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Physiological changes associated with caffeine consumption and the modulating effects of ascorbic acid in pregnant Wistar rats

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Abstract

Pregnant women commonly consume caffeine to avoid fatigue or as a habit. However, it has not been clearly determined what is its side effects on their bodies. The aim of this study was to evaluate the physiological changes associated with caffeine consumption and the modulating effects of ascorbic acid in pregnant Wistar rats. Forty nulliparous female Wistar rats were mated with male rats in a ratio of 2:1. Then they were monitored for pregnancy and randomly divided into four groups of ten rats each. Group A received distilled water while group B received caffeine at 0.3 g/l in drinking water. Group C received ascorbic acid at 100 mg/kg while group D received both caffeine and ascorbic Acid at 0.3 g/l and 100 mg/kg respectively. Their body weights were recorded every 3 days throughout the gestation period, while water consumption was monitored and recorded daily as well as the gestation length. At parturition the birth weight and litter size of the pups were recorded. There was a significant ($p < 0.05$) decrease in weight gain and gestation length but a significant ($p < 0.05$) increase in water consumption in the Dams administered caffeine when compared with the control. A significant ($p < 0.05$) decrease in birth weight of the pups was observed in the caffeine treated group as well as a significant ($p < 0.05$) decrease in litter size in the group that was administered only ascorbic acid when compared with the control. Ascorbic acid was able to modulate these changes in Wistar rats.

Keywords: Caffeine; Ascorbic acid; Gestation length; Litter size; Weight; Wistar rats

1. Introduction

Caffeine (1,3,7-trimethylxanthine) is a psychostimulant purine-like alkaloid, which is found naturally in coffee, tea, cacao beans guarana, mate, and kola nuts, though it has been identified in more than 60 plant species [1]. Caffeine's action is thought to be mediated via several mechanisms: the antagonism of adenosine receptors, the inhibition of phosphodiesterase, the release of calcium from intracellular stores, and the antagonism of benzodiazepine receptors [2]. Caffeine is frequently consumed by pregnant women [3], of which coffee, tea, soda, and chocolate are the main sources of caffeine consumption among pregnant women [4].

During pregnancy, caffeine is commonly consumed to avoid drowsiness or as a habit [5, 6]. Pregnancy is a unique process in which a woman's body constantly works to protect itself and the growing fetus [7] thereby maintaining homeostasis. While the impact of caffeine on adult physiology has been well-studied, its influence on reproductive outcomes, particularly litter size, in animal models like rats is less understood. [8, 9]. Pregnant women have a slower caffeine metabolism, with 1.5 to 3.5 times longer half-life needed to eliminate caffeine, compared to non-pregnant woman [10, 11]. There are reports that caffeine changes estrogen levels in women [12]. Since estrogen has a neuroprotective or neurotrophic effect and regulates the dopamine system of the black striatum [13], estrogen regulates the effect of caffeine on the dopamine system and suggests that a complex interaction between caffeine, estrogen, and

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dopamine exists in the basal ganglia system [14]. Furthermore, caffeine is a stimulant for the central nervous system that can penetrate biological membranes, including the blood–brain barrier and placental barrier, and it maintains arousal function in the brain as a nonspecific potent inhibitor of the A_1 and A_{2A} adenosine receptors that promote drowsiness [15].

Epidemiological data reported that the daily average caffeine intake during pregnancy varies from 300 to 500 mg, which is equivalent to approximately three to five cups of coffee a day [16, 17]. Many worldwide health agencies recommend that the maximum daily maternal consumption of caffeine should not exceed 200–300 mg/day [18]. Yet, consensus on the safe dose of daily caffeine intake during pregnancy remains a concern [19, 20].

Ascorbic acid (AA) is the most widely used vitamin supplement throughout the world [21]. Ascorbic acid is an essential micro-nutrient required for many physiological functions and is considered as an important neuroprotective agent [22]. Ascorbic acid has three biological actions of particular relevance to reproduction, each dependent on its role as a reducing agent: it is required for the biosynthesis of collagen, for the biosynthesis of steroid and peptide hormones, and to prevent or reduce the oxidation of biomolecules [23, 24]. It is widely used for the scavenging of free radicals, strengthening the immune system and prevention of chemical carcinogenesis induced by a number of xenobiotics [25, 26].

