Iranian Journal of Basic Medical Sciences

ijbms.mums.ac.ir

IJBMS

Baicalein, a flavonoid causes prolonged estrus and suppressed fertility output upon prenatal exposure in female mice

Sridevi Vaadala [1], Naveen Ponneri [1], Venkat Shashank Karnam [2], Ramachandra Reddy Pamuru [1*]

[1] Department of Biochemistry, Yogi Vemana University, Vemanapuram, Kadapa-516003, A.P, India
[2] West High School, Torrance - 90503, California, USA

ARTICLE INFO

Article type:
Short communication

Article history:
Received: Jul 14, 2018
Accepted: Dec 11, 2018

Keywords:
Baicalein
Estrus cycle
Infertility
Mice
Prenatal

ABSTRACT

Objective(s): Baicalein (BC), a phytoestrogen of the flavonoid family shows beneficiary and adverse effects. Effect of BC on reproduction is still not understood. Reproductive toxic effects on female mice were tested in this study.

Materials and Methods: Inseminated Wistar mice were divided into four groups and administered IP with 30, 60, and 90 mg/Kg body weight of BC on gestation daydays 11, 13, 15, and 17, and controls treated with DMSO. They were allowed to deliver pups and offspring were separated gender-wise on day 21. The stages of the estrus cycle and its lengthlengths of three successive cycles were measured from day 38 of young females. The mature female offspring of at 60 days age havewere cohabited with control males and the female reproductive endpoints and body weights of dams were measured.

Results: The BC exposure increased the length of the estrus cycle, especially the metestrus and diestrus phases were prolonged among other phases of the estrus cycle. Recorded significant reduction in the body weights (P<0.05) of prenatally BC exposed dams on 8 and 18 days of gestation. A significant increase in the conception time (P<0.0001), pre (53.42%) and post (8.82%) implantation loss, and resorptions (P=0.055), whereas a significant decrease in the number of implantations and live fetuses (P<0.0001) were found in BC exposed dams in a dose-dependent manner.

Conclusion: Prenatal BC exposure prolonged the estrus cycle due to the augmented length of metestrus and diestrus phase, and is reportingreported for the first time. Female fertility output in mice is affected severely by prenatal BC exposure.

► *Please cite this article as:*
Vaadala S, Ponneri N, Karnam VSh, Pamuru RR. Baicalein, a flavonoid causes prolonged estrus and suppressed fertility output upon prenatal exposure in female mice. Iran J Basic Med Sci 2019; 22:452-459. doi: 10.22038/ijbms.2019.33376.7972

Introduction

Recent studies indicate decreased fertility rates of humans and animals. Reasons for this are change in lifestyle, alcohol consumption, cigarette smoking, environmental estrogen pollution, intake of steroids, and consumption of excess amounts of phytoestrogens. Phytoestrogens, called phytochemicals are secondary metabolites produced in various parts of plants. These are structurally like 17β-estradiol, an estrogen (1). Phytoestrogens bind to the estrogen receptors (ER) and disrupt the endocrine system in mammals and affect development and reproduction in both humans and wildlife (2).

Mechanistically phytoestrogens bind to the target cell cytosolic ER (phytoestrogen-ER complex) and then enter into the nucleus where they bind to specific response element (SRE) (phytoestrogen-SRE complex). Later they bind to the estrogen response element located on DNA and stimulate the expression of genes involved in the agonist/antagonist action of estradiol. This leads to synthesis of special proteins, resulting in the alteration of the estrus cycle, sexual development, and reproduction (3). These phytochemicals are divided into various groups including flavonoids having a beneficiary role in preventing inflammation in the uterus and ovaries (4, 5) and adverse effects on reproduction (6).

Flavonoids are mainly present in flax seeds and act as endocrine disrupters when taken in excess amounts (7). Studies explained that exposure of endocrine disrupters in the neonatal period or in the early stages of fetus alter hormone functions through promoting physiological disorders in the brain and reproductive organs by passing through the placenta (8) and cause persistent anovulation by the exposure of diethylstilbestrol perinatally in mice (9). There is experimental evidence stating that phytoestrogens affect human health (10). In females, exposure to endocrine disruptors alters the estrus cycle length and pubertal timing besides causing uterine fibroids, ectopic pregnancy, and premature birth (11, 12).

Nowadays due to beneficiary effects, consumption of phytoestrogen-rich foods (viz., soy-based foods) has increased worldwide (13, 14). Soy foods are rich in isoflavonoids daidzein and genistein, which act as endocrine disruptors in humans and animals (15). Many pieces of evidence suggest that phytoestrogen exposure causes severe complications and abnormalities in unborn babies and infants (16, 17). Phytoestrogens like quercetin and genistein can cross the placenta and cause abnormalities after exposure during embryonic development (18). A study (19) reported disorders like longer menstrual bleeding in girls consuming soy foods.

**Corresponding author:* Ramachandra Reddy Pamuru. Department of Biochemistry, Yogi Vemana University Vemanapuram, Kadapa – 516 003; A.P., India. Tel: +91-8562-225425; Fax: +91-8562-225419; Email: prcrbio@yogivemanauniversity.ac.in

