Int J Physiol Pathophysiol Pharmacol 2020;12(4):115-127
www.ijppp.org /ISSN:1944-8171/IJPPP0114861

Original Article

Altered gestational outcomes and delayed pubertal onset in prenatally and early postnatally food restricted male and female rats: mitigation by quercetin and kaempferol

Kenneth Kelechi Anachuna, Ehitare Ikehuamen Ekhoye, Cordilia Iyare, Nkiru Katchy, Benneth Ben-Azu, Deborah Boluwatife Adeniyi, Tarela Melish Elias Daubry, Eghosa Iyare

Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta State, Nigeria

Received May 23, 2020; Accepted August 18, 2020; Epub August 25, 2020; Published August 30, 2020

Abstract: Nutrigenomic malnutrition during pregnancy and early postnatal life has serious consequences on original organ-programing, growth pattern, puberty and quality of life. The aim of this was to investigate the effect of two notable flavonoids, quercetin and kaempferol, with nutrigenomic potentials on prenatal and early postnatal food restrictions or both on gestational outcomes and the onset of puberty in male and females Wister rats. In three sets of experiments consisting of prenatal, postnatal food deprivations or both, rats were distributed into various treatment groups (n = 6). Prenatal food restriction (PrNFR) was initiated by 50% of *ad libitum* available diet in pregnancy (days 1-22) simultaneously with quercetin (50, 100 and 200 mg/kg, p.o./day) or kaempferol (50, 100 and 200 mg/kg, p.o./day) until delivery. However, postnatal food restriction (PsNFR) was simulated by litter-increment to 16 pups per mother from postnatal day 2 together with quercetin (50-200 mg/kg, p.o.) or kaempferol (50-200 mg/kg, p.o.) treatments until weaning (day 24) respectively. The last experiment encompasses both protocols with similar treatment protocols. Kaempferol attenuated PrNFR-induced alterations in gestational length compared to PrNFR-control. Quercetin and kaempferol significantly ($P < 0.05$) normalized nose-length of pups of rats exposed to PrNFR. Quercetin and kaempferol reduced the number of stillbirths due to PrNFR. Both also reduced the delay in pubertal onset as evidenced by normal onset of balanopreputial-separation and vaginal-opening in the PrNFR, PsNFR and PrNFR-PsNFR male and female rats respectively. Together, quercetin and kaempferol prevents prenatal and postnatal malnutrition-induced altered gestational outcomes and pubertal delays in rats.

Keywords: Gestational outcome, food restriction, quercetin, kaempferol, puberty onset, prenatal, postnatal

Introduction

The first resounding evidence that prenatal under nutrition could have a prolonged effect on human health emanated from the follow-up of off-springs who were in-utero during the Dutche Famine ('Hunger Winter') of 1944-1945 [1]. Of note, expanded bodies of epidemiological studies since then have shown that prenatal and postnatal nutritional status plays a critical role in postnatal growth and development [2, 3]. Various studies have shown that the quality and quantity of food during pregnancy and in early postnatal life has permanent consequences on the growth pattern and quality of life [2-4]. Specifically, prenatal food deprivation has long been reported to slow down the rates of cell division in tissues and organs, which may lead to altered programming of the structure and functions of body systems [2, 4]. Indeed, experimental data in mice have shown that intrauterine food deprivation may significantly reduce the number of puppies, increase the resorption of fetuses and neonatal mortality [5], retards intrauterine development of the fetus in sheep [3], and reduces the weight of pups at birth in rats [6]. Consequently, in humans, prenatal food deprivation has been associated with wide range of vulnerability risk factors for the development of various condi-

tions including preeclampsia, preterm birth, decreased body size and compositions, low birth weight that are predictive of heightened risk of the chronic lung and kidney diseases, metabolic and cardiovascular syndromes as well as decreased immune function in postnatal life [1-3].

Of note, chronic prenatal food deprivation is known to modify the duration of gestation thereby leading to cascades of alterations in the secretion of estradiol, progesterone and progestin necessary to induce reproductive motivation and pregnancy [2, 7]. Several findings have also shown the impacts of prenatal and early postnatal food deprivation on the onset of puberty and fertility [6, 8-10]. For example, in rodents, it is hypothesized that weight plays an important role in the regulation of puberty. Therefore, it has been suggested that in females, a critical body weight is needed for *menarche* to occur [6, 8, 11]. Moreover, previous studies have demonstrated that underfeeding during pregnancy resulted in growth retardation and a delayed onset of puberty including delayed balanopreputial separation in males and vaginal opening in female rats respectively [8]. Furthermore, it has also been noted that postnatal undernutrition in females causes delayed onset of puberty [8]. Thus, prenatal and postnatal food restrictions have increasingly become popular as animal models for exploring the relationship between nutritional fertility and pubertal development in both males and females [6, 8, 9]. However, proper maternal and long term pre- and post-natal dieting have fundamental roles in decreasing pregnancy complications, delayed maturation, onset of puberty and possible diseases in postnatal life [2, 13]. It is worthy of note that several studies have showed evidence of the role of oxidative stress in the pathogenesis of prenatal and postnatal food deprivation-induced complications including premature rupture of membranes, fetal growth restriction, negative birth outcomes and postnatal pubertal delay [14]. Accordingly, there are increasing evidences that support the potential of antioxidant supplementation in the prevention and treatment of prenatal and postnatal food deprivation related uterine underdevelopment and diseases [15, 16]. Hence, antioxidants with reproductive protective properties are popularly becoming one of the major targets in the search of new drugs and lead compounds for the prevention of possible metabolic disease conditions associated with prenatal and postnatal food-deprived victims [15, 16].

Quercetin and kaempferol are bioactive bioflavonoids abundantly distributed in many ethnomedicinal plants including apple, grape, orange, wild garlic, onion, red wine, black tea, broccoli, endive and wild leeks (ramps) [17, 18]. Quercetin and kaempferol are naturally occurring promising drugs and are generally regarded as very safe for human utilization, as they form essential components of our day-to-day diets in fruits and vegetables [17, 18]. Both of which have been reported to possess multifaceted therapeutic applications and pharmacological activities including: antioxidant [18, 19], anti-inflammatory-immunomodulatory [20], anticancer [21, 22], antidiabetic [23, 24], antihypertensive [25, 26], and reproductive enhancement [27, 28] properties, all of which currently underpins their international utility as food additive and neuroprotective compounds. [14, 29]. It is also important to note that recent *in vivo* and *in vitro* studies had reported that quercetin [23] and kaempferol [24] prevent many metabolic syndromes and their complication via inhibition of oxidative stress and inflammatory processes [18, 30]. Several clinical studies conducted on the antihypertensive and antidiabetic properties of quercetin [31, 32] and kaempferol [18, 27], all suggest significant beneficial effects. Moreover, some studies have also shown that quercetin and kaempferol protects against pregnancy-induced cardiovascular dysfunctions in rodents and humans [18, 33]. Notably, quercetin has been shown to protect against the toxic potential of chemotherapeutic agents during pregnancy via antioxidant-mediated mechanisms [34]. Despite the overwhelming evidence of long term effects of prenatal and postnatal food deprivations on physical characteristics, pubertal onset, and the possible mechanistic pathways involved, there are few studies on the effects of antioxidant compounds on prenatal and postnatal food deprivations-induced pubertal delay. In line with this, we hypothesized that quercetin and kaempferol supplementations could mitigate the deleterious effects of prenatal and postnatal food deprivations in rats. Hence, this study was designed to investigate the effect of quer-

cetin and kaempferol on prenatal and postnatal food deprivation-induced delayed onset of puberty in rats. Also, the effects of quercetin and kaempferol on duration of gestation, litter size, nose-tale length in rats exposed to prenatal food restriction were also evaluated.