Studies have reported inconsistent conclusions about the effects of caffeine intake during pregnancy [27] and considering the prevalence of caffeine consumption by pregnant women, a slight elevation in risk could produce a significant impact at the population level. As a result of paucity of reports on caffeine consumption among pregnant animals in the past decade, and limitations in previous studies [4], this study was designed to investigate the effect of caffeine consumption on some physiological parameters in the pregnant animals and determine how ascorbic acid can ameliorate these effects using Wistar rats.

2. Materials and method

2.1. Materials

Caffeine was purchased from Sigma Aldrich, Canada, Ascorbic acid was purchased from Nature's Field USA,

2.2. Method

Sixty Parent animals were used for this experiment and they consist of twenty males and forty nulliparous female Wistar rats (90-120 days old) weighing between 133 g - 218 g. They were obtained from the animal house of the College of Medical Sciences, Benue State University, Makurdi, Nigeria. They were housed in the laboratory of the Department of Pharmacology and Toxicology, College of Veterinary Medicine, Joseph Sarwuan Tarka University, Makurdi, Nigeria. The animals were maintained in a 12L: 12D cycle and provided with standard food (Chikun growers mash) and water *ad libitum*. The animals were allowed to acclimatize to laboratory conditions for a period of two weeks before the commencement of the experiment.

The females were housed overnight with the male rats and the presence of vaginal plug on the perineum was registered as an index of pregnancy and was referred to as gestational day 0 (GD 0). Pregnant females were weighed and housed individually in standard cages and were randomly assigned to one of the four (4) groups and treated as follows:

Group A - Control (n = 10) - The rats received only distilled water. Group B - The rats in this group were given caffeine at a dose of 0.3 g/l in drinking water (n = 10). Group C - Rats in this group received AA at a dose of 100 mg/kg in drinking water (n =10). Group D - Rats in this group received Caffeine + AA at a dose of 0.3 g/l and 100 mg/kg respectively in drinking water (n = 10).

Maternal weight was recorded on different gestational days (0, 3, 6, 9, 12, 15, 18). Drinking water was changed and the amount consumed was recorded daily. Briefly, 100mls of water was placed in the drinking troughs of the dams daily. After 24 hours, the amount of water left in the trough was subtracted from 100mls. This accounted for the amount of water consumed each day. Gestation length was determined on the day of parturition, and twenty-four hours after parturition, all pups were weighed and the number of pups per litter (litter size) was recorded.

3. Results

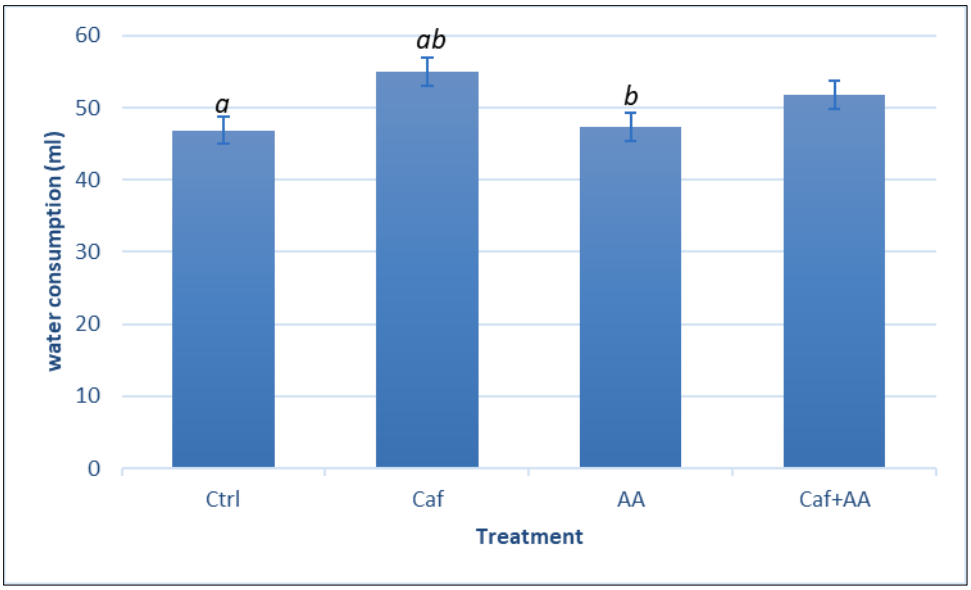
The results of investigating the Effects of Caffeine and Ascorbic acid administration on maternal weight of Dams is presented in Table 1. There was a significant (P<0.05) decrease in weight of dams in the caffeine treated group (215±4.74 g) on GD18 compared to the weight of dams in the control and the AA treated group which was 243±8.2 g and 252±6.90 g respectively. (Table 1)

