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Research Article
Histopathologic and Toxic Effects of Artificial Sweeteners (Caffeine and Saccharin) on Some Pregnancy Outcome Variables

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Abstract

Background and Objective: Pregnancy is characterized with craving for varieties of drinks, most of which containers artificial sweeteners. This study was carried out to determine and compare, by histological examination of the maternal spleen and foetal records, the effects of combined intake of varied concentrations of caffeine and saccharin on pregnant white albino rats. **Materials and Methods:** Four groups of 20 mated male and female rats were used in the study. Combined caffeine/saccharin syrup was administered as a single dose to three groups: Group 1 (19.20 and 1,420 mg kg^{-1} b.wt., of caffeine and saccharin, respectively), group 2 (38.40 and 2,840 mg kg^{-1} b.wt.,) and group 3 (76.80/5,680 mg kg^{-1} b.wt.,). The syrups were administered from gestation days (GD) 6-19. The fourth group (Control) had distilled water only. All rats were fed *ad libitum*. Histological analyses were carried out on maternal spleens on GD20. The weights of the maternal rats were recorded daily, while the weights and numbers of foetus from each treatment were recorded. **Results:** Histological analyses showed dose-dependent decrease in numbers and sizes of lymphoid follicles, increase in red pulp, with gradual loss of marginal zone and germinal centre. Higher dosages elicited vascular congestions. There was significant decrease in maternal and foetal body weight and number of foetuses as concentration increased. **Conclusion:** Combined intake of caffeine and saccharin during gestation produced significant inflammatory response. Hence, posing imminent danger to expectant mothers and developing foetuses, such sweeteners should be avoided entirely during pregnancy.

Key words: Saccharin, caffeine, gestation days, toxicity, spleen

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The desire for chocolatey and/or flavoured drinks in food materials have necessitated the development of flavouring agents. More so, apart from the enhanced tastes, consumers have reported 'inward satisfaction' derived from their consumption[1]. Among these teeming consumers are the pregnant mothers, who due to their physiological conditions crave for and eat these tasty chocolatey foods, drinks and flavoured drinks. Unfortunately, consumption of these food materials during the gestational period, can exposing them and their developing foetus to the deleterious effect of these chemicals[2]. Lately, food and drink companies have come under the sledge for the inclusion of sweetening agents, which have potentials in causing harmful effect on the health of consumers. Among these sweeteners are caffeine and saccharin. There are greater possibilities of the pregnant mothers to consume these materials in combination due to the presence of these in most food materials surrounding them.

Caffeine, present in chocolatey products, is an odourless, white powder widely known for its savoury taste in instant coffee. Its natural source includes leaves and beans of the coffee tree, tea, yerba mates, guarana berries and in small quantities in cocoa, the kola nuts and the yaupon holly[3]. Artificial sources include milk chocolate, sweet dark chocolates, cola type beverage[4]. Quantitatively, tea contains somewhat more caffeine per serving than coffee, while cola drinks contains about 10-50 mg of caffeine per serving[4]. Further, energy drinks such as Red Bull contains as much as 30 mg of caffeine per serving. The caffeine in these drinks originated either from the ingredients used or as an additive derived from the product of decaffeination or from chemical synthesis[5]. Saccharin, on the other hand, is a white crystalline powder and the first sweet organic compound to be identified that exhibit sweetness potency significantly greater than that of sucrose[5].

During pregnancy, the spleen performs so much important function[6]. For example, it is a reservoir of blood for the circulatory system, essentially concerned in phagocytosis, immune responses, cytopoiesis and erythrocytes storage. In developing foetus, the spleen plays an important role in haemopoesis[7]. Histologically, the parenchyma of the spleen is soft and pulpy. It is usually one in number and made up of the red and the white pulps[8].

Toxicological studies indicate that caffeine administered alone causes changes in growth rates, eating and drinking habits, nephritis, polydipsia, dieresis, thyroiditis, occasional dermatitis and loss of red pulp in the spleen[9-13]. Evaluation of the effect of dietary caffeine throughout pregnancy and lactation showed that offspring of successive pregnancy had reduction in growth, however, teratogenicity was not observed but after four pregnancies, offspring growth and neonatal mortality was severely reduced[2]. Although, it is assumed that only extremely large quantities daily intake of caffeine could affect foetus and that apart from inducing hypoglycaemia[14], saccharin, may not produce deleterious effects on blood sugar, kidney functions, vitamin utilization, blood coagulation or enzyme activities in man. It is pivotal that quantitative studies on the effects of these two substances is investigated to assist in making informed decisions on their daily intake.