The phytoestrogen baicalein (BC; 5, 6, 7-trihydroxyflavone), belongs to the flavonoid family found abundantly in roots of Scutellaria baicalensis and Scutellaria lateriflora. It shows beneficiary effects in treating many disorders. In Asian countries, BC is predominantly used as herbal medicine for the treatment of various disorders (20). Especially BC administered to the patients of memory disorders (21) and gastrointestinal dysfunction developed due to usage of ritonavir, a protease inhibitor drug used in AIDS therapy (22). Functionally, BC inhibits the formation of α-synuclein fibrils, aggregation of these fibrils leads to Parkinson's disease (23). Together BC and its metabolite baicalin display anti-dengue viral activity in vitro (24). Anticancer activity of BC was recorded on human ovarian cancer cells (25). Antidepressant effects have also been shown by baicalein in rats (26). It also acts as an inhibitor of CYP2C9 (27), an enzyme of the cytochrome P450 system that metabolizes drugs in the body. BC acts as a positive allosteric modulator of the benzodiazepine site and/or a non-benzodiazepine site of the GABA receptor (28, 29). Daily intake of BC reduces cataracts and age-related macular degeneration in animals (30). BC acts as an antagonist to the estrogen receptor (31). Since structurally BC mimics estradiol, it goes and binds to the estrogen receptors and acts as an antagonist and results in reproductive abnormalities. However, there is a lack of information on the effects of the flavonoid baicalein in reproduction and development in females exposed prenatally. In view of this, the present study aimed to evaluate the reproductive toxicity of BC during prenatal exposure in the female offspring using Wistar mice as a test model.

Materials and Methods

Test chemical

Baicalein (BC), purchased from Sigma Aldrich, USA. BC (5, 6, 7- trihydroxyflavone) is a flavonoid derived from a common class of phytoestrogens, isolated from the roots of S. baicalensis. The molecular weight of BC is 270.241 g/mol, the chemical formula is $C_{15}H_{10}O_5$ and is soluble in 100% dimethyl sulfoxide (DMSO).

Experimental mice

Healthy adult female Wistar mice weighing 29 ± 2 g (35–40 days old) were purchased from Sri Venkateswara Enterprises, Bangalore, India and used as experimental mice. Mice were maintained in a clean polypropylene cage with sterilized paddy husk as the bedding material, in a well-ventilated and air-conditioned room (12 hr:12 hr light:dark cycle) at 25 ± 2 ˚C with a relative humidity of 50 ± 5%. Tap water and sterilized feed (purchased from Sri Venkateswara Enterprises, Bangalore, and Karnataka, India) were provided ad libitum. The mice were acclimatized for a week before experimentation. Experiments were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision on Animal Experiments, Government of India CPCSEA (32). This study was also carried out according to the guidelines for the care and use of laboratory animals (NRC, 1996) and approved by the Institutional Animal Ethical Committee at Yogi Vemana University, Kadapa, India (resolution no: 1841/GO/Re/S/15/CPCSEA dt. 18-11-2015).

Experimental design

Inseminated control females mated with control male mice were identified through the vaginal smear cytology method (33, 34) reviewed by Cooper *et al* (35). Four groups of four inseminated mice each, were maintained in separate cages individually (one pregnant mouse per cage). Mice of group 1 were designated as controls and were treated similar to experimental mice with 100% DMSO (injection volume similar to the highest volume given for experimental mice). Groups 2, 3, and 4 were administered IP with 30, 60, and 90 mg/kg body weight (BW) of BC dissolved in 100% DMSO, respectively on gestation days (GD) 11, 13, 15, and 17, a critical period of reproductive organ development (36) during the experiment. The dosage of BC in this study, was in accordance with previous studies (30–100 mg BC/kg BW) (37, 38). General signs of toxicity, weight gain, and mortality rate were recorded in pregnant females during the gestation period. After the completion of the weaning period males and females of control and BC exposed mice were separated and maintained in distinct cages and fed with normal pellet diet and water ad libitum. A schematic representation for the experimental design is represented in Figure A.1.

Estrus cycle measurement

BC exposed females (F1) were patterned for three successive estrus cycles. The estrus cycle was measured using vaginal cytology described by Allen (33) and Caligioni (34) reviewed by Cooper *et al* (35). Four types of phases were identified in the estrus cycle such as proestrus, estrus, metestrus, and diestrus as described by Long and Evans (39) and Freeman (40). Three successive cycles were measured to know the irregularities in the estrus cycle. Estrus cycle length of normal female mice is about 4 days (41). In the vagina, nucleated epithelial cells appear at proestrus, cornified cells at estrus, both cornified and epithelial along with leukocytes at metestrus, and only leukocytes at diestrus.

Vaginal smear cytology was performed early in the morning at 6:00 am or in the evening at 6:00 pm by following Zarrow et al (42) and reviewed by Cooper *et al* (35). Vaginal fluid was pipetted out using a Pasteur pipette by injecting 50 to 60 µl of saline (0.9% NaCl) into the vagina of females on a clean glass slide. A thin smear was prepared and the cells were allowed to settle down. The slides were observed under a phase contrast microscope (BX 43, Olympus, Japan) immediately before drying the slide. Cells of distinct types of separate phases of the estrus cycle were observed and recorded in control and experimental mice individually for three consecutive cycles.

Reproductive parameters

Fertility studies

The reproductive performance of exposed female mice was determined by considering the reproductive endpoints such as conception time, mating index, fertility index, number of live and dead fetuses, resorptions, and number of pre- and post-implantations per mice. For this, six matured exposed female offspring from each group were cohabited with the control males and control females vs control males (2 females: 1 male). Inseminated F1 females were moved into separate cages

and housed individually from the day 1 of gestation. Signs of toxicity and mortality were recorded in each group.

The conception time is the interval between the first day of cohabitation and the day vaginal plug and/or sperm in vaginal smear was observed.

$$\text{Mating Index (\%)} = \frac{\text{No. of sperm positive females}}{\text{No. of pairing}} \times 100$$

Fertility index was determined by counting the pregnant mice from each experimental and control group using the following formula:

$$\text{Fertility Index (\%)} = \frac{\text{No. of pregnant females}}{\text{No. of sperm positive females}} \times 100$$

Autopsy and reproductive performance of F_1 females

On days 8 and 18 of gestation three F1 females from each group (control and experimental) were weighed and sacrificed by cervical dislocation. Mice were laparotomized and the uterus taken out and immediately number of live and dead fetuses, implantations (pre- and post-implantations), resorptions and number of corpora lutea on both gestation days were counted. The number of live pups was counted only on GD 18.