Materials and methods

Experimental animals

Male and female Wistar rats (170-200 g; 12-16 weeks old) were obtained from the Animal Facility of the Faculty of Basic Medical Sciences, Delta State University, Abraka Delta State, Nigeria. Animals were accommodated in transparent plastic transparent cage (27 × 30 × 42 cm) in a 25 ± 1°C environment with light/dark cycle of 12:12. Animals were given standard rodent pellets and water *ad libitum* throughout the investigations. Animals were acclimatized for a period 7 days before the start of experiments. Afterward, animals were randomly distributed into various treatment groups. Guide from National institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) in accordance with the animal rights laws of the University were strictly followed.

Drugs and chemicals

Quercetin - QCET (50, 100 and 200 mg/kg), kaempferol - KFAM (50, 100 and 200 mg/kg) were procured from Sigma-Aldrich, St. Louis, USA. They were dissolved in DMSO (0.1 M) and administered by gavage with an oral cannula. Animals in prenatal food deprivation experiment were given quercetin or kaempferol once daily for 21-22 days depending on delivery date, whereas rats in postnatal food deprivation experiment received once daily administration of quercetin and kaempferol for 22 days (until weaning period). The DMSO (10 mL/kg, p.o.) (0.1 M) solution was also administered orally by gavage with an oral cannula in a volume of 10 mL/kg per body weight, as normal control. The doses of quercetin [24] and kaempferol [23] used in this study were based on results obtained from preliminary and previous studies.

Ethical recommendation

Ethical approval was sought and obtained from the Faculty of Basic Medical Sciences, Delta State University, Abraka Delta State REC/FBMS/DELSU/19/077.

Experimental design

Experiment 1: Prenatal food deprivation: Virgin female Wistar rats were used in this study. A constant pattern of vaginal cyclicity was established during the last 10 days' baseline period. Rats were housed individually in a standard rat cage containing wood shavings as beddings with 25 ± 1°C environment, with light/dark cycle of 12:12 and they had water *ad libitum.* Timed mattings were carried out on the Wister rats by monitoring the stage of oestrus of the female rats before the introduction of the male Wister rats. Day 1 of pregnancy was confirmed by the presence of spermatozoa after a vaginal smear. After confirmation of pregnancy, the animals were randomly grouped into various treatment groups (n = 6). Group 1, which served as control was fed *ad libitum* with a standard rodent diet for 21 days, while group 2 was food deprived for 21 days and served a negative control. Groups 3-5 were food deprived but received quercetin (50, 100 and 200 mg/kg, p.o./day), and groups 6-8 were also food deprived and received kaempferol (50, 100 and 200 mg/kg, p.o./day) until delivery date which was between days 21-22. Prenatal food restriction (PrNFR) protocol was initiated according to previously described model with brief modification [35] by 50% reduction in the quality and quantity of rodent pellets *ad libitum*, which was determined by the amount of food consumed by the control group on the previous days, from day 1 of gestation onward to the end of the experiment (days 21-22) [6]. However, a control group was fed *ad libitum* with a standard rodent diet for 21 days. At day 21-22, pups were delivered naturally. The duration of gestation, litter size and number of still births were measured across all treatment groups after birth. The litters (males and females) were housed together with the mothers in standard rat cages containing wood shavings as beddings with free access to food and water. They were maintained at constant temperature and 12 h light and dark cycle. Onward, nose-tail length was measured at postnatal day (PND) 1 and PND 22.

Experiment 2: Postnatal food deprivation: Postnatal food deprivation (PsNFR) protocol was done by increasing the litters to 16 pups

per mother from PND 2 until weaning (day 24), that is a total period of 22 days of postnatal under-nutrition [8]. Postnatally food deprived rats were grouped into different treatment groups (n = 6). Animals in group 1 were fed by one mother (i.e, 6 pups per mother) *ad libitum* until weaning (22 days) and they served as normal control. Group 2 was postnatally food restricted (among 16 pups per mother) and served a negative experimental control. Groups 3-5 were postnatally food deprived (among 16 pups per mother) and but received quercetin (50, 100 and 200 mg/kg, p.o./day), while groups 6-8 were also postnatally food deprived (among 16 pups per mother) and received kaempferol (50, 100 and 200 mg/kg, p.o./day).

Experiment 3: Prenatal and postnatal food deprivations: Timed mattings were carried out in the Wister rats by monitoring the stage of oestrus of the rats before the introduction of the male Wister rats as described in *experiment 1*. Day 1 after confirmation of pregnancy by the presence of spermatozoa after a vaginal smear, prenatal food deprivation was initiated according to previously described model with brief modification [35] by 50% reduction in the quality and quantity of rodent pellets *ad libitum*, which was determined by the amount of food consumed by the control group on the previous days, from day 1 of gestation onward to the end of gestation (days 21-22). At day 21-22, pups were delivered freely. Thereafter, postnatal food deprivation was achieved by enlarging the litters to 16 pups per mother from PND 2 until weaning (day 22) as previously described above. Together, the prenatally and postnatally food restricted (PrNFR-PsNFR) rats were convened into different treatment groups (n = 6). The PrNFR-PsNFR animals in group 1 was fed by one mother (i.e, 6 pups per mother) *ad libitum* until weaning (22 days) and served as normal control. PrNFR-PsNFR group 2 had vehicle (10 mL/kg, p.o.) and served a negative experimental control. PrNFR-PsNFR groups 3-5 received quercetin (50, 100 and 200 mg/kg, p.o./day), while rats in PrNFR-PsNFR groups 6-8 were treated with kaempferol (50, 100 and 200 mg/kg, p.o./day) for 22 days respectively.

Measurements

The litter sizes were measured across all treatment groups after birth in *experiment 1.* Onward, nose-tail lengths (Ano-genital distance) were also determined at PNDs 1 and 22 with the aid of a thread and were read off on a meter rule in centimeter (cm) [8, 35].

Onset of puberty

After weaning on day 24, animals were kept in group cages with close weight range (two males or three females per cage) according to treatment groups, with free access to food and water. From day thirty (30) onwards, the females were investigated once daily for vaginal opening (VO), and from day 36 of the male rats, pubertal onset was assessed by checking for balano-preputal separation (BPS) as established from the control groups. Thereafter, pubertal onset was defined as the age (in days) at which VO and BPS took place [8].

Statistical analysis

After normality check, data were expressed as Mean ± S.E.M. (standard error of mean). All data were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni *post-hoc* test for multiple comparisons where appropriate. Data were analyzed by Graph Pad Prism software version 5 (GraphPad Software, Inc. La Jolla, CA 92037 USA). A level of $P < 0.05$ was considered as statistically significant for all tests.

