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RESEARCH ARTICLE

EFFECTS OF *IN-UTERO* EXPOSURE TO GENISTEIN ON REPRODUCTIVE PHYSIOLOGY OF ADULT FEMALE LABORATORY MOUSE

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ABSTRACT

Purpose: The purpose of the present study was to check deleterious effects of genistein on the reproduction of albino female mouse *Mus musculus*.
Methods: Pregnant mice were treated with genistein at 0, 0.5 and 10 mg/ kg body weight (BW) by a daily subcutaneous injection from the tenth day of gestation till the dams delivered their pups.
Results: Genistein exposed pups showed accelerated body growth and onset of puberty as compared to control. At the adult stage genistein exposed mice found with altered estrous cyclicity and hormone levels as compared to control mice. Histological observation revealed presence of less number of healthy follicles and some abnormal follicles, like cystic follicle as well as more atretic follicles in ovary and decreased lumen size and less proliferation of uterine glands in the uterus of genistein exposed mice as compared to control mice. During fertility test genistein exposed dams were found with significantly low litter size and hormone levels.
Conclusion: The results of the present study substantiate that developmental exposure to genistein induces abnormal development and affects reproductive physiology of female mice at adult stage.

INTRODUCTION

Exposure to estrogens during critical periods of development has numerous long-term consequences on the reproductive system of males and females of many species including rodents and humans (Bern *et al*., 1992; Colborn *et al*., 1993). The most well known example is the synthetic estrogen, diethylstilbestrol (DES) that has been documented to cause benign and malignant reproductive tract abnormalities in prenatally-exposed males and females of several species. In particular, malformations of the female reproductive tract, alterations in the onset of puberty, alterations in estrous cyclicity, subfertility/infertility and reproductive tract lesions have been reported in experimental animals and humans (McLachlan *et al*., 1982, Newbold *et al*., 1996, Newbold *et al*., 1990). Since numerous chemicals in our environment possess estrogenic activity (Shelby *et al*., 1996, Newbold *et al*., 2001, Jefferson *et al*., 2002), the possibility exists that some of these chemicals may disrupt normal processes of development, differentiation and subsequent function of the reproductive system similar to the adverse effects caused by DES (Colborn *et al*., 1996).

Genistein is a phytochemical that occurs naturally in the diet and is found in a wide variety of plant-derived foods.The structure of genistein resembles that of endogenous estrogens and it has the capability of binding to the estrogen receptor- β, but with a much lower affinity than estradiol. Once bound to the estrogen receptor genistein can increase the expression of estrogen responsive genes (Miksicek, 1995; Hsieh *et al*., 1998; Wang *et al*., 1996). Because of its ability to exert estrogenic activity, genistein and related compounds are referred to as phytoestrogens.

Genistein may act as an estrogen receptor agonist or antagonist. Genistein binds to the estrogen receptor with an affinity of 100 to 1000-fold less than that of estradiol and can compete with estradiol and displace it from its binding sites and this activity can be blocked by the estrogen receptor antagonist tamoxiphen (Wang *et al*., 1996). Thus, it has been demonstrated that genistein and related isoflavones have estrogenic activity mediated though the estrogen receptor and one would expect to see the physiologic effectsof an estrogenic compound, although at a reduced potency as compared to estradiol.

Human fetuses and neonates can be exposed to high levels of genistein if their mothers consume excessive amounts of soy (Foster *et al*., 2002).

The concentrations of genistein and other isoflavones found in soy based supplements far exceeds the amount found in an

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adult diet; one study estimates that infants fed soy-based formulas consume approximately 6-9 mg/kg/day of genistein compared to 1 mg/kg/day for an adult vegetarian (Setchell *et al*., 1997). Soybeans also have extremely variable isoflavone content depending on variety and environmental conditions such as growing season and location (Wang *et al*., 1994) and the USDA reports variable amounts of genistein in various soy products (USDA, 1999).