Table 1 Effects of Caffeine and Ascorbic acid administration on maternal weight (g) of Dams

Groups	A	B	C	D	p value
Gestation period					
GD0	167±9.4	170±7.4	172±8.0	163±8.7	>0.05
GD3	177±8.3	177±7.1	185±5.9	174±8.1	>0.05
GD9	192±7.2	190±8.3	201±6.7	198±6.7	>0.05
GD15	226±9.4	203±6.7	231±7.8	208±5.7	>0.05
GD18	243±8.2^{a}	215±4.7^{a*}	252±6.9^{*b}	223±6.9^{b}	<0.01

^{a,b*} = Means with different superscript letters in each row are significantly (P < 0.05) different; GD means gestational day; A - Control ; B – Caffeine; C - Ascorbic acid; D – Caffeine + Ascorbic acid

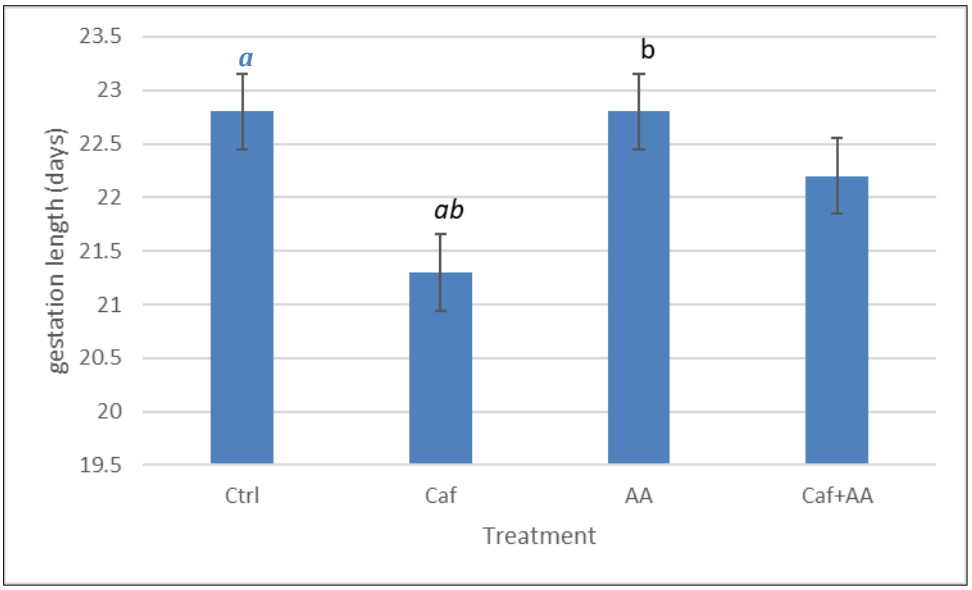
The present study showed a significant (P<0.05) increase in water consumption for the caffeine treated groups when compared to the other groups (Fig 1). The group consumed an average of 55.0±1.95 ml of fluid daily while groups A, C and D consumed 46.9±0.77 ml, 47.3±1.64 ml and 51.80±1.41 ml respectively.