Results

Effects of quercetin and kaempferol on duration of gestation in rats exposed to prenatal food restriction

The effects of quercetin and kaempferol on duration of gestation in rats exposed to prenatal food restriction are shown in **Figure 1**. One-way ANOVA revealed that chronic PrNFR did not cause significant ($P > 0.05$) delay on the duration of gestation in rats [F (7,40) = 2.659, P = 0.0234] when compared with vehicle control group. However, Bonferroni post-hoc test showed that only kaempferol (100 and 200 mg/kg, p.o.) significantly ($P < 0.05$) reduced the duration of gestation relative to PrNFR rats (**Figure 1**).

Effects of quercetin and kaempferol on prenatal food restriction-induced stillbirth and decreased litter sizes of rats

The effects of quercetin and kaempferol on prenatal food restriction-induced stillbirths and decreased litter size of rats are shown in **Figure**

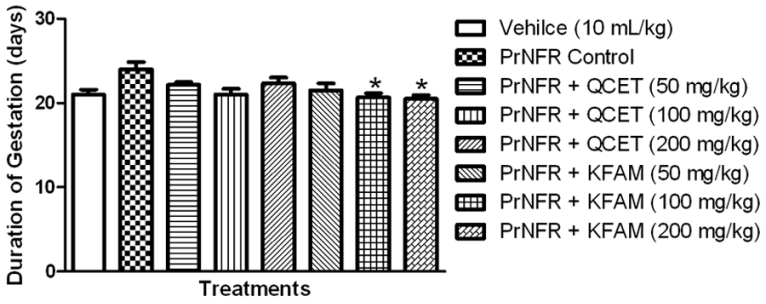

Figure 1. Effect of quercetin and kaempferol on duration of gestation in rats exposed to prenatal food restriction. Bars represent the mean ± SEM of 6 animals per group. $^{*}P < 0.05$ compared to PrNFR control group (One-way ANOVA followed by Bonferroni *post-hoc* test). PrNFR = Prenatal food restriction, QCET = Quercetin, KFAM = Kaempferol.

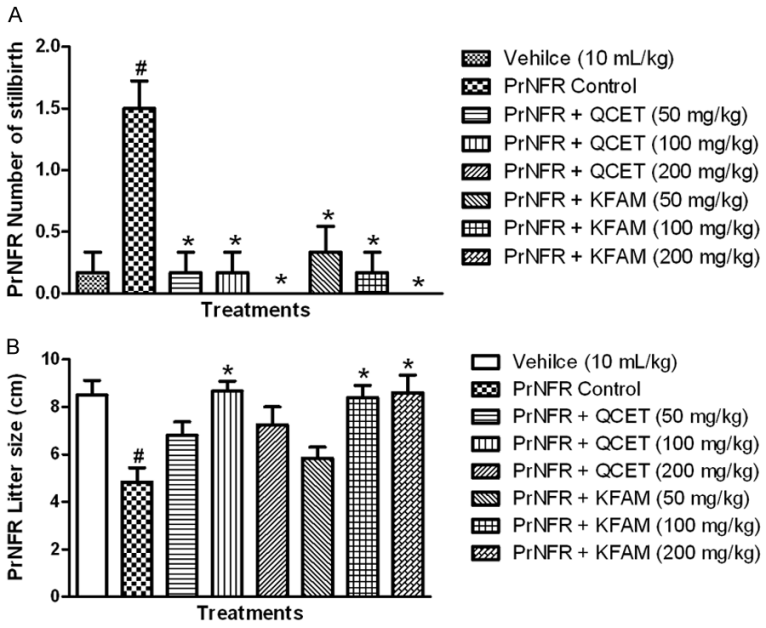

Figure 2. Quercetin and kaempferol reduce the effects of prenatal food restriction on (A) stillbirths and (B) litter size of rats. Bars represent the mean ± SEM of 6 animals per group. $^{\#}P < 0.05$ compared to vehicle group; $^{*}P < 0.05$ compared to PrNFR control group (One-way ANOVA followed by Bonferroni *post-hoc* test). PrNFR = Prenatal food restriction, QCET = Quercetin, KFAM = Kaempferol.

2A, **2B**. One-way ANOVA revealed that there were significant differences between various treatment groups in the number of stillbirths [F (7,40) = 9.402, $P < 0.0001$] and litter size in rats ($P < 0.001$) [F (7,40) = 6.594, $P < 0.0001$]. The PrNFR significantly ($P < 0.05$) increased the number of stillbirths when compared with vehicle control group. However, treatments with quercetin (50, 100 and 200 mg/kg, p.o.) and kaempferol (50, 100 and 200 mg/kg, p.o.) significantly reduced the number of stillbirths when compared with PrNFR control group (**Figure 2A**). PrNFR significantly ($P < 0.001$) reduced the litter size of pups rats when compared with PrNFR control rats. But, treatments with quercetin (100 mg/kg, p.o.) and kaempferol (100 and 200 mg/kg, p.o.) significantly ($P < 0.05$) increased the litter size when compared with PrNFR control group. On the other hand, quercetin (50 and 200 mg/kg, p.o.) and kaempferol (50 mg/kg, p.o.) failed ($P > 0.05$) to protect against PrNFR-induced decrease in litter sizes relative to PrNFR-exposed rats alone (**Figure 2B**).

Quercetin and kaempferol increase nose-tail length of rats submitted to prenatal food restriction

One-way ANOVA revealed that there were significant differences between various treatment groups at PND 1 [F (7,40) = 3.164, P = 0.0093] (**Figure 3A**) and PND 22 [F (7,40) = 4.396, P = 0.0011] (**Figure 3B**). PrNFR significantly reduced the nose-tail length of pups at PND 1 ($P < 0.05$) and PND 22 ($P < 0.001$) in comparison with vehicle control groups respectively. But, post-hoc analysis with Bonferroni showed that treatments with high doses of both quercetin (100 and 200 mg/kg, p.o.) and kaempferol (100 and 200 mg/kg, p.o.) significantly prevented PrNFR-induced decreased nose-tail lengths of rats at PND 1 (**Figure 3A**) and PND 22 (**Figure 3B**) when compared to PrNFR controls.

Quercetin and kaempferol reduce prenatal food restriction-induced delayed onset of puberty of male and female rats

Figure 4A, **4B** shows the effects of quercetin and kaempferol on the onset of puberty of male and female rats exposed to prenatal food