A recent study has shown that neonatal exposure to genistein at adose of 50 mg/kg/day on days 1-5 leads to an increased incidence of uterine adenocarcinoma in mice later in life; the incidence of uterine tumors in genistein treated mice (35%) was similar to the incidence found in mice treated with an equal estrogenic dose of DES [0.001 mg/kg/day (31%)] (Newbold *et al*., 2001). Although genistein was administered as subcutaneous injections, the levels of genistein used in these studies produced circulating serum levels similar to the range of that found in infants consuming soy-based formulas (Doerge *et al*., 2002). Therefore, the dose of genistein to the target tissue was comparable between subcutaneous injections and oral exposures. Similar findings have also been reported in neonatal rats using a dose of 40 mg/kg/day (Lewis *et al*., 2003). Further studies have shown adverse effects on the developing rat following genistein exposure including brain function, estrous cyclicity and reproductive behaviour (Awoniyi *et al*., 1998; Kouki *et al*., 2003). Studies using other phytoestrogens including coumestrol (Elias and Kincaid, 1984; Whitten and Patisaul, 2001), daidzein (Setchell *et al*., 2001; Kouki *et al*., 2003) and red clover (Morley *et al*., 1966; Kallela *et al*., 1984) have also demonstrated disruptions in reproduction at different reproductive endpoints, supporting the concept that phytoestrogens, although weaker than DES or 17β-estradiol. Phytoestrogens can cause adverse effects on the developing reproductive tract (Jefferson *et al*., 2002). Also, some of these effects may not be apparent until later in life similar to those caused by DES.

In the present study, adverse effects of genistein on development and sexual maturation of albino mouse have been studied.

MATERIALS AND METHODS

Animals and treatment

All the experiments were performed in accordance with institutional practice and within the framework of revised Committee for the Purpose of Control and Supervision of Experiments on Animals; CPCSEA Act of 2007 of Govt. of India on animal welfare. All experimental protocols were approved by Institutional Animal Ethical Committee, Faculty of Science Banaras Hindu University, Varanasi, India (Ref. F.Sc. / IAEC/ 2014-15/ 0334). All surgical processes were performed under mild anaesthesia.

Parke's strain female mice of 3 Months old and 26±3 gm weight, used in experiments were housed under controlled temperature and light 12 L: 12 D with free access to mice feed and water *ad libitum*. Vaginal smears of each mouse were recorded daily and only mice showing at least 2 consecutive 4-day cycles were used in the experiments. Virgin and cycling female mice of same age and weight kept for mating with a healthy male. Mating was confirmed by presence of vaginal plug. The day of appearance of plug was considered as 0 day of pregnancy. Plug positive mice divided into three groups as follows:

1. Control group: treated with vehicle
2. Low dose group : treated with 0.5 mg/Kg BW of genistein
3. High dose group: treated with 10 mg/ Kg BW of genistein

Mice of different groups were treated with vehicle or genistein from 10^{th} day pregnancy up to one day before delivery of pups to check the effect of *in- utero* exposure of genistein on development and reproductive physiology at adult stage.

Body growth and Sexual maturation

Body growth of mice was evaluated by observing rate of body weight gain by pups at different ages i.e. post-natal day (PND) 1, 7, 21, at the time of vaginal opening and at adult stage. Sexual maturation was confirmed by vaginal opening which is considered as sign of onset of puberty in female mice.

At adult stage ovarian weight and uterine weight were measured and histology of these organs was done to check the development of major reproductive organ i.e. ovary and uterus.

Estrous cycle monitoring

At the age of 8 weeks, mice were randomly selected for estrous cyclicity check. The vaginal smear slides were examined for following estrous stages according to cytology of the vaginal smear. After 30 days of observation, diestrus and estus indexes which show frequency of diestrus and estrus during observation respectively were calculated by the following formula:

$$\text{Diestrus Index} = \frac{\text{Number of days with clear diestrus smear}}{\text{Total duration of observation}} \times 100$$

$$\text{Estrus Index} = \frac{\text{Number of days with clear estrus smear}}{\text{Total duration of observation}} \times 100$$

Hormone Assay

To determine the circulating level of progesterone and estradiol, blood was collected by heart puncturing method. Serum was isolated and stored at-80 °C for further analysis. Serum levels of progesterone and estradiol were measured by using respective ELISA kit (Dia Metra, Italy).

