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Effect of Anthocyanin Extracts in Reproductive and Metabolic Parameters in Pregnant Rats

Octavio Aguirre-López[1,2], Elizabeth Lagunas-Mayoral[2], Francisco Javier Barrera-Cobos[2], Isaac Andrade-González[3], Guillermo Nolasco-Rodríguez[2], Jacinto Bañuelos-Pineda[2], Elia Margarita Rodríguez-Chávez[2], Ricardo Cuéllar-Pérez[2]

[1]Department of Sustainable Rural Development, University Centre for Biological and Agricultural Sciences (CUCBA), University of Guadalajara, Jalisco, Mexico
[2]Department of Veterinary Medicine, University Centre for Biological and Agricultural Sciences (CUCBA), University of Guadalajara, Jalisco, Mexico
[3]Department of Postgraduate Studies and Research, Technological Institute of Tlajomulco, National Technological Institute of Mexico, Jalisco, Mexico
Email: ricardo.cuellarp@academicos.udg.mx

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Abstract

Hibiscus sabdariffa (HS), commonly known in Mexico as "jamaica", is an annual plant of the Malvaceae family, which has been utilized in traditional medicine for its antihypertensive, anti-inflammatory and hypoglucemic properties, as well as a diuretic. This paper aims to analyze metabolic parameters during pregnancy and somatometric characteristics of the offspring of rats treated with anthocyanin extract or HS infusion during gestation to establish if any risk occurs from the consumption of phytochemical compounds or from the fraction of elements that constitute HS during pregnancy. An extract of the anthocyanin fraction or an infusion from the calyces of *Hibiscus sabdariffa* was administered to pregnant and non-pregnant female rats. Body weight, food intake, and serum levels of glucose, cholesterol and triglycerides were measured, as well as somatometric data of 21-day-old fetuses. The results showed that the administration of the extract and the HS infusion modified body weight, food intake and blood glucose concentration of pregnant female rats; on the other hand, blood lipid levels were not modified. In the offspring, a reduction in body weight and size was observed due to the consumption of HS infusion during pregnancy; likewise, the anthocyanin extracts significantly reduced their head circumference. HS consumption alters food intake, induces metabolic changes in pregnant female rats, and reduces somatometric proportions of their fetuses. Consequently, there is a need to evaluate the mechanisms associated with these somatometric changes and determine whether there are long-term functional effects in neonates.

Keywords

Hibiscus sabdariffa, Anthocyanins, Pregnancy, Metabolism, Fetus

1. Introduction

Hibiscus sabdariffa (HS) or jamaica, as it is commonly known in Mexico, is an annual plant belonging to the Malvaceae family, which has been used in traditional medicine for its antihypertensive [1], anti-inflammatory [2], hypoglycemic [3] [4] and diuretic properties [5]. However, scientific evidence has also confirmed that HS has antioxidant [6] [7], phytoestrogenic [8], neuroprotective [9], hepatoprotective [10] and antibacterial effects [11]. These properties are generated by the broad phytochemical profile of HS, which includes fractions of phenolic acids, flavonoids, organic acids and particularly anthocyanins [12]-[14].

The consumption of bioactive plant-derived compounds has been associated with the control of gestational health [15]. For instance, the supplementation of products rich in polyphenols during pregnancy can prevent the development of gestational obesity and cardio-metabolic disorders in the mother [16]. Another example is the supplementation of anthocyanins to women with gestational diabetes, generating an insulin-mimetic effect and a decrease in pro-inflammatory signals [17]. On the other hand, models with pregnant rats show an increase in the body weight of fetuses with the consumption of anthocyanin extracts, which is associated with an enhanced glucogenic activity in the liver and improved placental efficiency [18]. Despite evidence demonstrating the health benefits of HS consumption, information on the effects generated by its consumption during pregnancy is limited and contradictory.

During pregnancy, women experience a series of physiological changes, including alterations in hormonal patterns, modifications in metabolic activity and nutritional requirements, as well as changes in body weight [19]. As such, the nutritional status of the mother is crucial for the optimal development of the physiological processes associated with pregnancy, as well as the correct consolidation of the physiology and morphology of the embryo. Therefore, the normal development of pregnancy depends on the precise regulation of these changes [20]. Several studies have shown that nutritional deficiencies during pregnancy are related to the induction of premature births, alterations in fetal development, malformations, and a high probability of developing chronic-degenerative diseases during the adult life of the offspring [16] [21] [22]. As a result, optimally maintaining nutritional and metabolic status of the mother during pregnancy is of great importance.

Some studies warn that the consumption of the phytochemicals from the HS plant during pregnancy may have potential adverse effects on the offspring; for example, a delay in puberty-related characteristics such as vaginal opening has been observed. [23] [24], while other studies suggest positive effects from its inclusion

in the diet [25] [26], this has led to the fact that research on the effects of HS on gestational health remains debatable. For this reason, it is necessary that recommendations for the consumption of *Hibiscus sabdariffa* infusions or derivatives during pregnancy be established based on greater evidence, confirming that they do not cause metabolic alterations, modify the gestational health of the mother or alter the gestational development of the embryo. Thus, the main objective of this study was to analyze metabolic and reproductive parameters in rats that consumed HS or its extract of the anthocyanin fraction during gestation, to establish whether there is any risk by their consumption.

2. Materials and Methods

2.1. Preparation of the Anthocyanin Fraction and *Hibiscus sabdariffa* Calyx Infusion

The anthocyanin extract fraction derived from the calyces of the Nigerian variety of *Hibiscus sabdariffa* was prepared based on the recommendations of Farias-Cervantes *et al.* [27]. In brief, 2.0 kg of calyces of HS were processed; the sample was washed with distilled water and macerated. Once prepared, it was added to a 30% ethanolic solution for 7 days with occasional stirring, avoiding light and kept at room temperature. Following the resting period, the sample was filtered (150 μm diameter/pore) and the solution obtained was concentrated by rotary evaporation (BÜCHI®, USA) at a pressure of 170 bars, at 40˚C and 25 rpm. The pH of the concentrate was adjusted to 3.4 and kept refrigerated at 4˚C until it was freeze-dried (GEA Niro® Production mirror, Denmark). Maltodextrin (50:50) and agave fructans (Agave tequilana weber) were used as a vehicle, at a concentration of 0.5% (w/v).

The HS infusion was prepared according to the traditional method in Mexico for consumption. For this, 50 g of dried HS calyxes were weighed and processed by boiling in 1 L of purified water for 10 minutes. The infusion obtained was filtered (coarse pore, 20 μm) and stored at 4˚C for its later administration.