Corpora lutea were counted by taking the uterus of female mice. The edges of uterus consists of ovaries. Ovaries were removed and then squeezed gently in saline (0.9% NaCl) in which corpora lutea releases out from the ovaries, and then they were counted with the naked eye and the number of ovulated eggs determined. Using implantations, pre- and post-implantation loss was calculated. Resorption index was calculated using number of resorptions. The implantations, implantation loss, resorption, and resorption index were calculated by using following formulas individually for each group.

$$\text{Implantation (\%)} = \frac{\text{No. of corpora lutea} - \text{No. of resorption sites}}{\text{No. of corpora lutea}} \times 100$$

$$\text{Pre-implantation loss} = \frac{\text{No. of corpora lutea} - \text{No. of implantations}}{\text{No. of corpora lutea}} \times 100$$

$$\text{Post-implantation loss} = \frac{\text{No. of implantations} - \text{No. of live fetuses}}{\text{No. of implantations}} \times 100$$

$$\text{Resorption (\%)} = \frac{\text{Total no. of resorption sites}}{\text{Total no. of corpora lutea}} \times 100$$

$$\text{Resorption index} = \frac{\text{Total no. of resorption sites}}{\text{Total no. of implantation sites}} \times 100$$

Statistical analysis

The data were statistically represented with two-tailed ANOVA with Bonferroni post-test to compare replicate means by row using the statistical software GraphPad Prism (ver. 5.0.3.477). Results were expressed as mean ± SEM. $P<0.05$ was considered statistically significant.

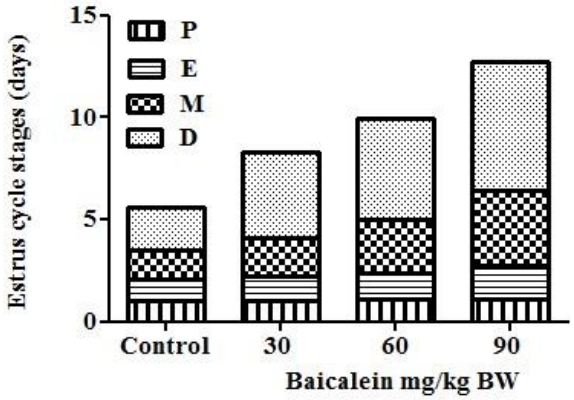

Figure 1. Prenatal exposure effect of baicalein on proestrus (P), estrus (E), metestrus (M) and diestrus (D) of estrus cycle in mice.
The M and D phases were prolonged in 30, 60 and 90 mg/kg body weight (BW) baicalein exposure over the control mice. The number of mice used from each group is six

Results

No clinical signs of toxicity were observed in pregnant mice and offspring exposed to BC during experimentation and none of the mice were excluded from the study. No offspring showed apparent overt signs during the postnatal period.

Developmental landmarks of F1 young females

Significant reduction in the weights of the pups, number of live pups, survival index of pups on postnatal day 4 and 21, and also early vaginal opening and puberty were recorded in F1 mice exposed prenatally to 30, 60, and 90 mg BC when compared to the control pups. Prenatal BC exposure decreased body weights in F1 young mice on postnatal days 1, 7, 14, and 21 reported in our earlier report (43).

Estrus cycle measurement

The mean length of the estrus cycle in control offspring (F1) was 5.29±0.14 days and 7.84±0.28, 9.4± 0.36, and 12.08±0.41 days, respectively in mice exposed prenatally to 30, 60, and 90 mg BC. Significant increase in estrus cycle length (F=103.5; $P<0.0001$) was recorded in BC exposed offspring compared with corresponding controls (Table 1). Further prolonged estrus cycle length with increased metestrus and diestrus phases were shown in BC exposed offspring compared with controls (Figures 1 and A.2).

Body weight F1 dams

The mean body weights of control dams (pregnant mice) was 31.8±0.54 g on day 8, 46.31±0.75 g on day 18; and 29.66±0.45, 28.93±0.38 and 27.53±0.54 g on the 8th day, 42.43±0.6, 39.88±0.33 and 37.37±1.07 g on the 18th day in F1 dams exposed prenatally to 30, 60 and 90 mg BC, respectively. Compared to controls, the body weights of BC exposed dams showed a significant decrease on days 8 (F=12.01; $P=0.0017$) and 18 (F=24; $P=0.0001$). Conversely, mice exposed prenatally to BC at 30 and 60 mg showed no significant difference in body weights on day 8 (Table 2).

Reproductive function studies of female offspring

The reproductive function assessment parameters

Table 1. Prenatal exposure effect of baicalein on the length of the estrus cycle in female mice

S. No.	Group	Estrus cycle length
1	Control	5.289 ± 0.13
2	Baicalein 30 mg/kg body weight	7.842 ± 0.27 (-48.25) *P*<0.0001
3	Baicalein 60 mg/kg body weight	9.394 ± 0.35 (-77.61) *P*<0.0001
4	Baicalein 90 mg/kg body weight	12.078 ± 0.40 (-128.35) *P*<0.0001
	F value	F=103.5 *P*<0.0001

Values are mean±SEM of six individuals. Values are cumulative of three successive cycles. BW: body weight. Values in the parentheses are percent change from control. *P*-value at <0.05 is considered significant