Fertility test

Fertility test was done to check the reproductive performance of mice which were exposed to genistein through their mothers (genistein exposed mice). For this test two adult cycling female

mice were kept with a healthy male for mating. After confirmation of mating by presence of vaginal plug female mice were separated from male and were kept individually in cage. After completion of pregnancy period mice delivered pups. Pups were counted to calculate litter size and left with dams (mothers) up to weaning. After weaning at PND 21 on PND 22 dams were sacrificed. Blood of these mice was collected by heart puncture method and serum was separated for the measurement of estradiol and progesterone levels. Serum hormone levels were checked by the procedure described in main materials and methods.

Histology of ovary and uterus

Ovaries and uteruses of 5 animals of each group were taken and fixed in Bouin's fluid, embedded in paraffin, serially sectioned at 5 µm thicknesses and stained with hematoxylin and eosin. Serial sections were observed under light microscope to check number of healthy or atretic follicles, lumen size and proliferation of uterine glands in ovary and uterus respectively.

Statistical Analysis

The results were expressed as Mean ± SE (SEM), analyzed through one-way ANOVA, followed by the post hoc Dunnett's test for comparison of various treatments using the SPSS 16.0. Differences were considered statistically significant at $p<0.05$.

RESULTS

Body growth and sexual maturation

Body growth was found elevated in genistein exposed mice as compared to control mice. The rate of body weight gain was increased significantly ($p< 0.001$) in low dose exposed mice while in high dose exposed mice it was significant ($p< 0.01$) at PND 7 as compared to control mice (Fig- 1 A, B & C). Age of vaginal opening was arrived significantly ($p< 0.01$) earlier in low dose exposed mice but the difference was not significant in high dose exposed mice as compared to control mice (Fig.2 A).Ovarian weight and uterine weight of genistein exposed mice were found decreased when compared with control mice at adult stage. The difference was statistically significant ($p< 0.05$; $p<0.01$) and dose dependent as compared to control mice in case of ovarian weight (Fig-2 B). In case of uterine weight the effect was dose dependent but statistically significant ($p< 0.05$) at high dose exposed mice only as compared to control mice (Fig-2 C).

Estrous cycle monitoring

Estrous cyclicity observation showed less number and abnormal reproductive cycles in genistein exposed mice as compared to control. Diestus index was calculated significantly decreased ($p< 0.001$; $p< 0.05$) but the difference was more significant in low dose exposed mice as compared control (Fig-3 A). Estrus index was found significantly ($p< 0.001$) elevated in low dose exposed mice but significantly ($p< 0.05$) decreased in high dose exposed mice as compared to control (Fig-3 B).