^{a,b} = Means with different superscript letters in the chart are significantly (P < 0.05) different; Ctrl - Control group, Caf – Caffeine group, AA - Ascorbic acid group, Caf+AA – Caffeine + Ascorbic acid

Figure 1 Effects of Caffeine and AA administration on water consumption in Pregnant Rats

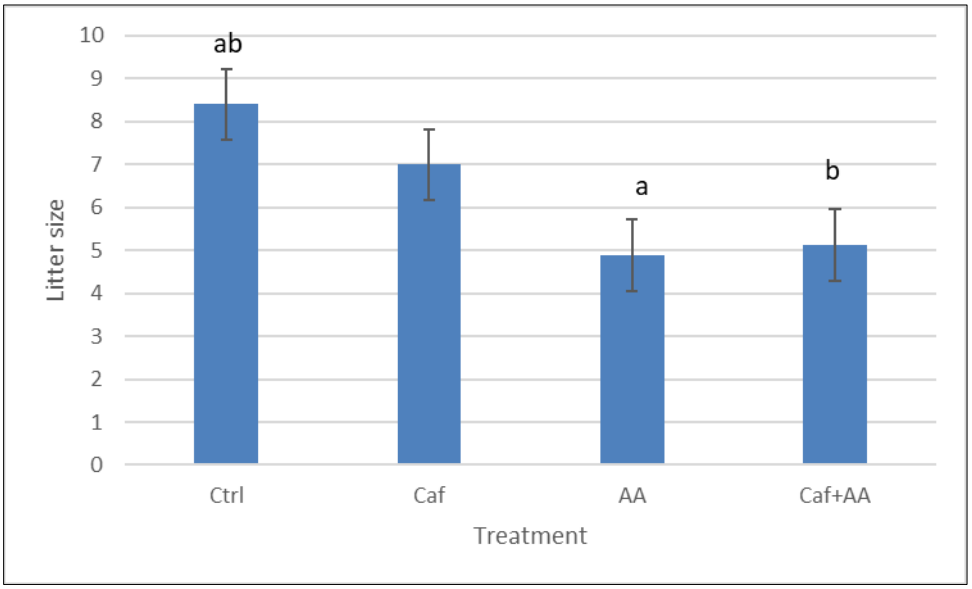
In this study, the caffeine treated group had the shortest gestation length (21.33±0.33 days) and it was significantly (P<0.05) shorter than the gestation length of dams in the control group (22.78±0.28 days) and the AA (22.78±0.28 days) treated groups (Fig. 2)



[a,b] = Means with different superscript letters in the chart are significantly (P < 0.05) different

Figure 2 Effects of Caffeine and AA administration on gestation length in Pregnant rats

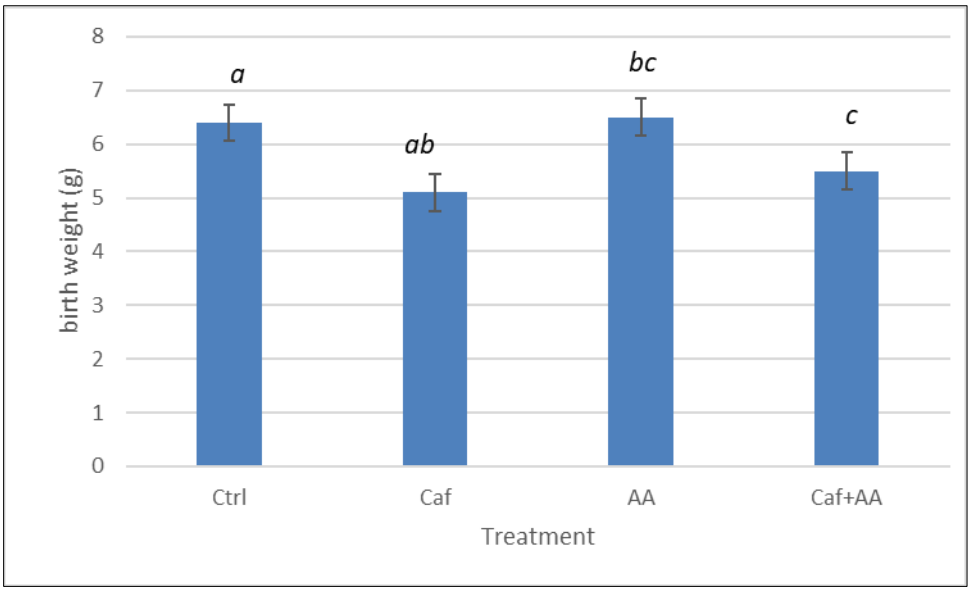
There was a significant (P<0.05) decrease in the litter size for the groups administered AA when compared to the control group. Group AA animals had a litter size of 4.88±0.61 while group Caf+AA had 5.13±0.64 and the control group had a litter size of 8.4±0.63 (Fig. 3)