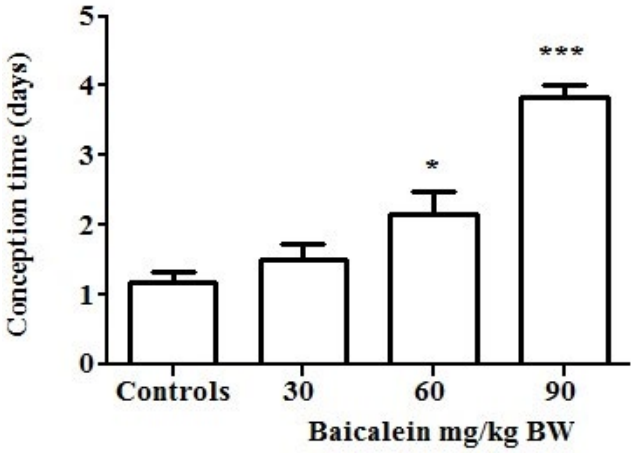

Figure 2. Effect of baicalein on the conception time of baicalein exposed females with control males.
Baicalein at 60 and 90 mg/kg body weight (BW) exposure significantly prolonged conception time compared to control mice. Conversely, no significant difference was recorded between 30 mg/kg BW exposed mice and controls. *, and *** represent significantly different from controls at *P*<0.05 and *P*<0.001, respectively. The number of mice used from each group is six

such as conception time, mating index, fertility index, number of corpora lutea, implantations, live fetuses, resorptions per female offspring and pre- and post-implantation losses were studied (Table 3 and Figure 2). A significant increase (F=30.4; *P*<0.0001) in the mean conception time was recorded in 60 and 90 mg BC treated female offspring compared to controls (Figure 2). Although the mating index was 100% in all control 30 and 60 mg BC exposed females, it was 5 of 6 females in 90 mg BC exposed females, whereas fertility index was 100% only in 30 mg BC exposed females and 83.3% and 66.6% in 60 and 90 mg BC exposed females compared to controls. Control pregnant females mated with control males showed a mean number of 13.33±0.21 corpora lutea and 11.67± 0.33 implantations. Significant and dose dependent decrease in corpora lutea (F=4.39; *P*=0.021), number of implantations (F=87.78; *P*<0.0001), and number of live fetuses (F=90.7; *P*<0.0001) were observed in BC exposed F1 females compared to controls. Conversely, the mean number of corpora lutea was not significantly different in 30 mg BC exposed F1 females compared to controls. Pre-implantation loss in controls was 12.5%, compared with 18.42, 35.13, and 53.42%, respectively in mice exposed to 30, 60, and 90 mg BC prenatally (Table 3 and Figure 3). Significant dose-dependent increase (F=3.18: *P*=0.054) in the number of resorptions was observed in female mice

Table 2. Prenatal exposure effect of baicalein on body weights (g) in female mice

Body weight (g)	Control	Baicalein 30 mg/kg body weight	Baicalein 60 mg/kg body weight	Baicalein 90 mg/kg body weight	F- value
On 8th day	31.80±0.54	29.66±0.45 (-6.73) *P*=0.078	28.93±0.38 (-9.01) *P*=0.054	27.53±0.54 (-13.44) *P*=0.019	F=12.01 *P*=0.0017
On18th day	46.31±0.75	42.43±0.60 (-8.36) *P*=0.0319	39.88±0.33 (-13.88) *P*=0.0024	37.37±1.07 (-19.29) *P*=0.0070	F=24.00 *P*=0.0001

Values are mean±SEM of three individuals. BW: body weight. Values in the parentheses are percent change from control. *P*-value at <0.05 is considered significant

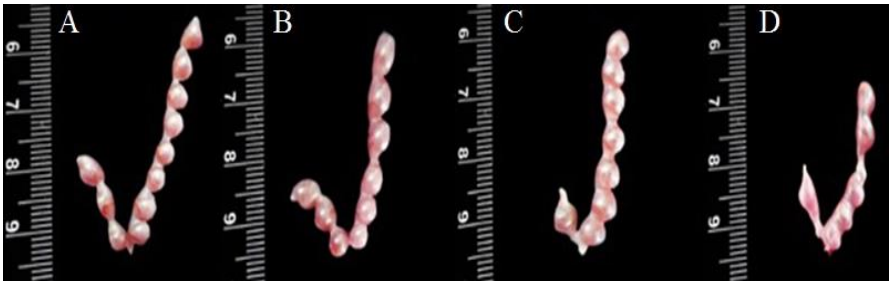

Figure 3. Uterus showing pre-implantations at day eight of control (A; 11.67±0.33), 30 (B; 10.33±0.21), 60 (C; 8.0±0.25), and 90 (D; 5.66±0.33) mg/kg body weight baicalein exposed prenatally mated with control males
Significant reduction in the implantations was shown in prenatally baicalein exposed female mice. The number of mice used from each group is three

Table 3. Prenatal exposure effect of baicalein on reproductive performance in female mice

Parameters	Control	BC 30 mg/Kg body weight	BC 60mg/Kg body weight	BC 90mg/Kg body weight	F-value
Mating index (%)	100(6/6)	100(6/6)	100(6/6)	83(5/6)	
Fertility index (%)	100%	100%	83.3%	66.6%	
No. of corpora lutea/mice #	13.33± 0.21	12.67 ± 0.21 (-5) *P*=0.025	12.33 ± 0.21 (-7.5) *P*=0.040	12.17 ± 0.40 (-8.75) *P*=0.0127	*P*=0.0209 F=4.389
Implantation %	100	94.65	93.16	86.24	
No. of implantations/mice	11.67±0.33	10.33± 0.21 (-12.67) *P*=0.042	8.00± 0.25 (-32.39) *P*=0.0005	5.66± 0.33 (-52.11) *P*<0.0001	*P*<0.0001 F=87.78
Pre-implantation loss # (%)	12.5	18.42	35.13	53.42	
No. of live fetuses/mice #	11.83± 0.307	10.50± 0.22 (-11.26) *P*=0.019	7.83± 0.30 (-33.80) *P*=0.0006	5.16± 0.307 (-56.33) *P*<0.0001	*P*<0.0001 F=90.70
Resorption % #	0	5.26	6.75	13.69	
No. of resorptions/ mice#	0	0.66±0.44 *P*=0.174	0.833±0.31 *P*=0.042	1.66±0.49 *P*=0.019	*P*=0.0546 F=3.182
Resorptions index (%)	0	6.66	10.25	28.25	
Post-implantation loss # (%)	0	1.61	2.08	8.82	