2.2. Quantification of Total Monomeric Anthocyanins by the Differential pH Method

Anthocyanin quantification was performed using the differential pH method [28]. From the infusion obtained, two dilutions were prepared, the first was made using a 0.025 M potassium chloride buffer solution pH 1 ± 0.05, the second was prepared using a 0.4 M sodium acetate buffer solution pH 4.5 ± 0.05. The dilution factor for the sample was 1:20. Both dilutions were centrifuged at 9500 rpm for 10 minutes at room temperature. The absorbances of both solutions were measured at 520 and 700 nm in a METASH UV-Vis spectrophotometer. The concentration of total monomeric anthocyanins was calculated with the following equation:

$$\mathrm{TAC}\left(\text{miliequivalents of cy-3-glu} \times 100\ \mathrm{g}^{-1}\right) = \frac{\mathrm{Ab}}{\mathrm{eL}} \times \mathrm{MW} \times \mathrm{D}\left(\frac{\mathrm{V}}{\mathrm{G}}\right) \times 100$$

where:

TAC = Total monomeric anthocyanins (milliequivalents of cy-3-glu/100 g of

sample).

Ab = pH 1.0 (absorbance 520 nm - absorbance 700 nm) - pH 4.5 (absorbance 520 - absorbance 700).

e = Molar absorptivity of cy-3-glu (26.900).

MW = Molecular weight of cy-3-glu (449.2 g/mol).

L = 1 (path length in cm).

D = Dilution factor.

V = Final volume of the solution.

G = Weight of the sample.

100 = Conversion factor.

2.3. Experimental Design

Sixty primiparous six month old female Wistar rats were used and randomly distributed into the following groups: non-pregnant females without treatment (Control, n = 12); non-pregnant females treated with the anthocyanin fraction of HS (Ext, n = 12); pregnant females without treatment (Preg, n = 12); pregnant females treated with the anthocyanin fraction (Preg/Ext, n = 12); and pregnant females treated with HS infusion (Preg/Inf, n = 12). During the entire experimental period, rats were housed in standard laboratory conditions at a temperature of 22˚C ± 1˚C and 60% relative humidity, with 12 h light/dark cycles. During the entire experimental period, they were allowed to consume commercial food (Nutricubes, Purina®) and handled according to the recommendations of the National Institute of Health Guide for the Care and Use of Laboratory Animals in its eighth edition and under the guidelines of the Mexican Official Standard NOM-062-ZOO-1999 for the use and management of experimental animals. On days 7, 14, and 19 of gestation, body weight and food consumption were recorded, and blood glucose was measured in all females from 7:00 to 9:00 a.m. using the Accu-Check® Active glucometer. Specifically, for blood glucose, capillary glucose in the tail was measured prior to the start of the different treatments and was considered as the baseline value.

2.4. Doses and Treatments

The anthocyanin extract fraction and the HS infusion were administered from day 1 to day 19 of gestation during the dark cycle to ensure daily consumption; meanwhile, during the light cycle, treatments were replaced with purified water. The anthocyanin fraction was prepared with purified water at a dose of 5 mg of anthocyanins per 100 g of body weight [8]. For the administration of the HS infusion, 30 mL of infusion was administered starting on day 1 of gestation. The quantification of polyphenols and anthocyanins was done for the infusion to determine daily consumption.

2.5. Acquisition of Pregnant Females

In females, staging of the estrous cycle was done, as well as identification of be-

havioral parameters such as acceptance toward the male, increased running activity, trembling of the ears and pronounced lordosis reflex after pelvic stimulation [29]. Once the estrous phase was confirmed, the female was housed with the male for 48 hours, monitoring for the presence of the vaginal plug. Once the vaginal plug was detected, this was deemed as day 1 of gestation [30]. Rats confirmed to be pregnant were separated from the males and randomly assigned to any of the groups of pregnant females.

2.6. Fetuses and Analysis of Somatometric Parameters

On day 19 of gestation, each pregnant female underwent thoracotomy to extract the uterine horns and fetuses; for this, they were anesthetized with sodium pentobarbital (50 mg/kg body weight, intraperitoneally Pisabental®, PiSA, Mex.). Body weight, height (cephalocaudal length) and head length (fronto-occipital length of the skull) were recorded, and morpho-anatomical abnormalities of each fetus were described.

2.7. Collection of Blood Samples and Quantification of Serum Lipids

During the thoracotomy, blood samples were obtained by direct intracardiac via. The samples were centrifuged at 7000 rpm for 10 minutes (DLab Mod. D1008) to obtain serum. To quantify the concentration of total cholesterol and triglycerides, the SPINREACT kit (Girona, Spain) was used.

2.8. Statistical Analysis

All data are presented as mean ± standard error and were analyzed using SPSS 21.0 software (IBM Corp.). Once the normality and homoscedasticity of the data were confirmed, the means were analyzed by one-way ANOVA and the Bonferroni pairwise comparison was used, using $p < 0.05$ to establish significant differences.

3. Results

3.1. Quantification and Consumption of Anthocyanins and Polyphenols from an Infusion Obtained with the Calyces of *Hibiscus sabdariffa*

The chemical quantification analysis of the infusion revealed an anthocyanin concentration of 0.042 ± 0.000206 mg per milliliter of infusion, while the total polyphenol content was 1.988 ± 0.0017 mg per milliliter of infusion. Thus, the average daily consumption of anthocyanins was 0.4239 ± 0.049 mg per 100 g of body weight and 20.06 ± 2.32 mg of polyphenols per 100 g of body weight in the Inf group (**Table 1**). The consumption of anthocyanin fraction of HS in the Ext and Preg/Ext groups was kept constant by preparing their daily dose adjusted to body weight.

Table 1. Relationship between the concentration and consumption of polyphenols and anthocyanins in the *Hibiscus sabdariffa* infusion and the daily intake per rat.

	Anthocyanins	Polyphenols
Concentration (mg/mL of sample)	0.042 ± 0.000206	1.988 ± 0.0017
Mean daily consumption per rat (mg/100 g body weight)	0.4239 ± 0.049	20.06 ± 2.32