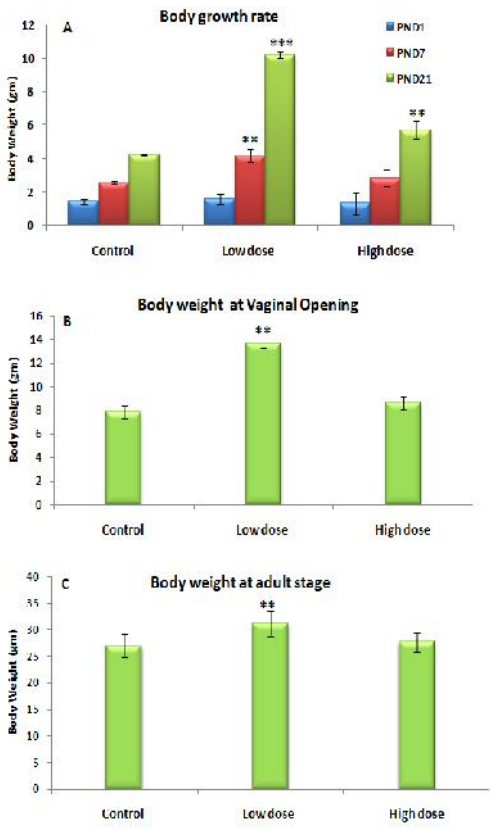

Figure 1

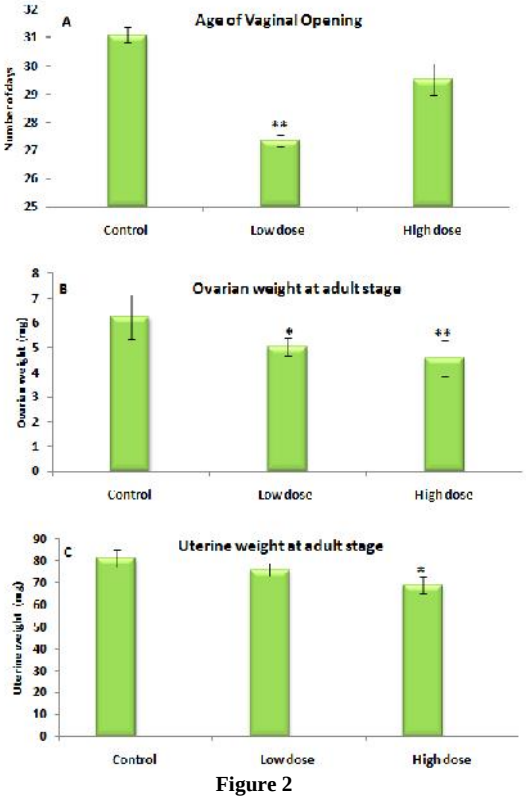

Figure 2

Hormone assay

In genistein exposed mice serum estradiol level was found significantly ($p< 0.01$) elevated as compared to control but the

difference was more significant ($p< 0.001$) in low dose exposed mice.The serum progesterone level was estimated significantly ($p< 0.05$; $p< 0.01$) low in a dose dependent manner as compared to control mice (Fig- 4 A & B).