[a,b] = Means with different superscript letters in the chart are significantly (P < 0.05) different; Ctrl - Control group, Caf – Caffeine group, AA - Ascorbic acid group, Caf+AA – Caffeine + Ascorbic acid

Figure 3 Effects of Caffeine and AA administration on Litter size

There was a significant (P<0.05) decrease in the birth weight of pups administered caffeine when compared to the control and AA groups. The caffeine treated group had the lowest birth weight of 5.12±0.36 g while the group administered only AA had the highest birth weight of 6.51±0.22 (Fig.4)



^{a,b,c} = Means with different superscript letters in the chart are significantly (P < 0.05) different

Figure 4 Effects of Caffeine and AA administration on birth weight of pups

4. Discussion

In the present study, the significant decrease in weight gain in the female rats treated with caffeine during gestation is in agreement with those of Owolabi and Shokunbi [28] especially as regards to GD18. Caffeine increases energy expenditure (EE) by 4-5% and 10-16% of fat oxidation and decreases the energy intake by the sympathetic nervous system (SNS) activation [29]. The activation of SNS has been shown to suppress hunger, enhance satiety, and stimulate EE, in part by increasing fat oxidation [30]. The intracellular signal, which produces increased lipolysis, heat production in skeletal muscle, and putative satiety signals in the liver, is dependent on the production and presence of cyclic adenosine monophosphate (cAMP). The reduction in weight gain seen in the present study may also be due to a reduction in placental weight as reported by Owolabi and Shokunbi [28], as caffeine has been reported to cause a reduction in placental blood flow following maternal consumption. Though placental weight was not determined in this study, Paula et al. [5] recently reported that caffeine has detrimental effects on placental vasculature.

Pretreatment with Ascorbic acid (AA) on the other hand caused a significant increase in weight gain when compared to all the other groups. There has been no study to this effect but some studies have shown that Vit. C or AA causes weight loss while other studies concluded that AA cause weight gain in individuals genetically predisposed to obesity [31, 32]. Garcia-Diaz et al. [33] suggests that vitamin C has the ability to modulate adipocyte lipolysis, regulate the release of glucocorticoids by the adrenal glands, inhibit glucose metabolism and leptin release by isolated adipocytes, reduce hyperglycemia and glycosylation in obese-diabetic models, and reduce the inflammatory response. Our study however involves pregnant animals with different physiological or humoural profile which could have caused the significant increase in weight gain.

In this study, the significant decrease in gestation length in the caffeine treated group is in agreement with Sengpiel et al. [34]. The mechanism by which caffeine causes this decrease is not well understood but in humans, parturition depends on physiological inflammatory reaction leading to cervical ripening and increased uterine tone. Caffeine is known to increase cortisol levels, and this could have contributed to shortening the gestation length [35]. AA is known to reduce cortisol levels and may be responsible for the slight increase in gestation length observed in the Caf+AA group.

In this study, the increase in water consumption in the Caffeine treated group when compared to other groups is in agreement with Zhang et al. [36]. Caffeine is generally recognized as a diuretic agent [36], however the underlining mechanism of caffeine induced diuresis is not clear. It has been postulated that methylxanthines such as caffeine can inhibit phosphodiesterases in the proximal tubule of the kidneys, which may contribute to the diuretic effect [37]. Evidence shows that caffeine acts on the kidneys by inhibiting sodium reabsorption in the proximal and distal tubules thus increasing the solute excretion and consequently free water excretion. Caffeine has also been reported to be metabolized slower in females than males, thus exerting diuretic effects longer in females than in males [36]. This may

explain in part the significant increase in water consumption, as an increase in diuresis may lead to an increase in fluid consumption.