3.2. Analysis of Body Weight

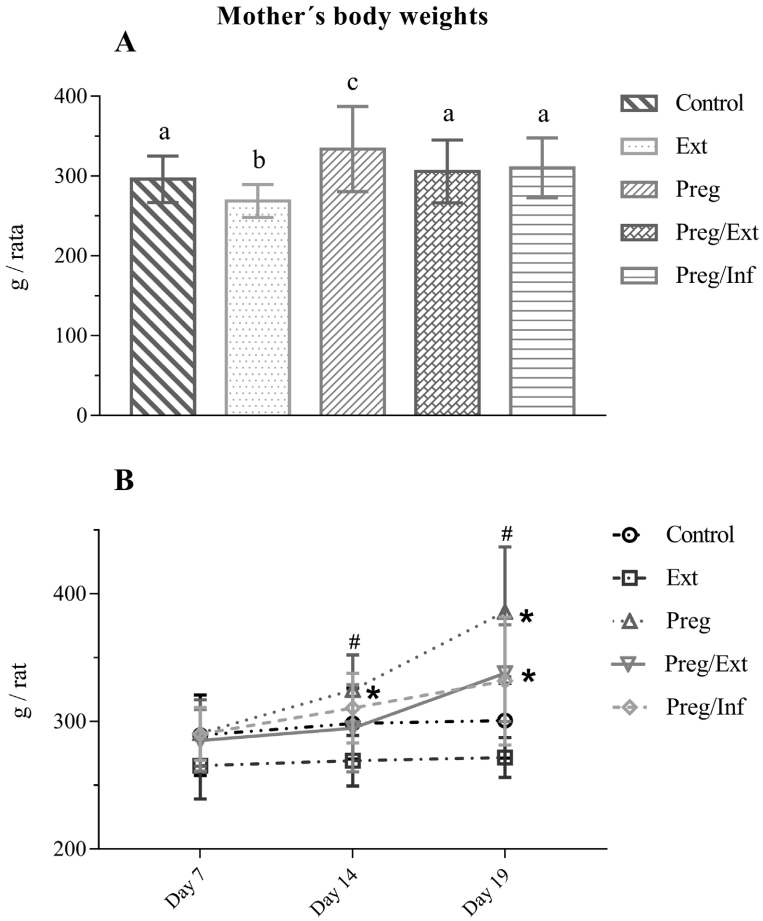

Figure 1. Body weight gain in all groups. (A) Body weight during pregnancy, the bars represent mean ± SEM. (B) Body weight on days 7, 14, and 19 of the gestational period, each point represents mean weight ± SEM. Different letters indicate significant differences between groups ($p < 0.05$). *$p < 0.05$ compared to the same group on a different day. #$p < 0.05$ indicates significant difference with the other groups on the same day ($p < 0.05$).

The analysis of the body weight of the females (**Figure 1(A)**) revealed significant differences between the groups ($F [4] = 23.510$; $p < 0.001$). The Preg group showed the highest body weight (333.85 ± 53.42 g) compared to the Control (296.08 ± 29.13 g), Ext (268.79 ± 18.81 g) Preg/Ext (310.07 ± 34.53 g) and Preg/Inf (310.19 ± 37.51 g) groups. The weight of the Ext group was significantly lower

than the average of all the groups ($p < 0.05$). When analyzing the variations in body weight throughout gestation (Figure 1(B)), it is observed that on day 7, there are no significant differences between the groups. On day 14, it was observed that only the rats in the Preg group had a significantly higher weight (324.77 ± 27.47 g) compared to day 7 ($p < 0.05$). Likewise, the Preg group had a higher body weight compared to the other experimental groups ($p < 0.05$). On day 19, the Preg (385.84 ± 51.0) and Preg/Ext (337.80 ± 37.98 g) groups had a higher body weight compared to their weight on days 7 and 14 ($p < 0.05$ respectively); also the Preg/Inf group had a higher body weight (331.69 ± 20.65 g) compared to day 7 ($p < 0.05$), and both groups had a higher weight compared to the Control and Ext groups ($p < 0.05$).

3.3. Food Consumption

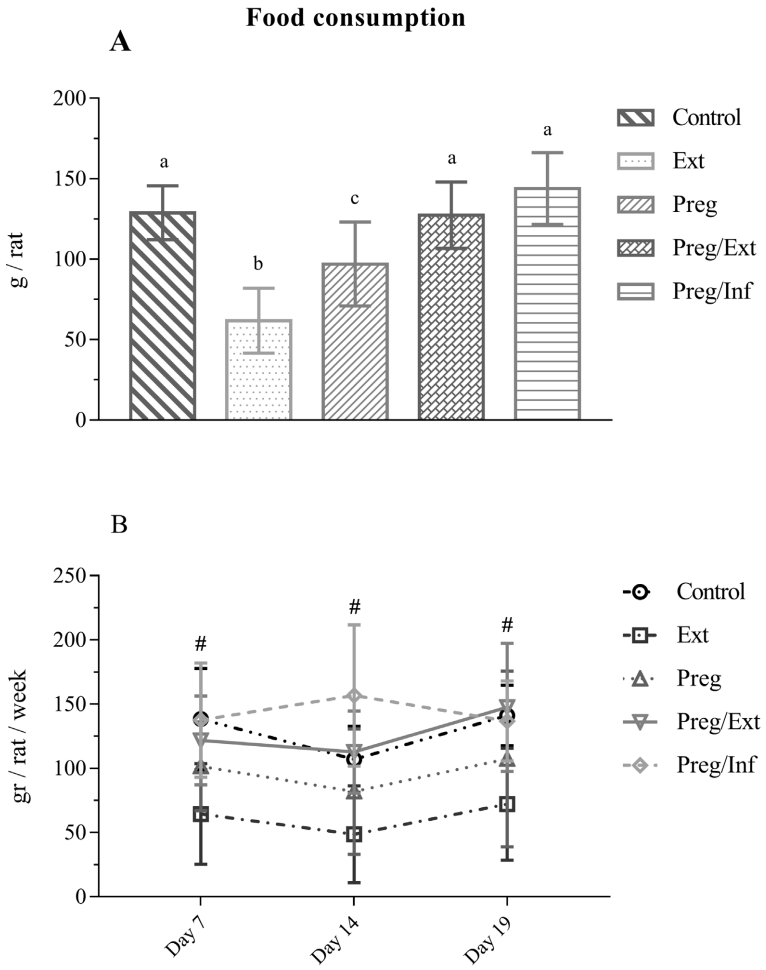

Figure 2. Amount of food consumed in all groups. (A) Food consumption during pregnancy, the bars represent the mean ± S.E.M. (B) Amount of food consumed on different days of the gestational period, each point represents the mean food consumed ± S.E.M. Different letters indicate significant differences between groups ($p < 0.05$). *$p < 0.05$ compared to the same group on a different day. #$p < 0.05$ indicates significant difference with the other groups on the same day ($p < 0.05$).

Statistical analysis of the mean feed consumption of the groups (**Figure 2(A)**) showed significant differences (F [4] = 23.9; $p < 0.001$). The control group (131.29 ± 27.38) had a similar feed consumption with the Preg/Ext (120.59 ± 26.45) and Preg/Inf (138.84 ± 40.71) groups ($p > 0.05$); while the Preg group consumed less food (96.95 ± 52.15) than the control and Preg/Inf groups ($p < 0.05$), and the Ext group showed lower food consumption (61.75 ± 40.28) compared to the other groups during gestation ($p < 0.05$).

When analyzing the variations in food consumption on days 7, 14 and 19 of gestation in all groups (**Figure 2(B)**), it was observed that within each group the amount of food ingested did not change significantly compared to the same group. On day 7, the Control (138.37 ± 39.47), Preg/Ext (101.60 ± 34.63) and Preg/Inf (119.51 ± 16.18) groups consumed a greater amount of food than the Ext (64.45 ± 39.15) and Preg (101.60 ± 34.62) groups ($p < 0.05$). Thus, the Ext and Preg groups had a significantly lower consumption ($p < 0.05$) and remained on days 14 and 19.

3.4. Blood Glucose Analysis

The glycemic analysis on the baseline day showed similar glucose concentrations between the groups (normoglycemia) (**Table 2**). However, when analyzing the data obtained from the glycemic concentration, it was observed that significant differences between the groups (F [4] = 2.427, $p < 0.05$). On day 14, the Preg/Ext group presented a decrease in glycemia (70.67 ± 3.12 mg/dL) compared to the Control group (92.08 ± 3.70 mg/dL) ($p < 0.05$).