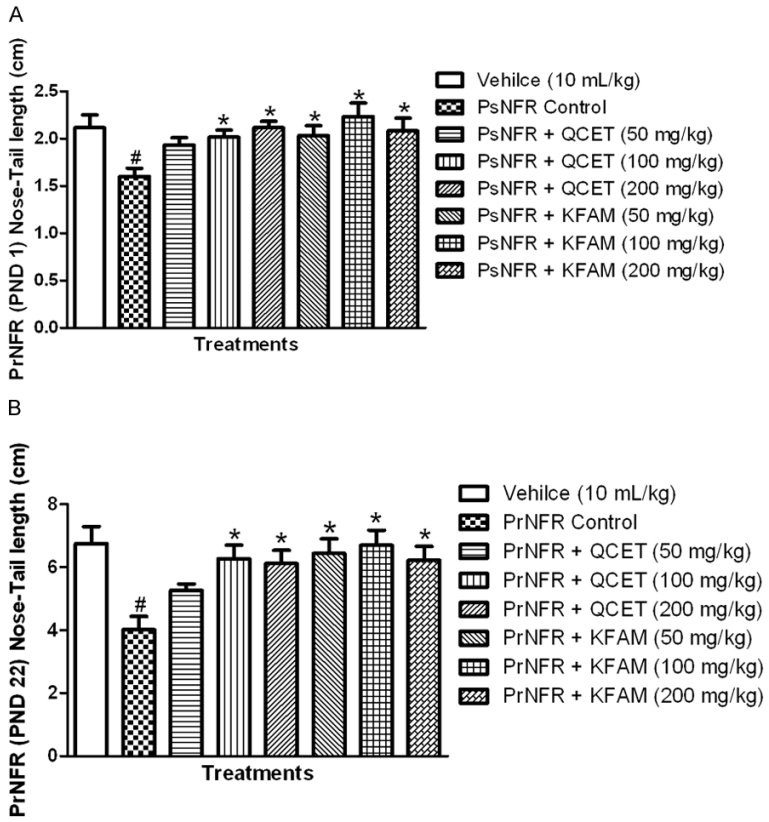

Figure 3. Quercetin and kaempferol increase nose-tail length of rats submitted to prenatal food restriction at postnatal day 1 (A) and postnatal day 22 (B). Bars represent the mean ± SEM of 6 animals per group. $^{\#}P < 0.05$ compared to vehicle group; $^{*}P < 0.05$ compared to PrNFR control group (One-way ANOVA followed by Bonferroni *post-hoc* test). PrNFR = Prenatal food restriction, QCET = Quercetin, KFAM = Kaempferol.

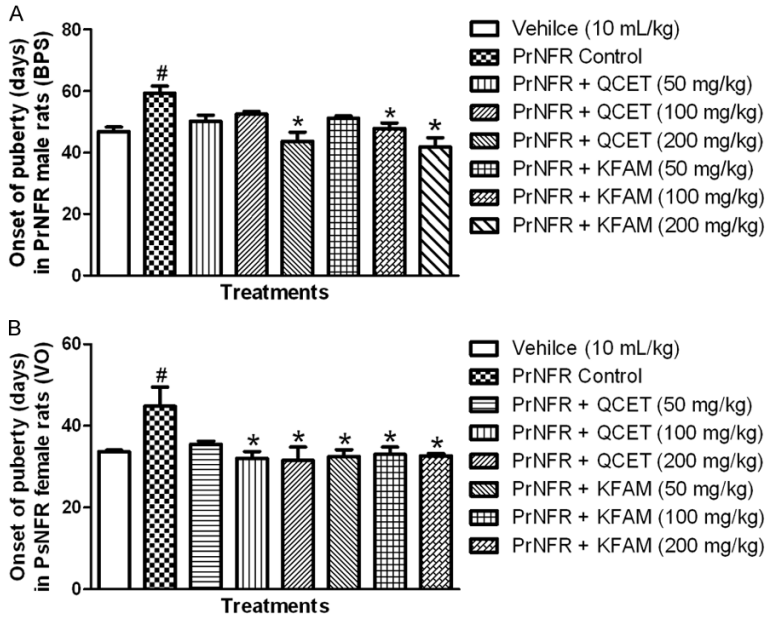

Figure 4. Quercetin and kaempferol reduce prenatal food restriction-induced delayed onset of puberty of male (A) and female (B) rats. Bars represent the mean ± SEM of 6 animals per group. $^{\#}P < 0.05$ compared to vehicle group; $^{*}P < 0.05$ compared to PrNFR control group (One-way ANOVA followed by Bonferroni *post-hoc* test). PrNFR = Prenatal food restriction, BPS = Balanopreputial separation, QCET = Quercetin, KFAM = Kaempferol, BPS = Balanopreputial separation.

restriction. One-way ANOVA indicates that PrNFR significantly ($P < 0.01$) delayed the onset of puberty of male [F (7,40) = 7.137, $P < 0.0001$] and female [F (7,40) = 3.538, $P = 0.0047$] rats when compared with PrNFR control groups respectively. Treatments with only quercetin (200 mg/kg, p.o.) and high doses of kaempferol (100 and 200 mg/kg, p.o.) significantly ($P < 0.05$) reduced the delayed onset of puberty of the male rats when compared with PrNFR control group (**Figure 4A**). Also, post hoc analysis with Bonferroni test showed that PrNFR significantly ($P < 0.05$) delayed the onset of puberty in the female rats when compared with PrNFR control group, which was reduced by quercetin (100 and 200 mg/kg, p.o.) and kaempferol (50, 100 and 200 mg/kg, p.o.) in an equipotent manner ($P < 0.05$) (**Figure 4B**).

Quercetin and kaempferol reduce postnatal food restriction-induced delayed onset of puberty of male and female rats

Analysis with one-way ANOVA showed that there were significant differences between various treatment groups in the onset of puberty of male [F (7,40) = 4.311, $P = 0.0012$] (**Figure 5A**) and female [F (7,40) = 3.538, $P = 0.0047$] (**Figure 5B**) rats exposed to

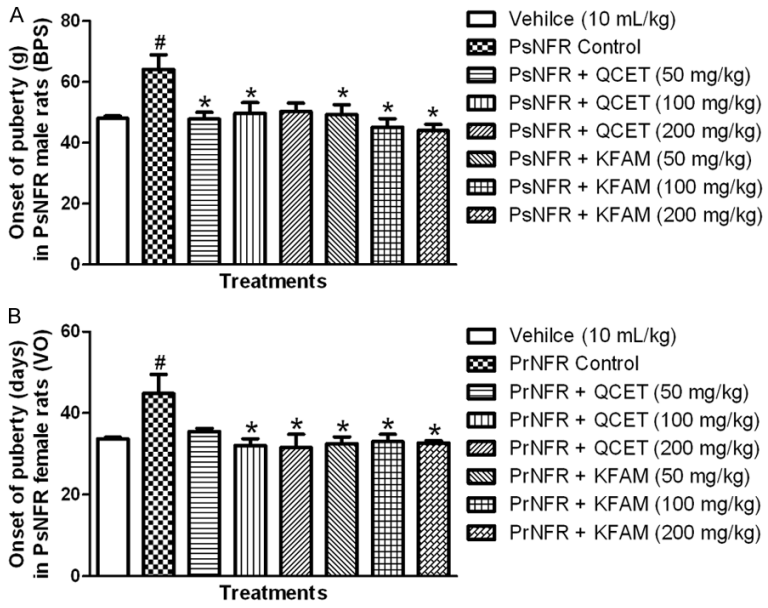

Figure 5. Quercetin and kaempferol reduce postnatal food restriction-induced delayed onset of puberty of male (A) and female (B) rats. Bars represent the mean ± SEM of 6 animals per group. #*P* < 0.05 compared to vehicle group; **P* < 0.05 compared to PsNFR control group (One-way ANOVA followed by Bonferroni *post-hoc* test). PsNFR = Postnatal food restriction, BPS = Balanopreputial separation, QCET = Quercetin, KFAM = Kaempferol.