This study was aimed at demonstrating the effects of gestational exposure of varied combined concentration of caffeine and saccharin on albino rats. This was achieved by (i) Histological examination of the maternal spleen (ii) Body weight increase during pregnancy (iii) The mean body weight and numbers of foetal number. The results obtained will update the present knowledge on the effects of these substances which are found abundantly in both natural and processed foods and drinks.

MATERIALS AND METHODS

Test articles: Caffeine was obtained in its extracted form (Tree of life products, London, UK) while Saccharin was purchased from retail outlets located. Stock solutions of caffeine and saccharin were prepared daily for administration. Briefly, 1 g of caffeine and 5 g of saccharin were dissolved, respectively, in 20 and 5 mL of distilled water.

Animal and husbandry: A total of 80 albino rats (40 males and 40 nulliparous females) were used. Following acclimation period of 2 weeks, the rats were weighed and individually identified by colour tattoo. The rats were kept in plastic cages at room temperature at 32±4°C and <30% relative humidity with a 12 h light/dark cycle. They had access to drinking water and standard laboratory diet (Vital feeds, Grand Cereals and Oil Mills, Ltd., Jos, Nigeria) *ad libitum*.

Experimental design: The female rats were cohabited with the male overnight (1:1) to induce mating. Evidence of mating was confirmed by a vaginal smear which indicate the presence of sperm and pregnancy, by the presence of vaginal plug around the vaginal orifice. The day of confirmation was

considered as gestation day zero (GD_0). The pregnant rats were weighed and randomly divided into 4 groups of 20 rats each. Group I served as the control and were administered municipal water equivalent to the highest volume of the test substance administered. Group II, III and IV were administered 19.20/1,420, 38.40/2,840 and 76.80/5,680 mg kg^{-1} caffeine/saccharin syrup, respectively. Dosage ranges were calculated[15,16] from the LD_{50} of 0.192 and 1.42 g kg^{-1}, respectively, for caffeine and saccharin. The rats were administered the test substance in a single dose, by gavage from GD_6-GD_{19}. During this period, the animals were closely observed for any sign of maternal toxicity. On GD_{20}, all female rats were weighed and humanely killed under anaesthesia and caesarean section performed. The maternal spleen was harvested for histological analysis and the weight and numbers of the foetuses noted.

Histological analysis: The harvested maternal spleens were fixed in 10% formalin and processed for light microscopy, sections were cut at 5 µm, stained with haematoxylin and eosin and sections were mounted on DPX. Light microscopic examination of the sections was carried out by an experienced histopathologist.

Ethical approval: The animal care local ethical committee of the Department of Anatomy, University of Maiduguri accepted all the procedures performed in this study.

Data analysis: Numerical data obtained were expressed as the mean value±standard error of mean. Differences among means of control and treated groups were determined using Statistical Package for Social Scientist (SPSS 11.0). A probability level of less than 5% ($p<0.05$) was considered significant.

RESULTS

The weight changes observed in all groups of rats from gestation day 6-19 are shown in Table 1. Results showed significant ($p<0.001$) increase in body weight in the control (group I), with high percentage increase in body weight. The group administered 19.20/1,420 mg kg^{-1}, also showed a significant ($p<0.05$) increase in mean body weight. The group administered 38.40/2,840 mg kg^{-1} also showed remarkable increase in body weight, though, difference in and percentage body weight gain were significantly lower than the control. Meanwhile, groups administered 76.80/5,680 mg kg^{-1} caffeine/saccharin syrup (i.e., the highest dosage tested) had the lowest values for difference in and percentage weight gain (Table 1).

Mean foetal body weights and litter sizes were also affected by all concentrations of the syrups, revealing a dose-dependent interaction. For example, the lowest foetal body weight and litter number were observed at 76.80/ 5,680 mg kg^{-1} caffeine/saccharin syrup. Though, there was no significant difference between the weights of foetuses at 19.20/1,420 and 38.40/2,840 mg kg^{-1} with significant reduction in the number of foetuses (Table 2).