Values are mean± SEM of six individuals; # n=3. Values in the parentheses are percent changes from that of control. Significance was considered at *P*<0.05

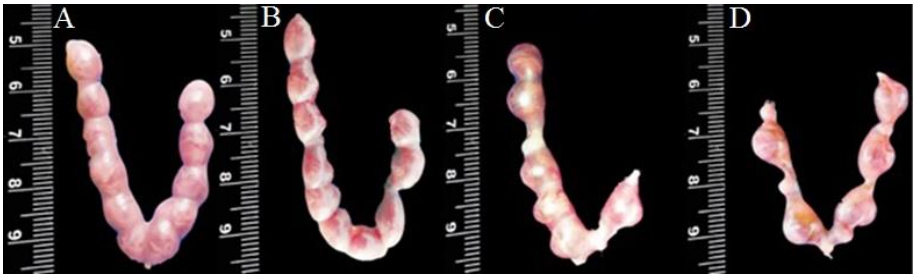

Figure 4. Uterus showing (post) implantations at day 18 of control (A), 30 (B), 60 (C), and 90 (D) mg/kg body weight (BW) baicalein exposed prenatally mated with control males
Significant reduction in the implantations was shown in prenatally baicalein exposed female mice. The number of mice used from each group is three

exposed prenatally to BC compared with controls. On the other hand, the number of resorptions in 30 mg BC exposed mice is not significantly different from controls. The resorption index decreased in a dose-dependent manner (6.66, 10.25, and 28.25% for 30, 60, and 90 mg BC exposure, respectively) in exposed female mice compared to controls. Zero post-implantation loss was observed in controls, whereas post-implantation loss was 1.61, 2.08, and 8.82% in mice exposed to 30, 60, and 90 mg/kg BW pre- and postnatally to BC respectively (Table 3 and Figure 4).

Discussion

No clinical signs of toxicity were observed due to the effect of BC at any dose level on dams and offspring; no abnormal behavior was recorded in BC exposed mice. In the present study, BC exposed offspring showed a significant increase (*P*<0.0001) in the estrus cycle length due to prolonged metestrus and diestrus phases. Like the present study, Nikaido *et al.* (44) reported prolonged estrus cycle with increased diestrus phase in mice exposed to genistein, resveratrol and bisphenol A or diethylstilbesterol. The same study also reported longer estrus cycle with prolonged estrus phase by zearalenone exposure. Menstrual cycle length is increased in pre-menopausal women by the intake of isoflavones (45). The exposure of genistein causes early vaginal opening and increased duration of estrus cycle length in female Sprague-Dawley rats (11). Jefferson *et al.* (46) stated alterations in the estrus cycle and a decrease in fertility of female mice exposed neonatally to genistein. In rats, perinatal exposure of genistein along with methoxychlor and diisononyl phthalate caused irregular estrus cycles (47). Perinatal exposure of bisphenol A causes irregular estrus cycles in rats (48). Similarly, gestation exposure of flaxseed meal altered length of the estrus cycle in rats (49). Previously we reported that prenatal BC exposure caused developmental abnormalities in F1 mice and reported significant decrease (*P*<0.05) in the number of live pups, survival index of pups on postnatal days 4 and 21, along with early vaginal opening and puberty in F1 female young ones exposed prenatally to BC (43). In continuation of our previous report, the present study was conducted. From the results of the present study,

the prenatal exposure to BC increases the estrus cycle length with prolonged metestrus and diestrus phases indicating endocrine disruption thereby hormonal imbalance in female mice. This is the first report showing increased metestrus and diestrus phases in the estrus cycle in mice.

Prenatal BC exposure decreased body weights in F1 young mice on postnatal days 1, 7, 14, and 21 reported in our earlier report (43). A similar trend is observed in F1 adult females exposed prenatally to BC in the present study. Weight was decreased in the treated mice compared to those of controls in a dose-dependent manner. Similarly, genistein 20 mg/kg/day exposed rats showed a decrease in the body weight with increased death rate of pups (50). The decrease in body weights of exposed mice were also linked with stress caused by the chemical. Increased production of corticosteroid a stress hormone is an indication for stress condition. Corticoid hormones are regulated by the hypothalamic-pituitary-adrenocortical (HPA) axis, which were activated in response to stress. It is reported that prolonged oxidative stress in pregnant women is responsible for reduced birth weight in full-term deliveries (51). Though we have not measured the levels of stress hormones in the present study, we presume that the oxidative damage and stress caused by BC exposure reduced body weight in female mice.