Table 2. Effect of *Hibiscus sabdariffa* consumption on blood glucose concentration.

	Blood glucose concentration mg/dL			
	Basal	Day 7	Day 14	Day 19
Control	83.17 ± 2.94	83.00 ± 4.40	92.08 ± 3.70	88.50 ± 4.38
Ext	84.92 ± 3.66	85.25 ± 1.81	78.33 ± 4.64	83.75 ± 4.52
Preg	89.69 ± 5.15	91.69 ± 3.38	83.15 ± 2.95	82.38 ± 3.47
Preg/Ex	87.00 ± 3.97	86.58 ± 4.54	70.67 ± 3.12*	79.50 ± 3.32
Preg/Inf	87.90 ± 4.22	87.00 ± 3.06	78.25 ± 2.17	82.45 ± 3.31

*Represents significant differences ($p < 0.05$) with respect to the control group.

3.5. Serum Lipid Concentration

The analysis of serum triglycerides (**Figure 3**) did not show differences between groups (F [4] = 1.673; $p = 0.172$), the Preg/Inf group had a slightly higher concentration (139.8±72.8 mg/dL) compared to the Control (64.3 ± 18.9 mg/dL), Ext (76.5 ± 23.5 mg/dL), Preg (121.3 ± 33.9 mg/dL) and Preg/Ext (103.0 ± 19.6 mg/dL) groups ($p > 0.05$).

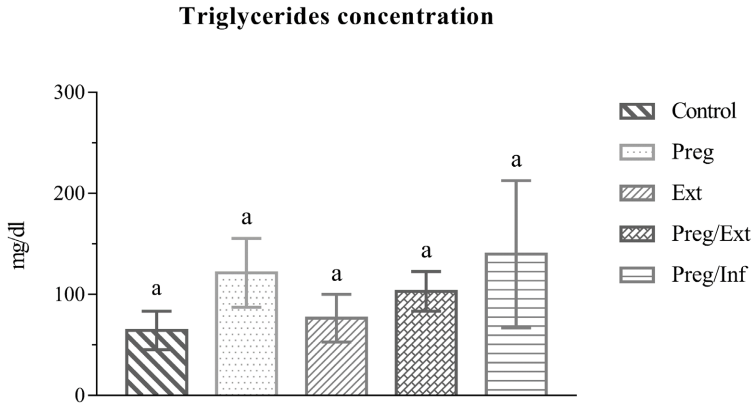

Figure 3. Serum triglyceride concentration in pregnant rats. Bars represent the mean ± S.E.M. of serum triglyceride concentration in different groups on days 7, 14 and 19. Different letters indicate significant differences between groups ($p < 0.05$).

Similar results were observed in the concentration of total serum cholesterol (**Figure 4**), with no differences between groups ($F [4] = 1.368$; $p = 0.259$). The Preg/Inf group exhibited a concentration (59.0 ± 9.4 mg/dL) similar to the Control (43.4 ± 12.2 mg/dL), Ext (34.6 ± 7.0 mg/dL), Preg (55.9 ± 25.9 mg/dL) and Preg/Ext (49.2 ± 12.2 mg/dL) groups.

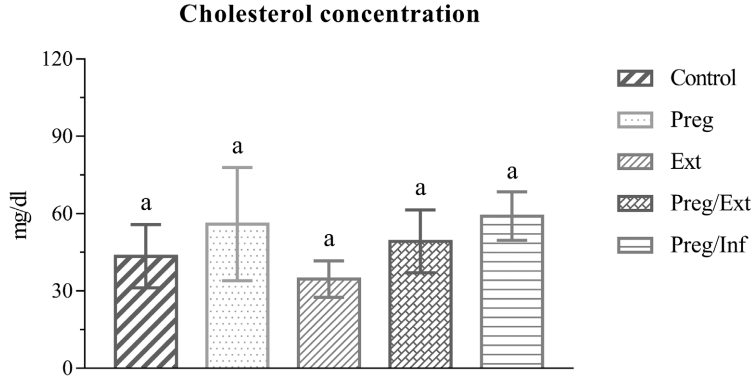

Figure 4. Total cholesterol concentration in blood serum. Serum cholesterol levels of the different groups, the bars represent the mean ± S.E.M of days 7, 14 and 19. Different letters indicate significant differences between groups ($p < 0.05$).

3.6. Somatometric Analysis of Offspring

The analysis of body weight of the fetuses (**Figure 5(A)**) showed significant differences between the groups of pregnant animals ($F [2] = 5.535$; $p < 0.05$). Differences were only observed in the offspring of the Preg/Inf group with lower weight (4.63 ± 0.88 g), compared to the weight of the Preg group (5.02 ± 0.74 g; $p < 0.05$). Meanwhile, the weight of the litter of the Preg/Ext group (4.86 ± 0.91 g) was similar to the other groups ($p > 0.05$). Regarding the size of the fetuses (**Figure 5(B)**) significant differences were found between the groups ($F [2] = 7.178$; $p < 0.05$). Fetuses in the Preg/Inf group had a smaller size (3.87 ± 0.29 cm) compared to those in the Preg (4.00 ± 0.27 cm) and Preg/Ext (3.98 ± 0.26 cm) groups. Likewise,

head length (Figure 5(C)) showed differences (F [2] = 10.022; $p < 0.01$), in fetuses of the Preg/Ext group which had the smallest length (1.56 ± 0.11 cm) compared to the Preg (1.61 ± 0.13 cm) and Preg/Inf (1.63 ± 0.10 cm) groups ($p < 0.05$).