Histological sections of maternal spleen in the group administered municipal water only showed normal lymphoid follicles containing lymphoid sheath within the periarterial

Table 1: Effect of ingestion (by gavage) of caffeine and saccharin on mean maternal body weight

Groups	Number of rats	Doses administered (mg kg^{-1}) Caffeine	Saccharin	Body weight (g) (GD6)	Body weight (g) (GD19)	Body weight difference (g) (GD19-GD6)	Body weight gain (%)
I	20	-	-	151.06±2.33[a]	198.74±0.91[c]	+47.68	23.99[a]
II	20	19.2	1,420	153.08±3.63[a]	171.94±4.69[a]	+18.86	10.97[b]
III	20	38.4	2,840	165.98±2.96[a]	182.46±2.18[b]	+16.48	9.03[c]
IV	20	76.8	5,680	171.50±3.11[a]	185.62±3.86[b]	+14.12	7.61[c]

GD: Gestation day, Results are presented as Mean±Standard error of mean, *Values followed by similar alphabets, in a column are not significantly different at p = 0.05

Table 2: Combined effect of caffeine and saccharin on mean foetal body weight and number of foetuses

Groups	Doses administered (mg kg^{-1}) Caffeine	Saccharin	Mean foetal body weight (g)	Mean numbers of foetus
I	-	-	4.70±0.95[c]	10.50±2.50[d]
II	19.2	1,420	3.82±0.23[b]	8.20±1.40[c]
III	38.4	2,840	3.31±0.83[b]	5.60±1.30[b]
IV	76.8	5,680	2.33±0.42[a]	2.70±0.40[a]

Results are presented as Mean±Standard error of mean, *Values followed by similar alphabets in a column are not significantly different at p = 0.05

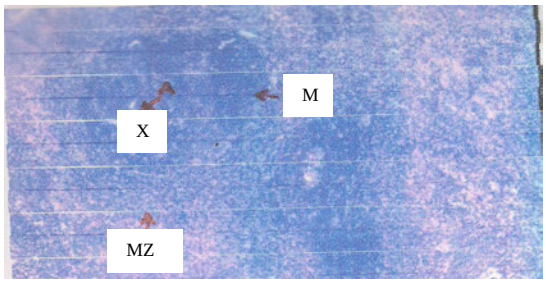

Fig. 1: Light photomicrograph of maternal splenic issue administered municipal water.
MZ: Marginal zone, M: Mantle layer, A: Central artery (H and E X200)

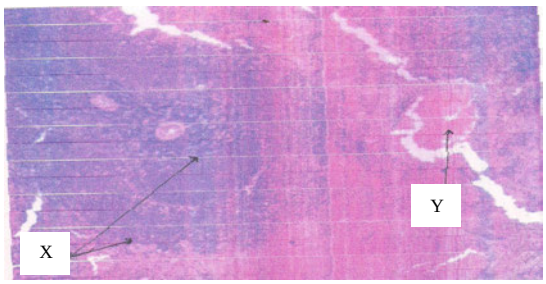

Fig. 2: Light photomicrograph of maternal splenic tissue administered 19.2/1,420 mg kg^{-1} of caffeine/saccharin showing a mild reduction in the numbers and sizes of the lymphoid follicles (X) of the white pulp and moderate vascular congestion (Y) in the red pulp
(H and E. X100)

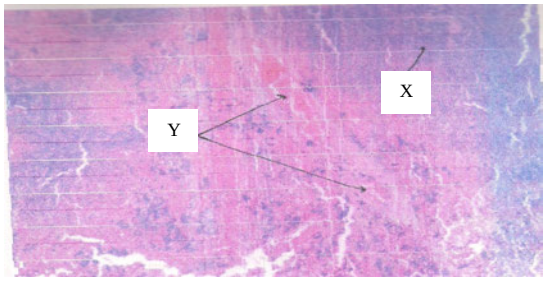

Fig. 3: Light photomicrograph of maternal splenic tissue administered 38.4/2,840 mg kg^{-1} of caffeine/saccharin showing loss of marginal zone of the white pulp (X) and vascular congestion (Y) in the red pulp
(H and E X100)

lymphoid sheath within the white pulp and the red pulp containing venous sinuses and splenic cord (Fig. 1). Histopathological analysis of maternal spleen from 19.2/1,420 mg kg^{-1} of caffeine/saccharin syrup showed mild