In this study the significant decrease in birth weight observed in the caffeine treated group when compared to the other groups is in disagreement with Yadegari et al. [38] who reported no significant difference in birth weight of pups born to dams administered with caffeine during gestation. However, it is in agreement with several other workers [39, 40, 4] who have reported significant decreases in birth weight of pups born to dams that consumed caffeine during gestation. Birth weight is influenced by both duration of gestation and rate of foetal growth, therefore, low birth weight can result from preterm delivery, insufficient foetal growth or both [4]. Hence, it seems likely that the association between maternal caffeine intake and the low birth weight in this study was due to both lower foetal growth and the shorter gestational length seen in our study. The exact mechanism through which caffeine impairs foetal growth remains unsettled. However, one of the hypothesized mechanisms is that caffeine increases the release of catecholamines, which may lead to vasoconstriction in the utero-placental circulation causing foetal hypoxia and eventually affect foetal growth and development. Also caffeine acts as an antagonist of adenosine, making adenosine unable to regulate the local blood flow during hypoxia.

The effects of caffeine on litter size in rats is not well understood [9], however there is an inverse relationship between fetal weight and litter size [41, 42]. Therefore, the significant decrease in litter size in the AA group and the CAF+AA groups maybe due to the larger fetal weight in this groups when compared to the caffeine treated group. Other factors influencing litter size such as age and genetic constitution of the dams were not considered in this study.

5. Conclusion

Caffeine administration to pregnant rats causes a significant decrease in weight gain and an increase in water consumption during the gestation period with AA modifying these changes. Caffeine also caused a significant decrease in gestational length with AA modifying this change marginally. It is therefore recommended that more work should be done to elucidate the mechanisms by which AA interacts or interferes with caffeine action.

Compliance with ethical standards

Disclosure of conflict of interest

The authors have no conflicts of interest regarding this investigation.

Statement of ethical approval

All procedures used in this study complied with the guidelines on animal care of the College of Veterinary Medicine Ethics Committee on the Use of Animals which follows the 'Principles of laboratory animal care' with ethical clearance number JOSTUM/CVMETHICS/2023(7).

References

[1] Willson, C. The clinical toxicology of caffeine: A review and case study. Toxicology Reports 2018; 5: 1140–1152

[2] Jee, H. J., Lee, S. G., Bormate, K. J. and Jung, Y. Effect of Caffeine Consumption on the Risk for Neurological and Psychiatric Disorders: Sex Differences in Human. Nutrients. 2020; 12, 3080

[3] Weng X, Odouli R, Li DK. Maternal caffeine consumption during pregnancy and the risk of miscarriage: a prospective cohort study. Am J Obstet Gynecol. 2008; 198(3):279.e1-8.

[4] Chen, L., Wu, Y., Neelakantan, N., Chong, M. F., Pan, A. and van Dam, R. B. (2014). "Maternal caffeine intake during pregnancy is associated with risk of low birth weight: a systematic review and dose-response meta-analysis". BMC Medicine. 12:174.

[5] Paula, T. M. D., Cardoso, L. C., Felicioni, F., Caldeira-Brant, A. L., Santos, T. G., Castro-Oliveira, H., Menezes, G. B., Bloise, E., Chiarini-Garcia, H., Almeida, F. R. C. L. Maternal chronic caffeine intake impairs fertility, placental vascularization and fetal development in mice. Reproductive Toxicology 2023; 121

[6] Butt, M. S. and Sultan, M. Coffee and its consumption: benefits and risks, Crit. Rev. Food Sci Nutr 2011. 51: 363–373.

[7] Lakin, H., Sheehan, P. and Soti, V. Maternal Caffeine Consumption and Its Impact on the Fetus: A Review. Cureus 2023. 15(11): e48266.

[8] Gaskins AJ, Chavarro JE. Diet and fertility: a review. Am J Obstet Gynecol. 2018 218(4):379-389.