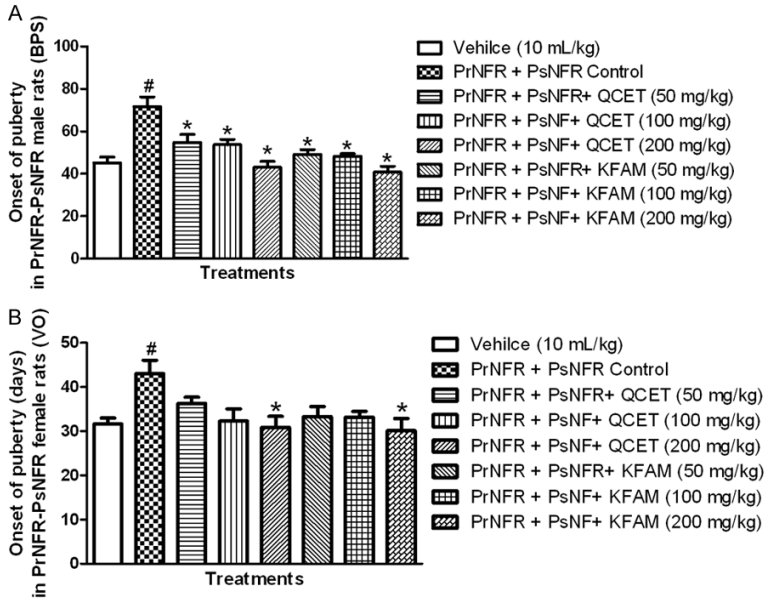

Figure 6. Effects of quercetin and kaempferol on both prenatal and postnatal food restrictions-induced changes in onset of puberty of male (A) and female (B) rats. Bars represent the mean ± SEM of 6 animals per group. #*P* < 0.05 compared to vehicle group; **P* < 0.05 compared to PrNFR-PsNFR control group (One-way ANOVA followed by Bonferroni *post-hoc* test). PrNFR = Prenatal food restriction, PsNFR = Postnatal food restriction, VO = Vaginal opening, QCET = Quercetin, KFAM = Kaempferol.

PsNFR compared with vehicle groups. Post hoc analysis revealed that PsNFR caused a significant (*P* < 0.05) delay in the onset of puberty of male and female rats when compared with PsNFR vehicle control groups. Treatments with quercetin (50, 100 and 200 mg/kg, p.o.) and kaempferol (50, 100 and 200 mg/kg, p.o.) significantly (*P* < 0.05) reduced the onset of puberty of male (**Figure 5A**) and female (**Figure 5B**) rats when compared with PsNFR control group in a dose-dependently respectively.

Effect of quercetin and kaempferol on both prenatal and postnatal food restrictions-induced changes in onset of puberty of male and female rats

The effects of quercetin and kaempferol on onset of puberty of male and female rats exposed to both prenatal and postnatal food restrictions (PrNFR-PsNFR) are shown in **Figure 6A**, **6B**. One-way ANOVA and Bonferroni post hoc test indicate that PrNFR-PsNFR protocols induced a profound significant (*P* < 0.001) delay in the onset of puberty of both male [*F* (7,40) = 8.371, *P* < 0.0001] (**Figure 6A**) and female [*F* (7,40) = 3.383, *P* = 0063] (**Figure 6B**) rats when compared with PrNFR-PsNFR control groups. Quercetin (50, 100 and 200 mg/kg, p.o.) and kaempferol (50, 100 and 200 mg/kg, p.o.) significantly (*P* < 0.05) reduced the delayed onset of puberty of male rats when compared with PrNFR-PsNFR control group (**Figure 6A**). However, only high doses of quercetin (200 mg/kg, p.o.) and kaempferol (200 mg/kg, p.o.) significantly (*P* < 0.05) reduced PrNFR-PsNFR-induced delayed onset of puberty of female rats relative to PrNFR-PsNFR control group respectively (**Figure 6B**).

Discussion

The results of this study revealed that both quercetin and kaempferol exhibit equipotent activities against PrNFR, PsNFR and/or PrNFR-PsNFR-induced alterations in gestational length, stillbirths, litter compositions and delayed onset of puberty respectively. Specifically, quercetin and kaempferol significantly reduced PrNFR-induced stillbirths. Although prenatal food restriction did not alter the duration of gestation, the study showed that administrations of higher doses of kaempferol significantly reduced gestational period relative to prenatal food restriction control group. However, quercetin and kaempferol profoundly attenuated prenatal food restriction-induced decrease in litter size. Also, the decreased in nose-tail lengths at PNDs 1 and 22 of pups were significantly normalized by quercetin and kaempferol. Importantly, the delay in the onset of puberty due to PrNFR, PsNFR- and/or PrNFR-PsNFR in the male and female rats in terms of balanopreputial separation (BPS) and vaginal opening (VO) were significantly attenuated in a dose-dependent manner in both male and female rats respectively.

Prenatal malnutrition of animals particularly in rapidly growing organisms could be a serious challenge to which the system begins to set priority of energy utilization and organogenesis including shutting off reproductive function [4, 8]. Previous studies have shown that nutritional infertility consisting of decreased quantity and quality of food during pregnancy may have some permanent consequences on the duration of gestation and postnatal quality of life [1, 4, 8, 9]. Specifically, prenatal food deprivation has long been reported to slow down the rates of cell division in tissues and organs, which may lead to altered programming of the structure and function of body systems [4]. Since a complete reproductive cycle of ovulation, conception, reproduction and lactation are one of the most energy consuming activities that female animals undergo, chronic prenatal food deprivation is known to modify the duration of gestation consequently leading to sequential alterations in the secretion of estradiol, progesterone and progestin necessary to induce reproductive behavior and pregnancy [7, 8]. Obviously, estrogen depletion and perturbations of gonadotrophin concentrations have been reported to cause alteration of bone mineralization, bone microstructure and osteoblast function in underfed pregnant mice [7] and ovariectomized rats [27]. In humans, prenatal food deprivation are often associated with wide range of adverse effects later in life including poorer human output (reduced stature, lower cognitive capacity) and increased vulnerability risk factors for development of several disease conditions such as diabetes, coronary heart disease, chronic lung and kidney disease and decreased immune function [1, 2]. In this study, repeated prenatal food restriction was found to produce a significant increase in the number of stillbirths in comparison with vehicle control, which indicates altered fetal programming. This finding further supports previous investigations, which showed that prenatal food deprivation causes stillbirths in rodents [5, 6, 8, 36]. Thus, the ability of quercetin and kaempferol to significantly reverse the increase in the number of stillbirths caused by prenatal food restriction suggests a beneficial effect in conditions associated nutritional infertility and prenatal food deprivation-induced maternal stress. Also, previous studies have confirmed that prenatal food deprivation causes alterations in programming as evidenced by prolonged duration of gestation in rodents. Although prenatal food restriction in this study produced a non-significant increase in duration of gestation, it is has been shown that delayed gestation is attributed to alterations in the levels of estradiol, progesterone and progestin thereby leading altered reproductive processes and prolonged pregnancy period [8]. However, the finding that treatment with kaempferol significantly reduced the duration of gestation of rats, further confirms its beneficial effects in conditions associated with nutritional infertility due to prenatal food deprivation.