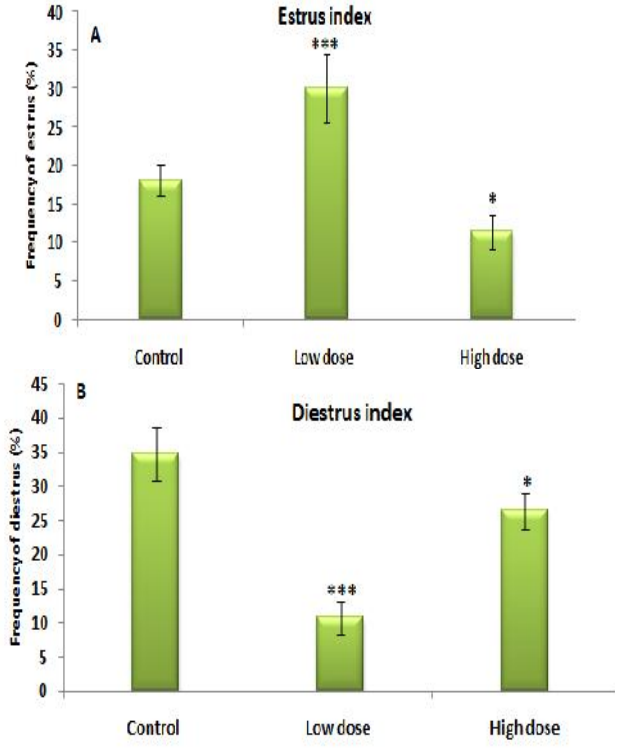

Figure 3

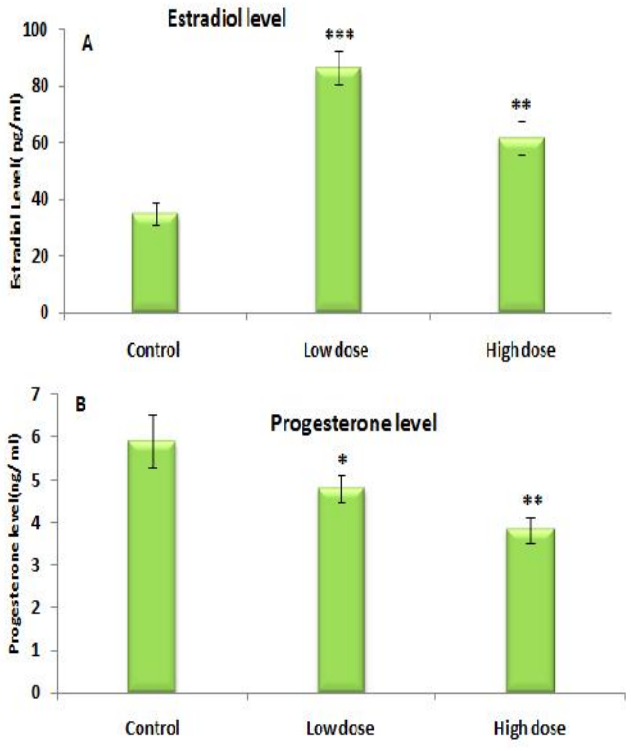

Figure 4

Fertility test

In genistein exposed mice litter size was found significantly ($p< 0.01$) low as compared to control mice (Fig-5 A). The serum levels of estradiol and progesterone of genistein exposed dams were estimated significantly ($p< 0.01$) low in a dose dependent manner as compared to control mice (Fig-5 B &C).

Histology of ovary and uterus

Histological observation revealed less number of healthy follicles and presence of some abnormal follicles, like atretic follicle and cystic follicle and a very few corpus luteum in histological sections of ovaries of genistein exposed mice compared with control mice (Fig-6 A, C & E; 7). The histological sections of uterus showed decreased lumen size and proliferation of endometrial glands in dose dependent manner in genistein exposed mice as compared to control (Fig-6 B, D & F).