Exposure to a range of endocrine disruptors during embryonic development causes adverse effects on the developing embryo. Especially exposure of these chemicals during the critical period of reproductive organs development (12 to 19 days of gestation in rats and mice) may alter the reproductive performance and other related activities of exposed mice at their adulthood (36). Alterations in reproductive performance and other related activities of female mice prenatally exposed to BC were identified in the present study. Conception time was increased, and the mating index was decreased in BC exposed females when compared with controls. We reported the same result with exposure of biochanin–A in female rats (52). And same reports observed that in rats and mice exposure to Proluton-Depot causes an increase in the conception time (53) and also observed a delay in the conception time in the married women of New Haven in the US due to caffeine-containing products (54). Effects of phytoestrogens also depend on the quantity ingested, at a lower concentration it does not cause any harm but when the concentration exceeds beyond the limit it mainly alters the reproductive physiology (55). It is supported by prolonged estrus cycle in women who were exposed to 45 mg genistein a day due to delay in luteinizing hormone surge (56). Similarly, consuming excessive amounts of soy food caused prolonged estrus cycle length in Japanese and Western Countrywomen (57). It is clear from the above reports and the present study that excessive amounts of phytoestrogens (BC) alter reproduction in mice. Moreover, exposure of offspring at the critical period of gestation disrupts regular development of reproductive tissues and organs, which continues in adulthood.

In the present study, a decrease in the number of live fetuses and pre- and post-implantation loss in prenatally BC exposed female mice was observed. Jefferson *et al.* (58) also observed numbers of live pups were decreased in adulthood of CD-1 mice by exposure to phytoestrogen genistein. A similar line of studies was performed with Biochanin-A in rats in this laboratory and a decrease in the number of live fetuses and loss of pre- and post-implantations was found (52). On the other hand, a decrease in the number of implantations and increases in resorptions were recorded in prenatally BC exposed females. Comparable results were reported in rats exposed to the isoflavones daidzin + daidzein, daidzein, genistin + genistein, and genistein at a concentration of 10 mg and 100 mg/kg BW each (59). Zearalenone exposure caused infertility in female heifers by increasing the resorption rate, which leads to early abortion (60). A study (61) reported that the inhibition of implantations and blockage of pregnancy by aflatoxin B1 exposure during pregnancy in female rats is primarily due to infiltration of leucocytes into the uterus, indicating inferior quality gametes production due to endocrine disruption by the exposure of such chemicals. Exposure of BC at the fetal stage and lactation might alter the regular process of reproductive system development by misbalancing the sexual hormones, which may lead to reduced number of female gametes and thereby decreased fertility.

Conclusion

The environmental phytochemicals primarily act on the steroidal signaling pathway and alter the hormones involved in the reproductive system of females. Since environmental phytoestrogens reach the fetus via placenta and young ones through milk, excess exposure of these chemicals during pregnancy, lactation, and at the time of puberty (9–14 years age of a female child in case of humans) may restrict its adverse effects on the offspring. We report that the maternal exposure of flavonoid BC prolongs the reproductive cycle and reduces fertility output in female mice. This study demonstrated the exposure of excess concentrations of environmental phytoestrogens at early life and its prolonged effects on adult life, which should not be overlooked. However, further establishing painstaking studies are needed and are undergoing in this laboratory.

Conflicts of Interest

The authors declare no competing financial interest.

Acknowledgment

Authors are highly thankful to Prof P Sreenivasula Reddy, Department of Zoology, Sri Venkateswara University, Tirupati, AP, India for guidance and encouragement in completing the study. Authors are thankful to Dr MGS Sowjanya for helping in experimentation. V Sridevi acknowledges Department of Science and Technology, New Delhi, India for awarding the INSPIRE Fellowship (code: IF131094). The results presented in this paper were part of Ms V Sridevi PhD thesis. This work was supported by the Department of Science and Technology, New Delhi, India in the form of DST-Inspire Research Fellowship to Ms V Sridevi [grant no DST/INSPIRE Fellowship/2013/1052].