For a long time, it has been hypothesized that metabolic pathway and fat stores play an important role in the litter size of pups [3]. In view of that, nutritional fertility and reproductive programming may still be an important determinant of the body compositions and litter sizes [6, 8]. In general, litter size which is an easily obtainable and widely known conventional measurement in rodent studies showing correlation with body mass index, anorexigenic hormones and other parameters of body composition [6, 12]. Notably, previous studies have

shown that litter size could serve us a useful marker for the determination of onset of puberty [6]. Also, it has been suggested that a critical weight and high body compositions are needed in the programming of the developmental processes as well as *menarche* and spermatogenesis to occur [11]. For example, in male rats, a study has reported a positive connection between reduced litter sizes and altered spermatogenesis at puberty [37]. Also, reduced litter sizes in females have also been negatively correlated with delayed pubertal vaginal opening [38]. From theoretical point of view, shuttling off of reproductive function or reduced organogenesis during pregnancy at time of food scarcity may be desirable for the survival of rodents. Besides, scientific data obviously showed a tight connection between the nutritional status and the pre-implantation stage of pregnancy, which is the period of increased vulnerability of the future embryo to miscarriage [39]. Thus, according to previous studies, it is possible to speculate that prenatal food deprivation attenuates reproductive function by suppressing sequential synthesis and secretion of estradiol, progesterone, progestin and gonadotropin secretatogues. Thus, leading to inhibition of macro- and micro-nutritional needs and consequently, reduced litter size [8]. Whereas on the other hand, increased prenatal food consumption is largely known to protects against reproductive failures and enhance normal "fetal programming" and developments. In this study, prenatal food deprivation was found to induce a significant decrease in litter size when compared with vehicle control, suggesting altered sequential secretion of reproductive hormones, abnormal fetal implantation, at least in part, due to inhibition of macro- and micro-nutritional needs. Together, this finding is congruent with previous studies showing that prenatal food restriction or deprivation decreases litter size [3, 13, 37]. However, treatment with quercetin and kaempferol dose-dependently prevented the effect of prenatal food restriction, as evidenced by increased litter sizes. Thus, the ability of quercetin and kaempferol to increase the litter sizes of food deprived prenatal rats, suggests their beneficial nutrigenomic effects in conditions associated with maternal stress due to prenatal food scarcity.

Furthermore, prenatal food deprivation is also known to affect gross physical features including nose-tail lengths [36]. Earlier studies showed variable impacts of prenatal food restriction on dental and long bone length, symmetry and body compositions [13, 36]. In general, results suggested that some type of prenatal stress including food deprivation could decrease body compositions and bone lengths [12, 13, 36], a condition that has been linked to increased activity of the adrenal gland [40]. Indeed, a number of mechanisms have been postulated to be involved in prenatal food deprivation-induced decreased litter size and decreased nose-tail length including elevated levels of glucocorticoids, which is known to cross the placenta and disrupt fetal programming and development [41]. Increase adrenal size and release of glucocorticoids due to prenatal stress might reflects effect on the central mediators of organogenesis and growth, such as the hypothalamic-pituitary adrenal axis, or direct effects on the affected tissues, such as bone cells [42]. Furthermore, fetal growth restrictions have long been linked to abnormal placentation and excessive maternal oxidative-inflammatory vascular response [15, 43]. Although the mechanisms underpinning the development of little size anomaly are not fully understood, dysfunction of vascular endothelium due to increased free radical generation and depleted antioxidant protective machineries are thought to be central to the pathogenesis of reduced little size and body status [44, 45]. Indeed, oxidative stress, which is a state of loss of balance between pro-oxidation and anti-oxidative machineries [15] has been postulated to be connected to reduced body compositions of prenatally food deprived rats via mechanism associated with trophoblastic invasion, leading to alteration in vascular remodeling and placental insufficiency [44, 46]. Moreover, since mitochondria are one of the major sources of oxidative stress, a small study has shown an association between increased mitochondrial protein in placental samples and prenatal food deprivation [44, 47]. Also, disturbances of normal homeostatic conditions, such as hypoxia- and glucose insufficiency-induced oxidative stress and cytotoxicity, have been postulated to lead to increased luminal and uterine misfolded proteins, which can lead to cellular apoptosis and reduced organogenesis [47]. Thus, it has been hypothesized that oxidative signaling, apoptotic pathway and protein misfolding at the placental interface may contribute to fetal growth restric-

tion that promotes reduced physical characteristics via mechanisms linked to oxidative and inflammatory cascades [16, 44].

Accordingly, the finding from this study mirrors those which showed that prenatal food deprivation induced significant decrease in nose-tail length of pups at PND 1 and up-to PND 22, which indicates disruption in bone mineralization, bone microstructure, osteoblast and physical characteristics. Again, the mounting evidence of oxidative stress playing a key role in the development of reduced litter size and decreased nose-tail length led to the hypothesis that antioxidant supplementation might have a role in mitigating these anomalies [15, 16]. Indeed, a large, randomized placebo-controlled trial investigated supplementation of pregnant women who underwent food scarcity with vitamins C and E, and discovered strong evidence that these antioxidants prevented the deleterious effect of prenatal food deprivation-induced oxidative stress-mediated premature rupture of membranes, fetal growth restriction and birth outcomes [14]. Accordingly, in this study, it was observed that quercetin and kaempferol, which are notable antioxidant compounds, significantly reversed the effects of prenatal food deprivation on litter size as well as nose-tail length. Of note, the beneficial effects of quercetin and kaempferol on bone mineral density, bone microstructure, and osteoblast function in estrogen deficiency-induced bone loss in ovariectomized rats have been reported [27]. Therefore, the protective activities of quercetin and kaempferol against prenatal food deprivation-induced fetal growth restriction might consequently suggest prevention of nutritional infertility-mediated uterine under-development and growth restriction.

Although the etiology of pubertal disturbances remains elusive, there have been growing amount of evidences from epidemiological, preclinical and clinical data supporting the role of prenatal and early postnatal under-nutrition in the pathogenesis of birth anomalies [8, 13]. In general, pubertal development such as balanopreputial separation in male rats and vaginal opening in female rats, which are the first visual signs that are easily obtainable and widely accepted parameters in rodent studies have been negatively correlated with prenatal and postnatal food deprivations as well as intrauterine growth retardation [6, 8, 13]. Remarkably, under-nutrition after weaning has been reported to cause growth retardation and a delayed vaginal opening in female rats [8]. Accordingly, it has been previously suggested that food intake and body weight could be initiating factors of puberty in rodents [8]. Clinically, a tight hypothetical association shows the delaying effects of food restriction and nutritional infertility on female *menarche*, *vice versa* the trend of early *menarche* due to good feeding have long been known [6]. The mechanisms regulating puberty and reproductive tempo are no doubt multi-complex. For example, circulating levels of the anorexigenic hormone, leptin, which is known to be secreted by the adipose tissue but acts in the brain [2] has been implicated to play central role in the relationship between fat stores, onset of puberty and the endocrine reproductive processes in the brain [6, 48]. Thus, prenatal and postnatal malnutritions may change the endocrine programming and therefore the timing of the onset of pubertal development.