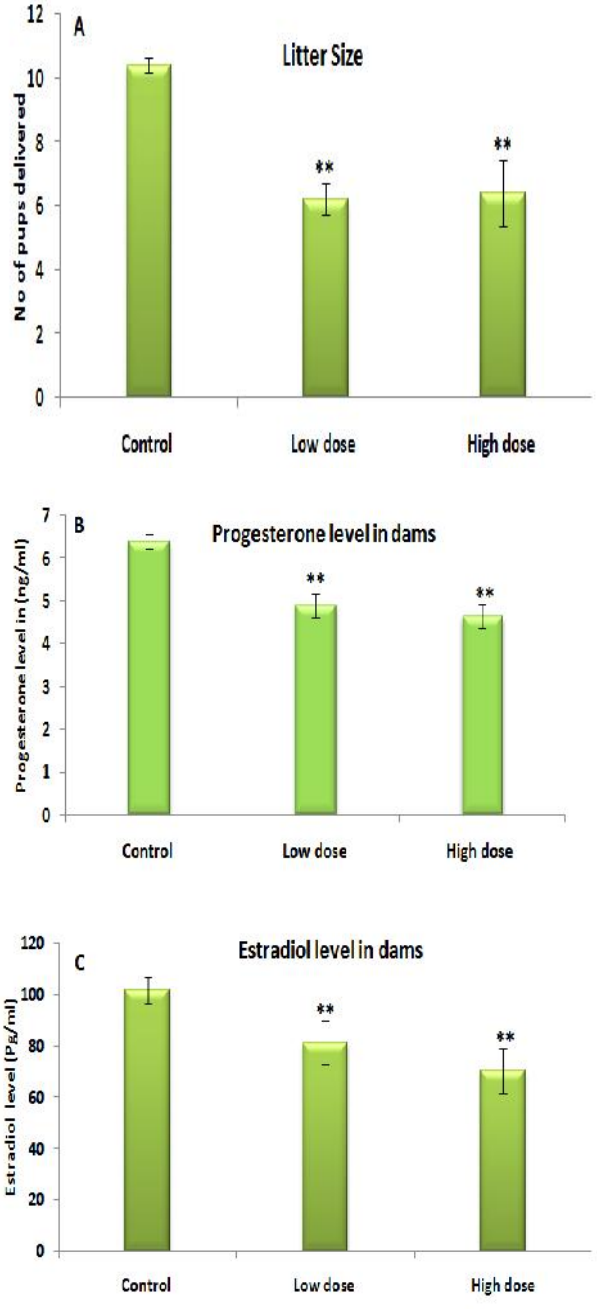

Figure 5

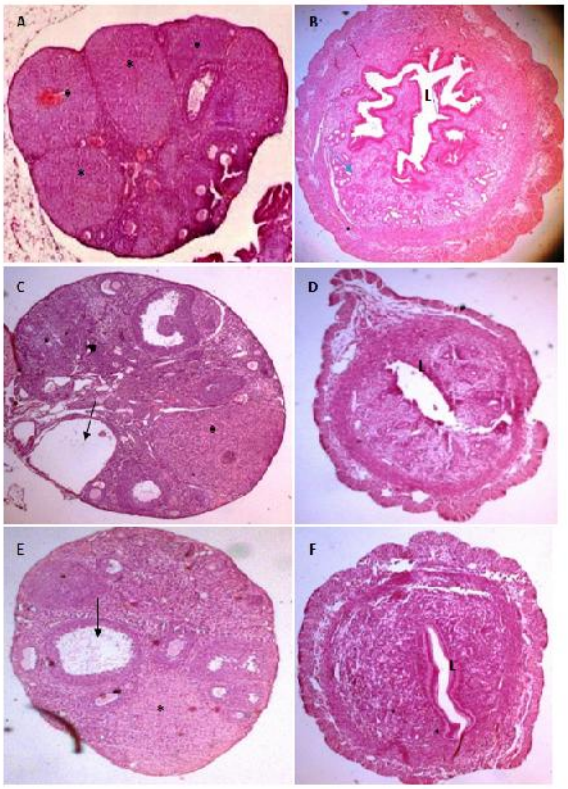

Figure 6

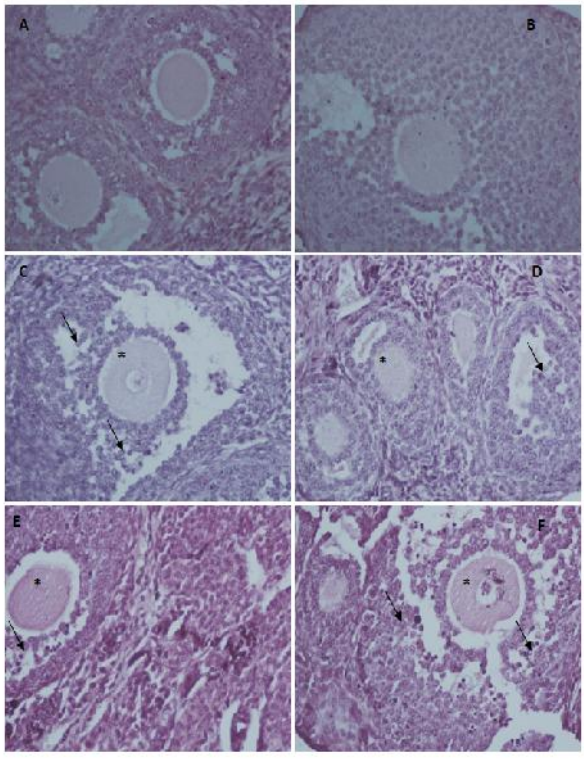

Figure 7

DISCUSSION

The adverse effects of phytoestrogen on fertility and development are well described in many animals (Klein, 1998). Development of reproductive system is precisely regulated by gonadal steroids and therefore is very sensitive to exogenous hormonal stimuli. Changes in the hormonal milieu during gestational period can affect reproductive development and trigger disorganization in later life. Slight alteration of steroid hormone during the prenatal period can accelerate reproductive tract development and growth. These phenomenon suggest that very low dose of estrogenic chemicals relevant to human exposure levels may also influence reproductive organs (Vom Saal *et al*., 1989, 1990).

We investigated whether exposure to genistein during the prenatal period affect female reproduction and sexual maturation. Present study found that *in-utero* exposure of mice to genistein at a low dose relevant to concentration present in normal human diet (0.5 mg/ kg BW) caused altered estrous cyclicity, abnormal development of reproductive organs and accelerated body growth. At high dose, relevant to concentration present in soy based supplement (10 mg/kg BW), similar effects were observed but these were not as intense as low dose level.

A high rate of weight gain in genistein exposed offspring at pubertal and post-pubertal stages of development may be due to high estradiol level as estradiol triggers pathway which enhances GH mediated IGF1 production by liver which enhances cell proliferation (Bourguignon, 1988; Marin *et al*., 1994). IGF1 plays an important role in childhood growth and continues to have anabolic effects in adults.

