B; C and D: 1000X).

Figure (2): Photomicrographs of a section in the thymus of adult female mice given 10mg genistein showing: (A) the thickness of the cortex was observed less than medulla. (B) The reverse of follicles, that mean mature T-lymphocyte (T cells) (arrow head) was observed in the center and the faint area was observed at the periphery (star). (C) This high magnification of the faint area which containing mature T cells (arrows) and few histiocytes (arrow head). (D) Treated with 10mg genistein caused dense increased congestion of blood vessels. (A: 100X; B and D: 400X; C:1000X), (C: cortex; M: medulla).

Figure (3): Photomicrographs of a section in thymus of adult female mice treated with 50mg genistein showing: (A) an increase of the thickness of the cortex more than medulla. (B) "Starry sky" appearance in the cortex and medulla (star). (C and D high magnifications of (B) the sparse epithelial cells (arrow) are overshadowed by abundant small lymphocytes that normally populate the cortex. (A and B:100X; C: 400X; D: 1000X) (C: cortex; M: medulla).

Figure (4): Photomicrographs of a section in the thymus of adult female mice treated with 50mg genistein showing: (A) Cortical region containing mature lymphocytes (T cells). (B) Medulla region consist of an increase number of the histocyte cells (arrow heads) which
3.2.2 Histological and histopathological studies of the effect of genistein on the thymus gland of postnatal (P22) female albino mice

In the control group of postnatal female albino mice, sections of the thymus gland indicate an infrastructurally well-developed compartment. The cortical and medullary epithelial cells were found to be at their maximum number, largest size and an appropriate shape. Despite this, cells in the cortical and medullary regions differed little. The cortex was found to be thicker than the medulla. T cells in various stages of development were found in the cortical and medullary regions. Due to a decrease in the number of T cells in the cortex region, the cortex was thinned. A dosage of 10 mg of genistein resulted in an increase in the number of T cells, causing the medulla to thicken (Fig.5A). In addition to the ‘Starry sky’ look in both cortical and medullary areas, the germinal center displayed reactive follicles (Fig.5A and B). Small lymphocytes are the most common cell in the cortex. After suffering apoptosis and being phagocytized by macrophages, these cells are removed via negative selection. The phagocytized lymphocytes are seen in “tingible body macrophages” in the thymic cortex (Fig.5C). Figure (5D) showed the existence of two neighbouring more characteristic Hassall’s corpuscles in the thymic medulla and exhibited retarded growth.

When given 50 mg of genistein, it caused the cortex to thicken unevenly and the medulla to thin (Fig.6A and B). Both the cortical and medullary areas resembled a starry sky (Fig.6A; B and C). This concentration also caused an increase congestion of the blood vessels which appeared like a channel of red blood cells (RBCs) (Fig.6D).

4. DISCUSSION

The thymus is the most significant lymphoid organ, and its function was discovered in 1961, according to (Miller, 2020). It's a lobule with a specific structure that runs through connective septa tissue (Krishna, 2020). This gland is found in the anterior superior mediastinum, behind the sternum, and in front of the heart in the upper front region of the chest. It is divided into two lobes, each with a center medulla and an outer cortex, and is encased in a capsule (Standring et al., 2008).

Because of the negative effects of phytoestrogens on the reproductive system as a result of increased use of soymilk and soy-based foods (Nago et al., 2001; Jefferson et al., 2002), the phytoestrogen genistein was utilized in this investigation. Soy milk was chosen for bottle feeding over cow's milk protein, according to Setchell et al., (1998). Genistein is the most abundant soy isoflavone identified in soybeans (Albulescu and Popovici, 2007).

An adult and postnatal (P22) female albino mouse was used as the treated animal model in this investigation. The age at P22 was chosen because, according to Raiser et al., (2006), it was a week before the body’s natural estrogen levels began to rise and two weeks before puberty began (around P35). Nago et al., (2001) and Jefferson et al., (2002) found that genistein exposure occurs often during the neonatal period. According to Md- Zin et al., (2013), genistein was given orally through oral gavage tube to imitate the common method of human exposure.

4.1 The effect of genistein on the weight of the thymus glands in adult and postnatal female albino mice

The thymus gland is vital to immunological function, and estrogens can cause thymic atrophies in neonatal or adult mice (Yellayi et al., 2002). T cells mature and are selected in the thymus gland. Immediately after birth, this gland reaches its full potential in terms of body weight. After reaching its
maximum size in adolescence, this gland undergoes involution, yet it continues to manufacture lymphocytes until old age (Junqueira and Carneiro, 2005). The current study found that treating adult female mice with genistein (10 and 50mg) resulted in a considerable increase in the weight of the thymus gland, with the impact being stronger at 50mg genistein than at 10mg genistein. This finding contradicts that of Yellayi et al., in (2002) who reported that a higher amount of genistein (200mg/kg) lowered thymus weight. They attributed the decrease to the action of genistein, which enhanced the rate of thymocyte cell death (apoptosis) (T cells). In contrast to these findings, both concentrations induced a substantial decrease in the weight of the thymus gland in postnatal female mice as compared to the control group, and the effect at 50mg genistein was greater than the effect at 10mg genistein in reducing the weight of this gland. This finding is consistent with that of Cimafranca et al., (2010), who found that neonatal genistein therapy reduced thymic weight relative to body weight.

4.2 Histopathological studies of the effect of genistein on the thymus gland in both adult and postnatal female mice

The current study found that treatment with 10 and 50 mg genistein generated a variety of alterations in the thymus tissue, including thickening of the cortical and medullary regions, creation of a 'Starry' sky, histiocyte hyperplasia, and increased blood channel congestion. These effects occurred because, as mentioned by Whitten and Patiaul (2001) and Nikitovic et al., (2003), isoflavones, particularly genistein, can bind to estrogen receptor (ERs) and cause numerous cellular alterations. As a result, treatment with genistein caused damage to the thymic tissues and cells. Thymus gland function controls thymocyte (T cell) development and general immunological development, and young animals are especially vulnerable to thymolytic effects of estrogen (Forsberg, 1996 and Yellayi et al., 2002). Others, such as Cimafranca et al., (2010), have corroborated the findings of this study. Their findings showed that exposing mouse pups to total blood concentrations of (25mg genistein/ml), which are comparable to those found in soy fed human newborns, significantly reduces thymic weight. It's unclear if these changes affect neonatal or adult immune function, but these developmental changes could lead to decreased immunological function, immune hypersensitivity, or higher vulnerability to autoimmune disorders in adulthood. In addition, Yellayi et al., (2002) found that genistein-induced declines in thymic weight occurred quickly, and were related with lower thymocyte counts and increased apoptosis. Apoptosis of thymocytes appears to be a key component of the mechanism of genistein's thymic actions. Despite the fact that genistein has an effect on other processes, such as cell proliferation, as shown in the current study, and depending on genistein concentration, this caused an increase in the thickness of the cortex or medulla, which is due to an increase or decrease in the number of T cells in these regions, in addition to histiocyte hyperplasia. In conclusion, because genistein affects thymic tissue negatively, it has the potential to create thymic and immunological diseases.

REFERENCES