In this present study, prenatal malnutrition was found to induce a significant delay in the onset of puberty in both male and female, as evidenced by delayed balanopreputial separation and vaginal opening in both male and female rats respectively. Also, early postnatal food deprivation was found to induce a delayed onset of puberty in both male and female in a similar manner. The combinational effects of prenatal and postnatal food deprivations also produced a marked delay on the balanopreputial separation and vaginal opening in the male and female rats respectively when compared with vehicular controls. However, these delays were attenuated following repeated treatment with quercetin and kaempferol in dose-dependent manners. Although in this study there was no significant difference between the delaying effect of prenatal food restriction and postnatal food restriction on the onset of puberty in both male and female rats, however it was observed that male rats exposed to combination of prenatal and postnatal food restrictions exhibited delayed balanopreputial separation than the female counterparts [8, 9]. It is important to state that since reproduction is initiated long before fertilization, male rats are particularly more vulnerable than female rats. Certainly, one of the needed requirements for

fertilization is the presence of germline and subsequent gametogenesis, which is usually finalized at puberty. In the male and female sex, germ cells are known to possess varied path of differentiation [13]. Males are believed to be more vulnerable to fetal life owing to the fact that in the male, germ cells DNA methylation is reacquired during spermatogenesis of fetal life. On the other hand, female gametes may be sensitive to disturbances during folliculogenesis, since the DNA methylation takes places through the growth and maturation of oocytes in the adult life [13]. Therefore, for a good fetal programming, maximum nutrition is critical not only during pregnancy, but also in early and late postnatal life from puberty [13, 49-51]. Therefore, the ability of quercetin and kaempferol to prevent the delay in the onset of puberty in male and female rats exposed to either prenatal, postnatal food deprivation or the combinational effects of both, suggests its potential utility in the management of pubertal failure that is associated with pre- or postnatal malnutrition.

Conclusion

The findings from our study provides evidence which showed that quercetin and kaempferol exhibit beneficial effects against rats exposed to prenatal, postnatal and/or the combination of both stress in rats. Therefore, these findings further support the notion that lead molecules with antioxidant property may be useful as adaptogens in providing resistance against the effect of prenatal and postnatal food deprivation-induced developmental anomalies.

Disclosure of conflict of interest

None.

Address correspondence to: Kenneth Kelechi Anachuna, Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Delta State University, Abraka, Delta State, Nigeria. E-mail: goldasaba20@gmail.com

References

[1] Fall CH. Fetal malnutrition and long-term outcomes. Nestle Nutr Inst Workshop Ser 2013; 74: 11-25.

[2] Sloboda DM, Howie GJ, Pleasants A, Gluckman PD and Vickers MH. Pre- and postnatal nutritional histories influence reproductive maturation and ovarian function in the rat. PLoS One 2009; 4: e6744.

[3] Ford SP and Long NM. Evidence for similar changes in offspring phenotype following either maternalundernutrition or overnutrition: potential impact on fetal epigenetic mechanisms. Reprod Fertil Dev 2011; 24: 105-111.

[4] Wade GN and Schneider JE. Metabolic fuels and reproduction in female mammals. Neurosci Biobehav Rev 1992; 16: 235-272.

[5] Gabory A, Attig L and Junien C. Developmental programming and epigenetics. Am J Clin Nutr 2011; 94: 1943-1952.

[6] Engelbregt MJ, van Weissenbruch MM, Popp-Snijders C, Lips P and Delemarre-van de Waal HA. Body mass index, body composition, and leptin at onset of puberty in male and female rats after intrauterine growth retardation and after early postnatal food restriction. Pediatr Res 2001; 50: 474-478.

[7] Rattner BA, Michael SD and Brinkley HE. Plasma gonadotrophins and progesterone concentrations during various degrees of underfeeding in pregnant mice. J Reprod Fertil 1979; 56: 587-591.

[8] Engelbregt MJ, Houdijk ME, Popp-Snijders C and Delemarre-van de Waal HA. The effects of intra-uterine growth retardation and postnatal undernutrition on onset of puberty in male and female rats. Pediatr Res 2000; 48: 803-807.

[9] Engelbregt MJ, van Weissenbruch MM, Popp-Snijders C and Delemarre-van de Waal HA. Delayed first cycle in intrauterine growth-retarded and postnatally undernourished female rats: follicular growth and ovulation after stimulation with pregnant mare serum gonadotropin at first cycle. J Endocrinol 2002; 173: 297-304.

[10] Gluckman PD and Hanson MA. Changing times: the evolution of puberty. Mol Cell Endocrinol 2006; 225: 26-31.

[11] Frisch ER and Revelle R. Height and weight at menarche and a hypothesis of menarche. Arch Dis Child 1971; 46: 695-701.

[12] Glass AR, Dahms WT and Swerdloff RS. Body fat at puberty in rats: alteration by changes in diet. Pediatr Res 1979; 13: 7-9.

[13] Castrogiovanni P and Imbesi R. The role of malnutrition during pregnancy and its effects on brain and skeletal muscle postnatal development. J Funct Morphol Kinesiol 2017; 2: 30.

[14] Spinnato JA 2nd, Freire S, Pinto e Silva JL, Rudge MV, Martins-Costa S, Koch MA, Goco N, Santos Cde B, Cecatti JG, Costa R, Ramos JG, Moss N and Sibai BM. Antioxidant supplementation and premature rupture of the membranes: a planned secondary analysis. Am J Obstet Gynecol 2008; 199: 433, e1-8.

[15] Agarwal A, Gupta S and Sharma RK. Role of oxidative stress in female reproduction. Reprod Biol Endocrinol 2005; 3: 28.