Ovarian weight is regulated by gonadotropin (Kulin and Reiter, 1973, Tagatz *et al*., 1970) whereas uterine weight is by estradiol (Edman, 1983). Decrease in healthy follicles corresponds to low FSH level (Gore- Langton and Danel, 1990). A significantly high level of estradiol in genistein exposed females may down regulate HPG axis which decreases secretion of gonadotopin (Zoeller *et al*., 1988). The low level of gonadotropin may lead to depressed folliculogenesis.

Genistein exposed females have shown early onset of puberty and persistence of estrus phase. Earlier puberty and consistent estrus might be a consequence of high level of estradiol in genistein exposed mice.

3β-HSD, 17β-HSD and aromatase are very important enzymes of steroidogensis in ovary. 3β-HSD and 17β-HSD convert pregnenolone, derived from cholesterol, into progesterone and androgen respectively. After that androgen is changed into estrogen by aromatase. Dietary genistein, relevant to human consumption enhances aromatase activity in ovarian cells (Ju *et al*., 2008). In an *in-vitro* study it was found that genistein inhibited basal and FSH – stimulated progesterone production, but did not alter estrogen accumulation (Nynca *et al*., 2006) and inhibits activity of 3β-HSD and 17β-HSD in ovarian cells (Whitehead *et al*., 2002). A decrease activity of 3β-HSD and increased activity of aromatase may be responsible for lower level of progesterone and higher level of estradiol in genistein exposed female mice respectively that were found in our results. Lower level of cholesterol in genistein exposed group is also in favour of lower level of progesterone because cholesterol is the precursor for steroid hormone. A low level of progesterone and estradiol in genistein exposed dams may be responsible for small litter size in these dams.

CONCLUSION

The results of present study demonstrate that *in-utero* exposure of genistein accelerates post-natal growth of mouse. It also affects sexual maturity, development of reproductive organs and estrous cyclicity. These findings suggest that high intake of soy based food product during pregnancy may lead to abnormal development which may disturb reproductive physiology of females. Therefore, the present study suggests that there is a need to limit and regulate the dose of genistein in diet of pregnant females to avoid such type of abnormalities in their offspring.

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References

Awoniyi CA, Roberts D, Veeramachaneni DN, Hurst BS, Tucker KE, Schlaff WD (1998). Reproductive sequelae in female rats after in utero and neonatal exposure to the phytoestrogen genistein. Fertil Steril, 70: 440-447.

Bern H, The fragile fetus. In: Colborn, T.; Clement, C., editors (1992). Chemically-induced alterations in sexual and functioal development: the wildlife/human connection. Princeton Scientific Publishing Co: Princeton.

Bourguignon, J.P., 1988. Linear growth as a function of age at onset of puberty and sex steroid dosage: therapeutic implications. Endocr Rev. 9, 467–488.

Colborn T, vom Saal FS, Soto AM (1993). Developmental effects of endocrine-disrupting chemicals in wildlife and humans. Environ Health Perspect, 101: 378–384.

Doerge DR, Twaddle NC, Banks EP, Jefferson WN, Newbold RR (2002). Pharmacokinetic analysis in serum of genistein administered subcutaneously to neonatal mice. Cancer Lett, 184: 21-27.

Foster WG, Chan S, Platt L and Hughes CL, Jr (2002). Detection of phytoestrogens in samples of second trimester human amniotic fluid. Toxicol Lett, 129: 199-205.

Gore-Langton, R.E., Daniel, S.A., 1990. Follicle-stimulating hormone and estradiol regulate antrum-like reorganization of granulosa cells in rat preantral follicle cultures. Biol. Reprod. 43, 65–72.

Hsieh C Y, Santell R C, Haslam S Z and Helferich W G (1998). Estrogenic effects of genistein on the growth of estrogen receptor-positive human breast cancer (MCF-7) cells in vitro and in vivo. Cancer Research, 58: 3833–3838.

Jefferson WN, Padilla-Banks E, Clark G and Newbold RR (2002). Assessing estrogenic activity of phytochemicals using transcriptional activation and immature mouse uterotrophic responses. J Chromatogr B Analyt Technol Biomed Life Sci, 777: 179-189.