References

1. Kanno S, Hirano S, Kayama F. Effects of phytoestrogens

and environmental estrogens on osteoblastic differentiation in MC3T3-E1 cells. Toxicology 2004; 196:137-145.
2. Gutendorf B, Westendorf J. Comparison of an array of *In vitro* assays for the assessment of the estrogenic potential of natural and synthetic estrogens, phytoestrogens and xenoestrogens. Toxicology 2001; 166:79-89.
3. Socorro RM, Horacio H, Jose Aifredo F, Minerva Munoz G, Gerardo D, Jesus V, *et al*. Effects of phytoestrogen on mammalian reproductive physiology. Tropical Subtrop Agroecosyst 2012; 15:129-145.
4. Chia JH, Kenton H, Tuanzhu H, Chuanfu L, Krishnaswam G, David SC. Baialein inhibits IL-1β- and TNF-α-induced inflammatory cytokine production from human mast cells via regulation of the NF- κB pathway. Clin Mol Allergy 2007; 5:1-10.
5. Lin CC, Shieh DE. The anti-inflammatory activity of Scutelleria rivularis extracts and its active components, baicalin, baicalein and wogonin. Am J Clin Med 1996; 24:31-36.
6. Aravinda kshan M, ChaUhan PS, Sundaram K. Studies on germinal effects of quercetin, a naturally occurinf flavonoid. Mutat Res 1985; 144:99-106.
7. Hodek P, Trefil P, Stiborova M. Flavonoids-potent and versatile biologically active compounds interacting with cytochromes P450. Chem-Biol Interac 2002; 139:1-21.
8. Todaka E, Sakurai K, Fukata H, Miyagawa H, Uzuki M, Omori M, *et al*. Fetal exposure to phytoestrogens - the difference in phytoestrogen status between mother and foetus. Environ Res 2005; 99:195-203.
9. Nakamura T, Katsu Y, Watanabe H, Iguchi HT. Estrogen receptor subtypes selectively mediate female mouse reproductive abnormalities induced by neonatal exposure to estrogenic chemicals. Toxicology 2011; 253:117-124.
10. Patisaul HB, Jefferson W. The pros and cons of phytoestrogens. Front Neuroendocrinol 2010; 30:400-419.
11. Delclos KB, Weis CC, Bucci TJ, Olson G, Mellick P, Sadovova N, *et al*. Overlapping but distinct effects of genistein and ethinyl estradiol (EE 2) in female sprague- dawley rats in multigenerational reproductive and chronic toxicity studies. Reprod Toxicol 2009; 27:117-132.
12. Fan W, Yanase T, Morinaga H, Gondo S, Okabe T, Nomura M, *et al.* Atrazine-induced aromatase expression is SF-1 dependent: implications for endocrine disruption in wildlife and reproductive cancers in humans. Environ Health Perspect 2007; 115:720-727.
13. Alexander VS, Halim harrath A. Phytoestrogens and their effects. Eur J Pharmacol 2014; 741:230-236.
14. Chen MN, Lin CC, Liu CF. Efficacy of phytoestrogens for menopausal symptoms: a meta- analysis and systematic review. Climacteric 2015; 18:260-269.
15. Patisaul HB, Todd KL, Mickens JA, Adewale HB. Impact of neonatal exposure to the ERα agonist PPT, Bisphenol-A or phytoestrogens on hypothalamic kisspeptin fiber density in maleand female rats. Neurotoxicol 2009; 30:350-357.
16. Cao Y, Calafat AM, Doerge DR, Umbach DM, Bernbaum JC, Twaddle NC, *et al*. Isoflavones in urine, saliva, and blood of infants: data from a pilot study on the estrogenic activity of soy formula. J Expo Sci Environ Epidemiol 2009; 19:223-234.
17. Rozman KK, Bhatia J, Calafat AM, Chambers C, Culty M, Etzel RA, *et al*. NTP-CERHR expert panel report on the reproductive and developmental toxicity of genistein. Birth Defects Res B Dev Reprod Toxicol 2006; 77:485-638.
18. Vanhees K, Godschalk RW, Sanders A, Barjestehvan S, van Schooten FJ. Maternal quercetin intake during pregnancy results in an adapted iron homeostasis at adulthood. Toxicology 2011; 290:350-358.
19. Strom BL, Schinnar R, Ziegler EE, Barnhart KT, Sammel MD, Macones GA, *et al*. Exposure to soy-based formula in infancy and endocrinological and reproductive outcomes in young adulthood. JAMA 2001; 286:807-814.
20. Wang CZ, Zhang CF, Chen L, Anderson S, Lu F, Yuan CS. Colon cancer chemopreventive effects of baicalein, an active enteric microbiome metabolite from baicalin. Int J Oncol 2015; 47:1749-1758.
21. Wang W, Wang F, Yang YJ, Li Hu Z, Hong Long L, Fu H, *et al*. Flavonoid baicalein promotes NMDA receptor-dependent long-term potentiation and enhances memory. Br J Pharmacol 2011; 162:1364-1379.
22. Mehendale S, Aung H, Wang CZ, Tong R, Foo A, Xie JT, *et al*. *Scutellaria baicalensis* and a constituent flavonoid, baicalein, attenuate ritonavir-induced gastrointestinal side-effects. J Pharm Pharmacol 2007; 59:1567-1572.
23. Zhu M, Rajamani S, Kaylor J, Han S, Feimeng Zhou, Anthony Fink L. The flavonoid baicalein inhibits fibrillation of α-synuclein and disaggregates existing fibrils. J Biol Chem 2004; 279:26846-26857.
24. Moghaddam E, Teong BT, Sin Sam S, Lani R, Hassandarvish P, Chik Z, *et al*. Baicalin, a metabolite of baicalein with antiviral activity against dengue virus. Sci Rep 2014; 4:5452-5460.
25. Pan Q, Xue M, Xiao SS, Wan YJ, Xu DB. A combination therapy with baicalein and taxol promotes mitochondria-mediated cell apoptosis: involving in Akt/β-catenin signaling pathway. DNA Cell Biol 2016; 35:646-656.
26. Xiong Z, Jiang B, Wu PF, Tian J, Shi LL, *et al*. Antidepressant effects of a plant-derived flavonoid baicalein involving extracellular signal-regulated kinases cascade. Biol Pharm Bull 2011; 34:253-259.
27. Dayong S, Wang Y, Zhou YH, Guo Y, Wang J, Zhou H, *et al*. Mechanism of CYP2C9 inhibition by flavones and flavonols. Drug Metab Dispos 2009; 37:629-634.
28. Carvalho R, Duarte F, Lima T. Involvement of GABAergic non-benzodiazepine sites in the anxiolyticlike and sedative effects of the flavonoid baicalein in mice. Behav Brain Res 2011; 221:75-82.
29. Zhang S, Obregon D, Ehrhart J, Deng J, Tian J, Hou H, *et al*. Baicalein reduces β-amyloid and promotes nonamyloidogenic amyloid precursor protein processing in an alzheimer's disease transgenic mouse model. J Neurosci Res 2013; 9:1239-1246.
30. Xiao JR, Do CW, To CH. Potential therapeutic effects of baicalein, baicalin, and wogonin in ocular disorders. J Ocul Pharmacol Ther 2014; 30:605-614.
31. Schwartz S. Psychoactive Herbs in Veterinary Behavior Medicine. John Wiley & Sons. 2008; pp. 13.
32. CPCSEA guidelines for laboratory animal facility. Indian J. Pharmacol 2003; 35:257-74.
33. Allen E. The oestrous cycle in the mouse. American Journal of Anatomy 1922;30:297–371.
34. Caligioni CS. Assessing reproductive status/stages in mice. Curr Protoc Neurosci 2009; 4:1-11.
35. Cooper RL, Goldman JM., Vandenbergh JG. Monitoring of the estrous cycle in the laboratory rodent by vaginal lavage. In Methods in Toxicology: Female Reproductive Toxicology 1993; New York pp. 45-54.
36. Supriya CH, Akhila B, Pratap Reddy K, Girish BP, Sreenivasula Reddy P. Effects of maternal exposure to aflatoxin B1 during pregnancy on fertility output of dams and developmental, behavioral and reproductive consequences in female offspring using a rat model. Toxicol Mech Methods 2016; 26:202-210.
37. Zhang Y, Li X, Ciric B, Gen Ma C, Gran, B, Rostami A, *et al*. Therapeutic effect of baicalin on experimental autoimmune encephalomyelitis is mediated by SOCS3 regulatory pathway. Sci Rep. 2015; 5:1-15.