[16] Duhig K, Chappell LC and Shennan AH. Oxidative stress in pregnancy and reproduction. Placenta 2016; 9: 113-116.
[17] Makris DP, Kallithraka S and Kefalas P. Flavonols in grapes, grape products and wines: Burden, profile and influential parameters. J Food Compos Anal 2006; 19: 396-404.
[18] Duthie G and Morrice P. Antioxidant capacity of flavonoids in hepatic microsomes is not reflected by antioxidant effects in vivo. Oxid Med Cell Longev 2012; 2012: 165127.
[19] Kampkötter A, Gombitang Nkwonkam C, Zurawski RF, Timpel C, Chovolou Y, Wätjen W and Kahl R. Effects of the flavonoids kaempferol and fisetin on thermotolerance, oxidative stress and FoxO transcription factor DAF-16 in the model organism Caenorhabditis elegans. Arch Toxicol 2007; 81: 849-858.
[20] Wijdan MD and Marra MV. Dietary quercetin and kaempferol: bioavailability and potential cardiovascular-related bioactivity in humans. Nutrients 2019; 11: 2288.
[21] Ozgen S, Kilinc OK and Selamoglu Z. Antioxidant activity of quercetin: a mechanistic review. Turkish J Agric - Food Sc and Technol 2016; 4: 1134-1138.
[22] Devi KP, Malar DS, Nabavi SF, Sureda A, Xiao J, Nabavi SM and Daglia M. Kaempferol and inflammation: from chemistry to medicine. Pharmacol Res 2015; 99: 1-10.
[23] Kashyap D, Sharma A, Tuli HS, Sak K, Punia S and Mukherjee TK. Kaempferol-A dietary anticancer molecule with multiple mechanisms of action: recent trends and advancements. J Funct Foods 2017; 30: 203-219.
[24] Hashemzaei M, Delarami F, Yari A, Heravi A, Tabrizian RE, Taghdisi K and Nikitovic D. Anticancer and apoptosis-inducing effects of quercetin in vitro and in vivo. Oncol Rep 2017; 38: 819-828.
[25] Khalid SA, Chandramohan C, Veeramani C and Alsaif MA. Ameliorative effect of kaempferol, a flavonoid, on oxidative stress in streptozotocin-induced diabetic rats. Redox Rep 2015; 20: 198-209.
[26] Prabhu S, Vijayakumar S, Kothandaraman S and Manogar P. Anti-diabetic activity of quercetin extracted from *Phyllanthus emblica* L. fruit: *in silico* and *in vivo* approaches. J Pharm Anal 2018; 8: 109-118.
[27] Juan D, Pérez-Palencia R, Vargas F, Ocete MA, Vizcaino FP, Zarzuelo A and Tamargo J. Antihypertensive effects of the flavonoid quercetin in spontaneously hypertensive rats. Br J Pharmacol 2001; 133: 117-124.
[28] Ożarowski M, Mikołajczak PL, Kujawski R, Wielgus K, Klejewski A, Wolski H and Seremak-Mrozikiewicz A. Pharmacological effect of quercetin in hypertension and its potential application in pregnancy-induced hypertension: review of *in vitro*, *in vivo*, and clinical studies. Evidence-Based Compl Alt Med 2018; 7421489.
[29] Trivedi R, Kumar A, Gupta V, Kumar S, Nagar GK, Romero JR and Chattopadhyay N. Effects of Egb 761 on bone mineral density, bone microstructure, and osteoblast function: possible roles of quercetin and kaempferol. Mol Cell Endocrinol 2009; 302: 86-91.
[30] Jamalan M, Ghaffari MA, Hoseinzadeh P, Hashemitabar M, and Zeinali M. Human sperm quality and metal toxicants: protective effects of some flavonoids on male reproductive function. Int J Fertil Steril 2016; 10: 215.
[31] Costa LG, Garrick JM, Roquè PJ and Pellacani C. Mechanisms of neuroprotection by quercetin: counteracting oxidative stress and more. Oxid Med Cell Longev 2016; 2986796.
[32] Crespo I, García-Mediavilla MV, Gutiérrez B, Sánchez-Campos S, Tuñón MJ and González-Gallego J. A comparison of the effects of kaempferol and quercetin on cytokine-induced pro-inflammatory status of cultured human endothelial cells. Br J Nutr 2008; 100: 968-976.
[33] Dower JI, Geleijnse JM, Gijsbers L, Schalkwijk C, Kromhout D and Hollman PC. Supplementation of the pure flavonoids epicatechin and quercetin affects some biomarkers of endothelial dysfunction and inflammation in (pre) hypertensive adults: a randomized double-blind, placebo-controlled, crossover trial. J Nutr 2015; 145: 1459-1463.
[34] Dower JI, Geleijnse JM, Gijsbers L, Zock PL, Kromhout D and Hollman PCH. Effects of the pure flavonoids epicatechin and quercetin on vascular function and cardiometabolic health: a randomized, double-blind, placebo-controlled, crossover trial. Am J Clin Nutr 2015; 101: 914-921.
[35] Geleijnse JM, Launer LJ, Van der Kuip DAM, Hofman A and Witteman JCM. Inverse association of tea and flavonoid intakes with incident myocardial infarction: the rotterdam study. Am J Clin Nutr 2002; 75: 880-886.
[36] Doğan Z, Kocahan S, Erdemli E, Köse E, Yılmaz I, Ekincioğlu Z, Ekinci N and Türköz Y. Effect of chemotherapy exposure prior to pregnancy on fetal brain tissue and the potential protective role of quercetin. Cytotechnol 2015; 67:1031-1038.
[37] Gualillo O, Caminos JE, Nogueiras R, Seoane LM, Arvat E, Ghigo E, Casanueva FF and Diéguez C. Effect of food restriction on ghrelin in normal-cycling female rats and in pregnancy. Obes Res 2002; 10: 682-687.
[38] Dancause KN, Laplante D, Oremus C, Fraser S, Brunet A and King S. Disaster-related prenatal

maternal stress influences birth outcomes: project ice storm. Early Hum Dev 2011; 87: 813-820.
[39] Glass A, Damon R, Herbert C and Anderson J. Fertility onset, spermatogenesis, and pubertal development in male rats: effect of graded. Pediatr Res 1986; 20: 11.
[40] Frisch RE. Pubertal adipose tissue: is it necessary for normal sexual maturation? Evidence from the rat and human female. Fed Proc 1980; 39: 2395-2400.
[41] Minkin MJ. Embryonic development and pregnancy test sensitivity: the importance of earlier pregnancy detection. Womens Health (Lond) 2009; 5: 659-667.
[42] Phillips DI, Barker DJ, Fall CH, Seckl JR, Whorwood CB, Wood PJ and Walker BR. Elevated plasma cortisol concentrations: a link between low birth weight and theinsulin resistance syndrome? J Clin Endocrinol Metab 1998; 83: 757-760.
[43] Lazinski MJ, Shea AK and Steiner M. Effects of maternal prenatal stress on offspring development: a commentary. Arch Womens Ment Health 2008; 11: 363-375
[44] Swolin-Eide D, Dahlgren J, Nilsson C, Wikland K Albertsson, Holmang A and Ohlsson C. Affected skeletal growth but normal bone mineralization in rat offspring after prenatal dexamethasone exposure. J Endocrinol 2002; 174: 411-418.
[45] Auten RL and Davis JM. Oxygen toxicity and reactive oxygen species: the devil is in the details. Pediatr Res 2009; 66: 121-7.
[46] Burton GJ, Yung HW, Cindrova-Davies T and Charnock-Jones DS. Placental endoplasmic reticulum stress and oxidative stress in the pathophysiology of unexplained intrauterine growth restriction and earlyonset preeclampsia. Placenta 2009; 30 Suppl A: S43-S48.
[47] Turpin CA, Sakyi SA and Owiredu WK. Association between adverse pregnancy outcome and imbalance in angiogenic regulators and oxidative stress biomarkers in gestational hypertension and preeclampsia. BMC Pregnancy Childbirth 2015; 15: 189.
[48] Karowicz-Bilinska A. Lipid peroxides concentration in women with intrauterine growth restriction. Ginekologia Polska 2004; 75: 6-9.
[49] Makhmudov ES, Akhmerov RN, Babayeva RN, Kulkaraev A and Rasulova Z. Effect of water and food deprivation in pregnant rats on mitochondrial respiration and the state of gluconeogenesis in the rat pup liver. J Evol Biochem Phys 2000; 36: 24-27.
[50] Quennell JH, Howell CS, Roa J, Augustine RA, Grattan DR and Anderson GM. Leptin deficiency and diet induced obesity reduce hypothalamic kisspeptin expression in mice. Endocrinol 2011; 152: 1541-1550.
[51] Bourc'his D and Proudhon C. Sexual dimorphism in parental imprint ontogeny and contribution to embryonic development. Mol Cell Endocrinol 2008; 282: 87-94.