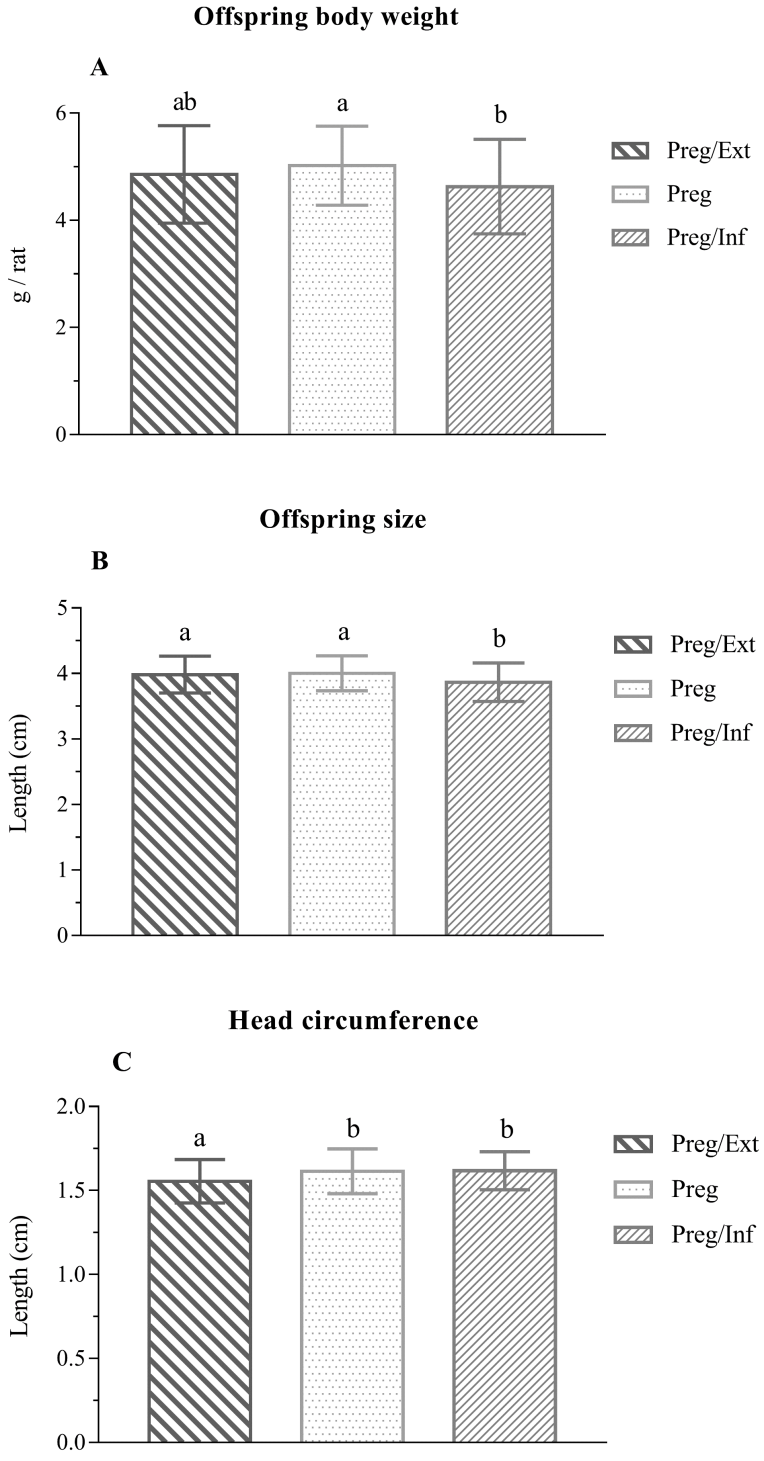

Figure 5. Somatometric analysis of fetuses on day 19 of gestation. (A) Average body weight of the fetuses. (B) Average size of the fetuses. (C) Average head circumference of the fetuses. Different letters indicate significant differences between groups ($p < 0.05$).

4. Discussion

Multiple studies have demonstrated the effects of polyphenols, such as anthocyanins, on metabolism [31]. This study evaluated the effects on body weight, food intake and glucose and lipid concentrations, as well as reproductive parameters of pregnant female rats after the ingestion of Polyphenols contained in *Hibiscus sabdariffa*, either in an infusion or from its extract with a high anthocyanin content. The results showed that the administration of the extract and the HS infusion modified the body weight of pregnant females, feed intake and the concentration of glucose in blood; on the other hand, blood lipid levels were not modified. Following the consumption of HS during gestation, the offspring showed a reduction of body weight and size; also, the anthocyanin extract significantly reduced their head circumference.

In this study, we observed a progressive increase in body weight that was significant in the Preg group compared to the Control group. In contrast, the Preg/Ant and Preg/Ext groups maintained body weights similar to the Control group and showed a reduction compared to the Preg group. These findings suggest that both the infusion and the anthocyanin fraction of *Hibiscus sabdariffa* (HS) may contain bioactive compounds associated with the regulation of weight gain during pregnancy, without inducing signs of malnutrition. The reduction in body weight may be attributed to the metabolic effects of HS polyphenols, as the anthocyanin extract administered to the Ext group also significantly decreased their body weight. In this sense, previous studies have shown that pregnancy induces chronic and systemic inflammation [32] [33], and sustained activation of the hypothalamic-pituitary-adrenal axis can alter glucose metabolism, reduce insulin sensitivity, and negatively affect the release of hormones involved in appetite and eating behavior [34] [35]. Possibly, the administration of both the infusion and the anthocyanin extract fraction of HS contributed to the attenuation of oxidative stress generated during gestation, accelerated lipid metabolism, prevented fat accumulation, and supported the regulation of intestinal microbiota and metabolic hormones in the mother [36] [37]. Furthermore, metabolic changes generated during pregnancy—such as excessive gestational weight gain—may imply an inappropriate development of pregnancy, increasing the risks of suffering from preeclampsia and post-pregnancy metabolic syndrome in the mother [38] [39]. Thus, the consumption of infusion or anthocyanin extract fraction of HS can prevent these effects due to the presence of its various polyphenol types: delphinidin 3,5-diglucoside, cyanidin-3-O-sambubioside, delphinidin-3-O-sambubioside, rutin, isoquercitrin, hyperoside and chlorogenic acid [40] [41]. Moreover, reports also indicate that the intake of *Hibiscus sabdariffa* infusion increases blood sodium levels, which could trigger an acute hypernatremia process, possibly caused by water imbalance due to the diuretic effect of HS. This condition may lead to a reduction in food intake [23], which could also contribute to the observed decrease in body weight.

Regarding blood glucose, only the Preg/Ext group reduced their blood glucose on day 14 compared to the control group. Golic *et al.* mention that, in pregnant rats,

blood glucose concentration is reduced, particularly after 7 days of pregnancy until the last days of gestation, due to a circadian variation [42]. This phenomenon could be related to the decrease in sensitivity in insulin receptors, with greater release of insulin by the β-cells of the pancreas, increased postprandial blood glucose levels and decreased blood glucose during fasting. This generates changes in the plasma levels of circulating free fatty acids, triglycerides, cholesterol and phospholipids [43] [44]. Interestingly, the decrease in insulin receptor sensitivity during pregnancy is induced by the increase in circulating blood levels of placental lactogen, progesterone, corticosterone, prolactin and 17-βestradiol [39] [45]-[47]. Evidence indicates that elements contained in HS calyces, such as anthocyanins, can interact with hormones such as estradiol and glucocorticoids, regulating their activity, expression levels or competing with their receptors [48] [49], without excluding the possibility of interaction with other endocrine elements. It is possible that the glycemic results in our work are related to the intake of HS extract with high anthocyanin content, due to changes in the activity patterns of these hormones, modifying the allostatic mechanisms of sensitivity in insulin receptors and reestablishing pro-inflammatory signals.