[9] Demir, Ebru Ofluoğlu, Demirtaş, Canan Yılmaz and Paşaoğlu, Özge Tuğçe. "The effects of caffeine on the renal antioxidant activity in rats / Ratlarda böbrek antioksidan aktivitesi üzerine kafeinin etkileri" Turkish Journal of Bichemistry. 2016; 41(3): 216-222

[10] Yu, T., Campbell, S. C., Stockmann, C., Tak, C., Schoen, K., Clark, E. A. S., Varner, M. W., Spigarelli, M.G. and Catherine M. T. Sherwin, Pregnancy-induced changes in the pharmacokinetics of caffeine and its metabolites. J. Clin. Pharmacol. 2016 56, 590–596.

[11] Knutti, R., Rothweiler H, and Schlatter C. Effect of pregnancy on the pharmacokinetics of caffeine. Eur J Clin Pharmacol, 1981; 21(2):121–6.

[12] Sisti, J. S., Hankinson, S. E., Caporaso, N. E., Gu, F., Tamimi, R. M., Rosner, B., Xu, X., Ziegler, R., Eliassen, A. H. Caffeine, coffee, and tea intake and urinary estrogens and estrogen metabolites in premenopausal women. Cancer Epidemiol. Biomark. Prev. 2015. 24, 1174–1183.

[13] Shulman, L. M. Is there a connection between estrogen and Parkinson's disease? Park. Relat. Disord. 2002. 8, 289–295.

[14] Cappelletti, S., Daria, P., Sani, G. and Aromatario, M. Caffeine: Cognitive and Physical Performance Enhancer or Psychoactive Drug? Curr. Neuropharmacol. 2015. 13, 71–88.

[15] Ribeiro, J. A. and Sebastião, A. M. Caffeine and Adenosine. J. Alzheimer's Dis. 2010; 20: 3–15.

[16] EFSA Scientific opinion on the safety of caffeine. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). EFSA J. 2015; 13:4102.

[17] Grosso, L. M. and Bracken, M. B. Caffeine metabolism, genetics, and perinatal outcomes: a review of exposure assessment considerations during pregnancy. Ann. Epidemiol. 2005; 15: 460–466,

[18] NHS Choices. (2016). Breastfeeding and diet. (https://www.nhs.uk/conditions/pregnan cy-and-baby/breastfeeding-diet) Accessed March 30th 2022

[19] Paula, T. M. D., Shang, F. L. T., Chiarini-Garcia, H. and Almeida, F. R. C. L. Caffeine intake during pregnancy: what are the real evidences? J. Pharm. Pharmacol. 2017; 5:249–260,

[20] Jahanfar, S. and Jaafar, S. H. Effects of restricted caffeine intake by mother on fetal, neonatal and pregnancy outcome, Cochrane Database Syst. Rev. 2015. (2) CD006965, ttps://doi.org/10.1002/14651858.CD006965.pub3.

[21] Naidu, K. A. Vitamin C in human health and disease is still a mystery? An overview. Nutr. J. 2003. 2: 1 - 10.

[22] Naseer, M. I., Ullah, N., Ullah, I.,Koh, P. O., Lee, H. Y., Park, M. S. and Kim, M.O. Vitamin C protects against ethanol and PTZ-induced apoptotic neurodegeneration in prenatal rat hippocampal neurons. Synapse 2011. 65, 562–571.

[23] Zreik, T. G., Kodaman, P. H, Jones, E. E, Olive, D. L. and Behrman, H. (1999). Identification and characterization of an ascorbic acid transporter in human granulosa lutein cells. Mol. Hum. Reprod. 5(4): 299-302.

[24] Luck, M. R., Jeyaseelan, I. and Scholes, R. A. Ascorbic acid and fertility. Biol. Reprod. 1995. 52: 262-266.

[25] Aydin, M. and Yuksel, M The effects of high-level ascorbic acid on pregnancy rate and litter size in rats. Journal of Medicinal Plants Research. 2011. 5(13), 2695-2698

[26] Turk, G., Sönmez, M., Aydin, M., Yüce, A., Gür, S., Yüksel, M., Aksu, E. H. and Aksoy, H. Effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats. Clin. Nutr. 2008. 27(2): 289-296.