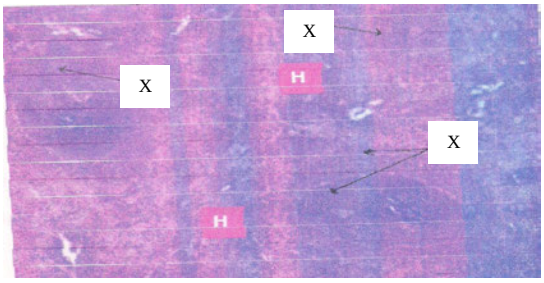

Fig. 4: Light photomicrograph of maternal splenic tissue administered 76.8/ 5,680 mg/kg of caffeine/saccharin showing loss of marginal zone, reduction in the germinal centre of the white pulp (X) and hyperplasia of the red pulp (H)
(H and E. X100)

reduction in the numbers and sizes of the lymphoid follicles, with the incidence of moderate congestion of vascular channels (Fig. 2). There was however an increased reduction in the numbers and sizes of lymphoid follicles, with the loss of the marginal zone of the white pulp and incidence of vascular congestion (Fig. 3) in the group administered 38.4/2,840 mg kg^{-1} of caffeine/saccharin. The histological analysis of the maternal spleen of the group administered 76.8/5,680 mg kg^{-1} of caffeine/saccharin showed a loss of marginal zone, a reduction of the germinal centre of the lymphoid follicles with hyperplasia of the red pulp (Fig. 4).

DISCUSSION

In Nigeria, a lot of food substances containing caffeine are taken by expecting mothers, especially as kola nuts, coffee, tea, chocolate drinks, cola drinks. Saccharin, on the other hand, gets to them through local confectionaries, local 'kunu' drinks, local 'zobo' drinks and other local flavoured drinks. In the present study, analysis showed significant decrease in maternal weights with increase in concentration of the combined syrup. These differences could be attributed to the dose dependent increase in the level of serum corticosterone as a result of stress[17]. For corticosterone, secreted from the cortex of the adrenal gland, cause the release of free fatty acid (FFA) from storage sites (Adipose tissue), i.e., lypolysis[18], thus leading to a loss of weight. More so, the relationship between caffeine and lipid homeostasis suggested elevation in serum cholesterol and phospholipids[19]. Earlier studies have reported increase in serum corticosterone level by caffeine intake[20,21], with some reduction in maternal weight gain during the 1st week. However, the weight increase observed in all gravid

rats may be attributed to the pregnancy status and unaltered eating behavioural habit[9].

The present study also revealed significant reduction in the number and weight of the foetuses as the concentrations of these additives increased. Earlier studies on saccharin administered independently to albino rats showed no foetal abnormalities, no reduction in litter size, growth, foetal mortality and weight[22]. Although, Nolen[23] reported higher incidence of unossified sternebrae at full instant coffee, suggesting a retardation of the foetal calcification, which results in lower weights. More so, Collins *et al.*[24] concluded that these effects were less after *ad libitum* than oral intubation.

In the present study, histological examination of maternal spleen showed an increase in the red pulp (hyperplasia) with gradual loss of the marginal zone and the germinal centre. Although, Boyd *et al.*[15] had described a loss of the red pulp due to caffeine intake. The difference may be due to combined intake with saccharin in our study. Early studies on similar gland (thymus gland), revealed its atrophy with progressive decrease in the cortical lymphocytes following increased intake of caffeine with scattered medullary lymphocytes of stromal cells. Other studies also revealed a decrease in lymphocytes counts in the peripheral blood as a result of the diminished lymphoid follicles[10,25,26].

Hyperplasia of the red pulp could occur due to an increase in the blood storage capacity of the splenic veins and increase in red blood cell proliferation[6]. The vascular congestion is a passive process resulting from impaired outflow of blood from the tissue. It may occur systemically or locally from an isolated venous obstruction[27]. Vascular congestion is a component of inflammation and could result to tissue hypoxia, vascular blockage or hyperaemia.

CONCLUSION

This study has shown that a combined intake of caffeine and saccharin pose imminent threat to the maternal immune system and weight of neonatal. It has potential of reducing foetal weight, increasing the risks of neonatal complications. Pregnant mothers should, therefore, entirely avoid the intake of the materials during such period.

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