During pregnancy, metabolic changes in cholesterol are essential for the correct development of the pregnancy and the fetus. The initial stages are characterised by an anabolic phase that enables the generation of energy reserves for future needs. In the final stage of pregnancy, lipid metabolism transitions to a catabolic phase that constantly generates a high concentration of cholesterol and triglyceride levels, which in non-pathological conditions initiates a process to reestablish plasma levels [50]. In our work, the quantification was carried out during the terminal phase of pregnancy, which could explain the fact that no differences were found between the groups, due to the restoration of basal levels. In addition, other studies have indicated that the consumption of anthocyanins can promote a decrease in serum lipid levels [51]. It should be noted that there are no reports describing the effect of anthocyanin extract consumption or HS infusion on total cholesterol or triglyceride concentration in a pregnancy model. Therefore, we assume that the trends observed in the Preg/Ext and Preg/Inf groups are related to the bioactive components present in HS. Among the proposed mechanisms for the allostatic maintenance of cholesterol levels during pregnancy is the regulation of metabolism by hormones such as estrogen and progesterone [52], since flavonoids such as anthocyanins have been observed to have a strong influence on estrogenic activity [8], and this can regulate lipid metabolic control through endocrine mediation.

Finally, we found changes in the somatometric measurements of the fetuses in the Preg/Inf and Preg/Ext groups. It is necessary to note that the changes in the offspring should not be considered a teratogenic effect or postnatal dysfunction; however, studies are needed to confirm whether the changes observed in fetuses are due to the interaction between the bioactive compounds and the hormonal and metabolic signaling of the pregnant woman. Although there are no long-term

studies describing the effects of these changes in individuals, it has been reported that low birth weight in neonates is associated with cognitive and social developmental disorders [53]. In this regard, there is evidence that shows an interaction of flavonoids consumed from grape seeds with the fetus [54]. Although the placental transport of polyphenols may be limited and inefficient, these compounds probably have a direct effect on fetal cells. In conclusion, the consumption of HS modifies the amount of food consumed, generates metabolic changes in pregnant female rats and reductions in the somatometric proportions of the fetuses were observed. All of this generates the need to continue studying the mechanisms associated with somatometric characteristics and to determine whether there are long-term functional changes in neonates.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Ahad, A., Raish, M., Bin Jardan, Y.A., Alam, M.A., Al-Mohizea, A.M. and Al-Jenoobi, F.I. (2020) Effect of *Hibiscus sabdariffa* and *Zingiber officinale* on the Antihypertensive Activity and Pharmacokinetic of Losartan in Hypertensive Rats. *Xenobiotica*, **50**, 847-857. https://doi.org/10.1080/00498254.2020.1729446

[2] Onuka, A.E., Emmanuel, M. and Egbengu, E.P. (2014) Anti-Inflammatory Activity of Methanol Extract of *Hibiscus sabdariffa* in Comparison with Aspirin. *The Pharma Innovation*, **3**, 42-47.

[3] Lans, C.A. (2006) Ethnomedicines Used in Trinidad and Tobago for Urinary Problems and Diabetes Mellitus. *Journal of Ethnobiology and Ethnomedicine*, **2**, Article No. 45. https://doi.org/10.1186/1746-4269-2-45

[4] Peng, C., Chyau, C., Chan, K., Chan, T., Wang, C. and Huang, C. (2011) *Hibiscus sabdariffa* Polyphenolic Extract Inhibits Hyperglycemia, Hyperlipidemia, and Glycation-Oxidative Stress While Improving Insulin Resistance. *Journal of Agricultural and Food Chemistry*, **59**, 9901-9909. https://doi.org/10.1021/jf2022379

[5] Alarcón-Alonso, J., Zamilpa, A., Aguilar, F.A., Herrera-Ruiz, M., Tortoriello, J. and Jimenez-Ferrer, E. (2012) Pharmacological Characterization of the Diuretic Effect of *Hibiscus sabdariffa* Linn (Malvaceae) Extract. *Journal of Ethnopharmacology*, **139**, 751-756. https://doi.org/10.1016/j.jep.2011.12.005

[6] Prenesti, E., Berto, S., Daniele, P.G. and Toso, S. (2007) Antioxidant Power Quantification of Decoction and Cold Infusions of *Hibiscus sabdariffa* Flowers. *Food Chemistry*, **100**, 433-438. https://doi.org/10.1016/j.foodchem.2005.09.063

[7] Sanou, A., Konate, K., Dakuyo, R., Kabore, K., Sama, H. and Dicko, M.H. (2022) *Hibis-*

cus sabdariffa: Genetic Variability, Seasonality and Their Impact on Nutritional and Antioxidant Properties. *PLOS ONE*, **17**, e0261924. https://doi.org/10.1371/journal.pone.0261924

[8] Lorenzana-Martínez, G., Santerre, A., Andrade-González, I. and Bañuelos-Pineda, J. (2020) Effects of *Hibiscus sabdariffa* Calyces on Spatial Memory and Hippocampal Expression of BDNF in Ovariectomized Rats. *Nutritional Neuroscience*, **25**, 670-680. https://doi.org/10.1080/1028415x.2020.1804095

[9] Owoeye, O. and Gabriel, M.O. (2017) Evaluation of Neuroprotective Effect of *Hibiscus sabdariffa* Linn. Aqueous Extract against Ischaemic-Reperfusion Insult by Bilateral Common Carotid Artery Occlusion in Adult Male Rats. *Nigerian Journal of Physiological Sciences*, **32**, 97-104.

[10] Obouayeba, A., Boyvin, L., MBoh, G., Diabat, S., Kouakou, T., Djaman, A., *et al.* (2014) Hepatoprotective and Antioxidant Activities of *Hibiscus sabdariffa* Petal Extracts in Wistar Rats. *International Journal of Basic & Clinical Pharmacology*, **3**, 774-780. https://doi.org/10.5455/2319-2003.ijbcp20141034 https://www.ijbcp.com/index.php/ijbcp/article/view/1084

[11] Fullerton, M., Khatiwada, J., Johnson, J.U., Davis, S. and Williams, L.L. (2011) Determination of Antimicrobial Activity of Sorrel (*Hibiscus sabdariffa*) on *Esherichia coli* O157:H7 Isolated from Food, Veterinary, and Clinical Samples. *Journal of Medicinal Food*, **14**, 950-956. https://doi.org/10.1089/jmf.2010.0200

[12] Da-Costa-Rocha, I., Bonnlaender, B., Sievers, H., Pischel, I. and Heinrich, M. (2014) *Hibiscus sabdariffa* L.—A Phytochemical and Pharmacological Review. *Food Chemistry*, **165**, 424-443. https://doi.org/10.1016/j.foodchem.2014.05.002

[13] Carvajal-Zarrabal, O., Hayward-Jones, P.M., Orta-Flores, Z., Nolasco-Hipólito, C., Barradas-Dermitz, D.M., Aguilar-Uscanga, M.G., *et al.* (2009) Effect of *Hibiscus sabdariffa* L. Dried Calyx Ethanol Extract on Fat Absorption-Excretion, and Body Weight Implication in Rats. *BioMed Research International*, **2009**, Article ID: 394592. https://doi.org/10.1155/2009/394592

[14] Montalvo-González, E., Villagrán, Z., González-Torres, S., Iñiguez-Muñoz, L., Isiordia-Espinoza, M., Ruvalcaba-Gómez, J., *et al.* (2022) Physiological Effects and Human Health Benefits of *Hibiscus Sabdariffa*: A Review of Clinical Trials. *Pharmaceuticals*, **15**, Article 464. https://doi.org/10.3390/ph15040464