38. Lai CC, Huang PH, Yang AH, Chiang SC, Tang CY, Tseng KW, *et al*. Baicalein, a component of *Scutellaria baicalensis*, attenuates kidney injury induced by myocardial ischemia and reperfusion. Planta Med 2016; 82:181-189.
39. Long JA, Evans HM. The oestrous cycle in the rat and its associated phenomena. Mem Univ Calif 1992; 6:1-148.
40. Freeman ME. The ovarian cycle of the rat. In: Knobil E, Neil J (eds.). Physiology of reproduction 1988; New York, pp. 1893-1928.
41. Mandl AM. The phases of the oestrous cycle in the adult white rat. J Exp Biol 1951; 28:576-584.
42. Zarrow MX, Yochim JM, McCarthy JL. Experimental endocrinology: A sourcebook of basic techniques 1964, New York.
43. Sridevi V, Sowjanya MGS, Ramachandra Reddy P. Pre and postnatal exposure of baicalein (flavonoid) on developmental landmarks of mice. J Infer Reprod Biol 2016; 4:199-207.
44. Nikaido Y, Danbara N, Tsujita-Kyutoku M, Yuri T, Uehara N, Tsubura A. Effects of Prepubertal Exposure to Xenoestrogen on Development of Estrogen Target Organs in Female CD-1 Mice. In Vivo 2005;19: 487-494.
45. Jefferson WN, Doerge D, Padilla-Banks E, Woodling KA, Kissling GE, Newbold R. Oral exposure to genistin, the glycosylated form of genistein, during neonatal life adversely affects the female reproductive system. Environ Health Perspect 2009; 117:1883-1889.
46. Jefferson WN, Couse JF, Padilla-Banks E, Korach KS, Newbold RR. Neonatal Exposure to Genistein Induces Estrogen Receptor (ER)α Expression and Multioocyte Follicles in the Maturing Mouse Ovary: Evidence for ERβ-Mediated and Nonestrogenic Actions. Biology of Reproduction 2002;67:1285–1296.
47. Nikaido Y, Yoshizawa K, Danbara N, Kyutoku MT, Yuri T, Uehara N, *et al*. Effects of maternal xenoestrogen exposure on development of the reproductive tract and mammary gland in female CD-1 mouse offspring. Reprod Toxicol 2004; 18:803-811.
48. Johansson HK, Jacobsen PR, Hass U, Svingen T, Vinggaard AM, Isling LK, *et al*. Perinatal exposure to mixtures of endocrine disrupting chemicals reduces female rat follicle reserves and accelerates reproductive aging. Reprod Toxicol 2016; 61:186-194.
49. Collins TF, Sprando RL, Black TN, Olejnik N, Wiesenfeld PW, Babu US, *et al.* Effects of flaxseed and defatted flaxseed meal on reproduction and development in rats. Food Chem Toxicol 2003; 41:819-834.
50. McClain RM, Wolz E, Davidovich A, Edwards BJ. Reproductive safety studies with genistein in rats. Food Chem Toxicol 2007; 45:1319-1332.
51. Ju kim Y, Chul Hong Y, Hee Lee K, Joo Park H, Ae Park E, Sung Moon H, *et al*. Oxidative stress in pregnant women and birth weight reduction. Reprod Toxicol 2003; 19:487-492
52. Soujanya MGS, Prathap Reddy K, Sridevi V, Ramachandra Reddy P, Sreenivasula Reddy P. In utero exposure of biochaninA alters female reproduction in rat. J Clin Mol Endocrinol 2016; 2:1-10.
53. Pushpalatha T. In utero exposure of proluton depot on male reproduction in rats. Thesis submitted to Sri Venkateswara University, Tirupati 2004, A.P., and India.
54. Elizabeth EH, Michaei BB. Association of delayed conception with caffeine consumption. Am J Epidemiol 1993; 138:1082-1092.
55. Keith RM, Christy LA. Polyphenols as dietary supplements: a double-edged sword. Nutr Diet Suppl 2009;2:1-12.
56. Cassidy A, Bingham S, Setchell K. Biological effects of isoflavones in young women: importance of the chemical composition of soyabean products. Br J Nutr 1995; 74:587-601.
57. Fujiwara T, Rieko N. Current problems of food intake in young women in japan: Their influence on female reproductive function. Reprod Med Biol 2004; 3:107-114.
58. Jefferson WN, Padilla-Banks E, Newbold RR. Adverse effects on female development and reproduction in CD-1 mice following neonatal exposure to the phytoestrogen genistein at environmentally relevant doses. Biol Reprod 2005; 73:798-806.
59. Romero V, Dela Cruz C, Pereira O. Reproductive and toxicological effects of isoflavones on female offspring of rats exposed during pregnancy. Anim Reprod 2008; 5, 83-89.
60. Kallela K, Ettala E. The oestrogenic fusarium toxin (zearalenone) in hay as a cause of early abortions in the cow. Nord Vet Med 1984; 36:305-309.
61. Salleh N, and Giribabu N. Leukemia inhibitory factor: Roles in embryo implantation and in nonhormonal contraception. ScientificWorldJournal 2014; 2014:1-10.