[27] Rhee, J., Kim, R., Kim, Y., Tam, M., Lai, Y., Keum, N., Oldenburg, C. E. Maternal Caffeine Consumption during Pregnancy and Risk of Low Birth Weight: A Dose-Response Meta-Analysis of Observational Studies. PLoS ONE 2015. 10(7): e0132334. doi:10.1371

[28] Owolabi, Lukman Femi; Shokunbi, Mathew Temitayo. Effect of caffeine on foetal morphometric parameters of embryonic Wistar rat (Rattus norvegicus). Nigerian Journal of Basic and Clinical Sciences 2016 13(2): 67-71,

[29] Glade, M. J. Caffeine-not just a stimulant. Nutrition. 2010. 26:932–938

[30] Astrup A, Toubro S, Cannon S, Hein P, and Madsen J. Thermogenic synergism between ephedrine and caffeine in healthy volunteers: a double-blind, placebo-controlled study. Metab Clin Exp. 1991; 40:323–329.

[31] Larsen SC, Angquist L, Ahluwalia TS, Skaaby T, Roswall N, Tjønneland A, Halkjær J, Overvad K, Pedersen O, Hansen T, Linneberg A, Husemoen LL, Toft U, Heitmann BL, Sørensen TI. Dietary ascorbic acid and subsequent change in body weight and waist circumference: associations may depend on genetic predisposition to obesity--a prospective study of three independent cohorts. Nutr J. 2014; 3:13 - 43.

[32] Molz, P., Rael, A. N., Fischer, M., Limberger, L. B. Prá, D. and Franke, S. I. R. (2017) Vitamin C decreases the obesogenic and hyperglycemic effect of invert sugar in prediabetic rats. Rev. Nutr., Campinas, 30(1):23-32.

[33] Garcia-Diaz DF, Lopez-Legarrea P, Quintero P, Martinez JA. Vitamin C in the treatment and/or prevention of obesity. J Nutr Sci Vitaminol (Tokyo). 2014; 60(6):367-79.

[34] Sengpiel, V., Elind, E., Bacelis, J., Nilsson, S., Grove, J., Myhre, R., Haugen, M., Meltzer, H. M., Alexander, J. and Jacobsson B. Maternal caffeine intake during pregnancy is associated with birth weight but not with gestational length: results from a large prospective observational cohort study. BMC Med 2013; 11:42.

[35] Lovallo WR, Whitsett TL, al'Absi M, Sung BH, Vincent AS, Wilson MF. Caffeine stimulation of cortisol secretion across the waking hours in relation to caffeine intake levels. Psychosom Med. 2005; 67(5):734-9.

[36] Zhang, Y., Aitor, Coca, Douglas J. Casa, Jose A, James M. G. and Phillip, A. B. Caffeine and diuresis during rest and exercise: A meta-analysis. J Sci Med Sport. 2015; 18(5): 569-74.

[37] Coulson, R. and Scheinman, S. J. Xanthine effects on renal proximal tubular functionand cyclic AMP metabolism. J Pharmacol Exp Ther. 1989; 248(2):589–595.

[38] Yadegari, M, Khazaei M, Anvari M, and Eskandari, M. Prenatal Caffeine Exposure Impairs Pregnancy in Rats. International journal of fertility and sterility. 2016; 9: 558-62.

[39] Bech BH, Obel C, Henriksen TB, Olsen J. Effect of reducing caffeine intake on birth weight and length of gestation: randomised controlled trial. BMJ. 2007; 334(7590): 409.

[40] Bakker R, Steegers EA, Obradov A, Raat H, Hofman A, Jaddoe VW. Maternal caffeine intake from coffee and tea, fetal growth, and the risks of adverse birth outcomes: the Generation R Study. Am J Clin Nutr. 2010 91(6):1691-1698

[41] Chahoud I, Paumgartten F. Influence of litter size on the postnatal growth of rat pups: Is there a rationale for litter-size standardization in toxicity studies? Environmental research. 2009. 109: 1021-1027.

[42] Romero A, Villamayor F, Grau MT, Sacristán A, Ortiz JA. Relationship between fetal weight and litter size in rats: application to reproductive toxicology studies. Reprod Toxicol. 1992; 6(5):453-456.