[15] Ramli, I., Posadino, A.M., Giordo, R., Fenu, G., Fardoun, M., Iratni, R., *et al.* (2023) Effect of Resveratrol on Pregnancy, Prenatal Complications and Pregnancy-Associated Structure Alterations. *Antioxidants*, **12**, Article 341. https://doi.org/10.3390/antiox12020341

[16] Osredkar, J. (2023) Impact of Endogenic and Exogenic Oxidative Stress Triggers on Pregnant Woman, Fetus, and Child. *International Journal of Molecular Sciences*, **24**, Article 5958. https://doi.org/10.3390/ijms24065958

[17] Scazzocchio, B., Filardi, T., Varì, R., Brunelli, R., Galoppi, P., Morano, S., *et al.* (2021) Protocatechuic Acid Influences Immune-Metabolic Changes in the Adipose Tissue of Pregnant Women with Gestational Diabetes Mellitus. *Food & Function*, **12**, 7490-7500. https://doi.org/10.1039/d1fo00267h

[18] Eghosa, I.E., Charles, E.O., Thomas, N. and Ifeoma, O.A. (2015) Effects of *Hibiscus sabdariffa* L. Consumption during Pregnancy on Biochemical Parameters and Litter Birth Weight in Rats. *African Journal of Traditional, Complementary and Alternative Medicines*, **12**, 79-83. https://doi.org/10.21010/ajtcam.v12i5.12

[19] Jouanne, M., Oddoux, S., Noël, A. and Voisin-Chiret, A.S. (2021) Nutrient Requirements during Pregnancy and Lactation. *Nutrients*, **13**, Article 692.

https://doi.org/10.3390/nu13020692

[20] Marshall, N.E., Abrams, B., Barbour, L.A., Catalano, P., Christian, P., Friedman, J.E., *et al.* (2022) The Importance of Nutrition in Pregnancy and Lactation: Lifelong Consequences. *American Journal of Obstetrics and Gynecology*, **226**, 607-632. https://doi.org/10.1016/j.ajog.2021.12.035

[21] Hambidge, K.M., Bann, C.M., McClure, E.M., Westcott, J.E., Garcés, A., Figueroa, L., *et al.* (2019) Maternal Characteristics Affect Fetal Growth Response in the Women First Preconception Nutrition Trial. *Nutrients*, **11**, Article 2534. https://doi.org/10.3390/nu11102534

[22] Yao, X., Liu, R., Li, X., Li, Y., Zhang, Z., Huang, S., *et al.* (2021) Zinc, Selenium and Chromium Co-Supplementation Improves Insulin Resistance by Preventing Hepatic Endoplasmic Reticulum Stress in Diet-Induced Gestational Diabetes Rats. *The Journal of Nutritional Biochemistry*, **96**, Article ID: 108810. https://doi.org/10.1016/j.jnutbio.2021.108810

[23] Iyare, E.E., Adegoke, O.A. and Nwagha, U.I. (2010) Mechanism of Delayed Puberty in Rats Whose Mothers Consumed *Hibiscus sabdariffa* during Lactation. *Pharmaceutical Biology*, **48**, 1170-1176. https://doi.org/10.3109/13880200903567120

[24] de Arruda, A., Cardoso, C.A.L., Vieira, M.D.C. and Arena, A.C. (2016) Safety Assessment of *Hibiscus sabdariffa* after Maternal Exposure on Male Reproductive Parameters in Rats. *Drug and Chemical Toxicology*, **39**, 22-27. https://doi.org/10.3109/01480545.2014.1003938

[25] Okasha, H.S., Helal, E.G.E., Mansoury, M.M.S., Alsrehy, G.M. and Khattab, H.A.H. (2024) The Effects of *Hibiscus sabdariffa* and *Salvia officinalis* Aqueous Extracts on Reproductive Function, Antioxidant Status, and Epididymal Sperm Quality in Male Rats. *Advancements in Life Sciences*, **11**, 104-111.

[26] Bako, I.G., Abubakar, M.S., Mabrouk, M.A. and Mohammed, A. (2014) Lactogenic Study of the Effect of Ethyl-Acetate Fraction of *Hibiscus sabdariffa* Linn (*Malvaceae*) Seed on Serum Prolactin Level in Lactating Albino Rats. *Advance Journal of Food Science and Technology*, **6**, 292-296. https://doi.org/10.19026/ajfst.6.26

[27] Farias-Cervantes, V.S., Chávez-Rodríguez, A., García-Salcedo, P.A., García-López, P.M., Casas-Solís, J. and Andrade-González, I. (2018) Antimicrobial Effect and *in Vitro* Release of Anthocyanins from Berries and Roselle Obtained via Microencapsulation by Spray Drying. *Journal of Food Processing and Preservation*, **42**, e13713. https://doi.org/10.1111/jfpp.13713

[28] Lee, J., Durst, R.W., Wrolstad, R.E., Eisele, T., Giusti, M.M., Hach, J., *et al.* (2005) Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the Ph Differential Method: Collaborative Study. *Journal of AOAC International*, **88**, 1269-1278. https://doi.org/10.1093/jaoac/88.5.1269

[29] Lohmiller, J.J., Swing, S.P. and Hanson, M.M. (2020) Reproduction and Breeding. In: Suckow, M.A., Claire Hankenson, F., *et al.*, Eds., *The Laboratory Rat*, Elsevier, 157-179. https://doi.org/10.1016/b978-0-12-814338-4.00006-4

[30] Zhou, P., Zhang, X., Xu, Z., Gao, S., Zheng, Q., Xu, M., *et al.* (2017) Staphylococcal Enterotoxin B Administration in Pregnant Rats Alters the Splenic Lymphocyte Response in Adult Offspring Rats. *BMC Microbiology*, **17**, Article No. 1. https://doi.org/10.1186/s12866-016-0921-2

[31] Lee, Y., Yoon, Y., Yoon, H., Park, H., Song, S. and Yeum, K. (2017) Dietary Anthocyanins against Obesity and Inflammation. *Nutrients*, **9**, Article 1089. https://doi.org/10.3390/nu9101089

[32] Fialová, L., Malbohan, I., Kalousová, M., Soukupová, J., Krofta, L., Štípek, S., *et al.* (2006) Oxidative Stress and Inflammation in Pregnancy. *Scandinavian Journal of Clinical and Laboratory Investigation*, **66**, 121-128. https://doi.org/10.1080/00365510500375230

[33] Holingue, C., Owusu, J.T., Feder, K.A. and Spira, A.P. (2018) Sleep Duration and C-Reactive Protein: Associations among Pregnant and Non-Pregnant Women. *Journal of Reproductive Immunology*, **128**, 9-15. https://doi.org/10.1016/j.jri.2018.05.003

[34] Bose, B. and Shenoy P, S. (2013) Non Insulin Producing Cell Line, MIA PaCa-2 Is Rendered Insulin Producing *in Vitro* via Mesenchymal Epithelial Transition. *Journal of Cellular Biochemistry*, **114**, 1642-1652. https://doi.org/10.1002/jcb.24506

[35] Ulrich-Lai, Y.M., Fulton, S., Wilson, M., Petrovich, G. and Rinaman, L. (2015) Stress Exposure, Food Intake and Emotional State. *Stress*, **18**, 381-399.