Ju YH, Allred C D, Allred K F, Karko K L, Doerge D R and Helferich W G (2001). Physiological concentrations of dietary genistein dose-dependently stimulate growth of estrogen-dependent human breast cancer (MCF-7) tumors implanted in athymic nude mice. Journal of Nutrition, 131(11): 2957–2962.

Kouki T, Kishitake M, Okamoto M, Oosuka I, Takebe M and Yamanouchi K (2003). Effects of neonatal treatment with phytoestrogens, genistein and daidzein, on sex difference in female rat brain function: estrous cycle and lordosis. Horm Behav, 44: 140-145.

Kulin, H.E., Reiter, E.O., 1973. Gonadotrophins during childhood and adolescene. A review. Pediatrics. 51, 260–271.

Lewis RW, Brooks N, Milburn GM, Soames A, Stone S, Hall M and Ashby J (2003). The effects of the phytoestrogen genistein on the postnatal development of the rat. Toxicol Sci, 71: 74-83.

Mc Lachlan J A, Newbold R R, Shah H C, Hogan M D and Dixon R L (1982). Reduced fertility in female mice exposed transplacentally to diethylstilbestrol (DES). Fertil Steril, 38: 364-371.

Miksicek R J, (1995). Estrogenic flavonoids: structural requirements for biological activity. Proceedings of the Society of Experimental Biology and Medicine, 208: 44–50.

Newbold R R and McLachlan J A, (1996). Transplacental hormonal carcinogenesis: diethylstilbestrol as an example. Prog Clin Biol Res, 394: 131-147.

Newbold R R, Banks E P, Bullock B and Jefferson W N (2001). Uterine adenocarcinoma in mice treated neonatally with genistein. Cancer Res, 61: 4325-4328.

Newbold R R, Bullock B C and McLachlan J A (1990). Uterine adenocarcinoma in mice following developmental treatment with estrogens: a model for hormonal carcinogenesis. Cancer Res, 50: 7677-7681.

Newbold R R, Jefferson W N, Padilla-Banks E, Walker V R and Pena D S (2001). Cell response endpoints enhance sensitivity of the immature mouse uterotropic assay. Reprod Toxicol, 15: 245-252.

Nynca, A., Ciereszko, R.E., 2006. Effect of genistein on steroidogenic response of granulosa cell populations from porcine preovulatory follicles. Reprod. Biol. 6, 31–50.

Setchell K D, Zimmer-Nechemias L, Cai J and Heubi J E (1997). Exposure of infants to phyto- oestrogens from soy-based infant formula. Lancet, 350: 23-27.

Shelby M D, Newbold R R, Tully D B, Chae K and Davis V L (1996). Assessing environmental chemicals for estrogenicity using a combination of in vitro and in vivo assays. Environ Health Perspect, 104: 1296-1300.

Tagatz, G.E., Fialkow, P.J., Smith, D., 1970. Hypogonadotropic hypogonadism associated with anosmia in the female. N. Engl. J. Me. 283, 1326–1329.

USDA, (1999).USDA-Iowa State University Database on the Isoflavone Content of Foods. In: United States Department of Agriculture.

Vom Saal F S, Montano M M and Wang M H (1992). Sexual differentiation in mammals, In: Chemically–Induced Alterations in Sexual and Functional Development: The

Wildlife/ Human Connection, pp. 17-83. (Colborn T and Clement C Eds.) Princeton Scientific Pub.

Vom, Saal, F.S., Quadagno, D.M., Even, M.D., Keisler, L.W., Keisler, D.H., Khn, S., 1990. Paradoxical effects of maternal stress on fetal steroids and postnatal reproductive traits in female mice from different intrauterine position. Biol Reprod. 43, 751–61.

Vom, Saal, F.S., 1989. Sexual differentiation in litter-bearing mammals: influence of sex of adjacent fetuses in utero. J. Anim. Sc, 67, 1824–40.

Wang, T T, Sathyamoorthy N and Phang, J M (1996). Molecular effects of genistein on estrogen receptor mediated pathways. Carcinogenesis 17, 271–275.

Zoeller, R.T., Young, W.S., 1988. Changes in cellular levels of messenger ribonuclei acid encoding gonadotropin-releasing hormone in the anterior hypothalamus of female rats during the estrous cycle. Endocrinol. 123, 1688–1689.

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