[36] Chaiittianan, R., Sutthanut, K. and Rattanathongkom, A. (2017) Purple Corn Silk: A Potential Anti-Obesity Agent with Inhibition on Adipogenesis and Induction on Lipolysis and Apoptosis in Adipocytes. *Journal of Ethnopharmacology*, **201**, 9-16. https://doi.org/10.1016/j.jep.2017.02.044

[37] Jayarathne, S., Stull, A.J., Park, O., Kim, J.H., Thompson, L. and Moustaid-Moussa, N. (2019) Protective Effects of Anthocyanins in Obesity-Associated Inflammation and Changes in Gut Microbiome. *Molecular Nutrition & Food Research*, **63**, Article ID: 1900149. https://doi.org/10.1002/mnfr.201900149

[38] Poblete, J.A. and Olmos, P. (2020) Obesity and Gestational Diabetes in Pregnant Care and Clinical Practice. *Current Vascular Pharmacology*, **19**, 154-164. https://doi.org/10.2174/1570161118666200628142353

[39] Wu, Y., Hankinson, S.E., Smith-Warner, S.A., Wang, M. and Eliassen, A.H. (2019) Flavonoid Intake and Plasma Sex Steroid Hormones, Prolactin, and Sex Hormone-Binding Globulin in Premenopausal Women. *Nutrients*, **11**, Article 2669. https://doi.org/10.3390/nu11112669

[40] Hopkins, A.L., Lamm, M.G., Funk, J.L. and Ritenbaugh, C. (2013) *Hibiscus sabdariffa* L. in the Treatment of Hypertension and Hyperlipidemia: A Comprehensive Review of Animal and Human Studies. *Fitoterapia*, **85**, 84-94. https://doi.org/10.1016/j.fitote.2013.01.003

[41] Yagi, S., Uba, A.I., Sinan, K.I., Piatti, D., Sagratini, G., Caprioli, G., *et al.* (2023) Comparative Study on the Chemical Profile, Antioxidant Activity, and Enzyme Inhibition Capacity of Red and White *Hibiscus sabdariffa* Variety Calyces. *ACS Omega*, **8**, 42511-42521. https://doi.org/10.1021/acsomega.3c05217

[42] Golic, M., Kräker, K., Fischer, C., Alenina, N., Haase, N., Herse, F., *et al.* (2018) Continuous Blood Glucose Monitoring Reveals Enormous Circadian Variations in Pregnant Diabetic Rats. *Frontiers in Endocrinology*, **9**, Article 271. https://doi.org/10.3389/fendo.2018.00271

[43] Drynda, R., Peters, C., Jones, P. and Bowe, J. (2014) The Role of Non-Placental Signals in the Adaptation of Islets to Pregnancy. *Hormone and Metabolic Research*, **47**, 64-71. https://doi.org/10.1055/s-0034-1395691

[44] Wang, M., Xia, W., Li, H., Liu, F., Li, Y., Sun, X., *et al.* (2018) Normal Pregnancy Induced Glucose Metabolic Stress in a Longitudinal Cohort of Healthy Women: Novel Insights Generated from a Urine Metabolomics Study. *Medicine*, **97**, e12417. https://doi.org/10.1097/md.0000000000012417

[45] Kirschbaum, C., Tietze, A., Skoluda, N. and Dettenborn, L. (2009) Hair as a Retrospective Calendar of Cortisol Production—Increased Cortisol Incorporation into Hair in the Third Trimester of Pregnancy. *Psychoneuroendocrinology*, **34**, 32-37. https://doi.org/10.1016/j.psyneuen.2008.08.024

[46] Nanashima, N., Horie, K. and Maeda, H. (2017) Phytoestrogenic Activity of Blackcurrant Anthocyanins Is Partially Mediated through Estrogen Receptor Beta. *Molecules*, **23**, Article 74. https://doi.org/10.3390/molecules23010074

[47] Wagner, L., Aurich, J., Melchert, M., Okada, C.T.C., Gautier, C., Kaps, M., *et al.* (2023) Low Progesterone Concentration in Early Pregnancy Is Detrimental to Conceptus Development and Pregnancy Outcome in Horses. *Animal Reproduction Science*, **257**, Article ID: 107334. https://doi.org/10.1016/j.anireprosci.2023.107334

[48] Casagrande, S., DeMoranville, K.J., Trost, L., Pierce, B., Bryła, A., Dzialo, M., *et al.* (2020) Dietary Antioxidants Attenuate the Endocrine Stress Response during Long-Duration Flight of a Migratory Bird. *Proceedings of the Royal Society B*: *Biological Sciences*, **287**, Article ID: 20200744. https://doi.org/10.1098/rspb.2020.0744

[49] Calfío, C., Donoso, F. and Huidobro-Toro, J.P. (2021) Anthocyanins Activate Membrane Estrogen Receptors with Nanomolar Potencies to Elicit a Nongenomic Vascular Response via NO Production. *Journal of the American Heart Association*, **10**, e020498. https://doi.org/10.1161/jaha.119.020498

[50] Formisano, E., Proietti, E., Perrone, G., Demarco, V., Galoppi, P., Stefanutti, C., *et al.* (2024) Characteristics, Physiopathology and Management of Dyslipidemias in Pregnancy: A Narrative Review. *Nutrients*, **16**, Article 2927. https://doi.org/10.3390/nu16172927

[51] Danielewski, M., Matuszewska, A., Szeląg, A. and Sozański, T. (2021) The Impact of Anthocyanins and Iridoids on Transcription Factors Crucial for Lipid and Cholesterol Homeostasis. *International Journal of Molecular Sciences*, **22**, Article 6074. https://doi.org/10.3390/ijms22116074

[52] Desoye, G., Schweditsch, M.O., Pfeiffer, K.P., Zechner, R. and Kostner, G.M. (1987) Correlation of Hormones with Lipid and Lipoprotein Levels during Normal Pregnancy and Postpartum. *The Journal of Clinical Endocrinology & Metabolism*, **64**, 704-712. https://doi.org/10.1210/jcem-64-4-704

[53] Zwicker, J.G. and Harris, S.R. (2008) Quality of Life of Formerly Preterm and Very Low Birth Weight Infants from Preschool Age to Adulthood: A Systematic Review. *Pediatrics*, **121**, e366-e376. https://doi.org/10.1542/peds.2007-0169

[54] Arola-Arnal, A., Oms-Oliu, G., Crescenti, A., del Bas, J.M., Ras, M.R., Arola, L., *et al.* (2013) Distribution of Grape Seed Flavanols and Their Metabolites in Pregnant Rats and Their Fetuses. *Molecular Nutrition & Food Research*, **57**, 1741-1752. https://doi.org/10.1002/mnfr.201300032