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Histological Assessment of Placental Development Following Intrauterine Exposure to Caffeine in Adult Wistar Rats

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ABSTRACT: In recent years, there have been concerns about human reproductive disorders. Physiological adaptations are crucial for optimal fetal development during pregnancy. The widespread consumption of caffeine by pregnant women raises questions about its impact on maternal physiology and fetal development. Hence, the objective of this study was to evaluate the histological assessment of placenta development following intrauterine exposure to caffeine in adult Wistar rats using appropriate standard techniques. On each gestational day (GD13, GD15, GD17, and GD19), five (5) animals were sampled from each group and their placentas were harvested for histological assessment. The Maternal weight, Fetal Crown Rump Length, Placental weight, Placental diameter major, Placental diameter minor, and fetal weight were taken on the harvested placenta. Results showed varying alterations in the histomorphology of the placenta ranging from delayed differentiation of glycogen cells, dilated and congested blood vessels, vacuolar degeneration of glycogen cell islands, poor development of the labyrinth zone, and dilated fetal capillaries. In conclusion, there is histomorphological evidence that caffeine administration has deleterious effects on the development of the placenta in Wistar rats.

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The placenta, a vital organ, plays a crucial role in facilitating the exchange of nutrients, gases, and waste products between the growing fetus and the mother's blood supply. Placental development involves complex molecular, cellular, and physiological changes (Charnock-Jones *et al.*, 2004), influenced by factors such as maternal diet, environmental factors, and maternal health. Caffeine, a methylxanthine alkaloid, is a widely used central nervous system psychostimulant that shares chemical bonds with adenine and guanine bases found in RNA and DNA, respectively (van Dam *et al.*, 2020). Historically, caffeine was discovered in 1819 by Friedrich Ferdinand Runge, and its synthesis was achieved by Emil Fischer in 1882 (Merck Index 2001). Caffeine is found in various plants and dietary sources, including coffee, tea, cocoa drinks, chocolate, soft drinks, energy drinks, and some medications (Weinberg and Bealer, 2004). Caffeine acts as a stimulant of the central nervous system by inhibiting adenosine, a neurotransmitter that promotes sleep and reduces wakefulness. While moderate caffeine consumption can boost focus, vitality, and alertness, excessive intake can lead to negative effects such as insomnia, nervousness, anxiety, and palpitations (Weakley *et al.*, 2023). Pregnant women metabolize caffeine at a slower rate, potentially leading to its accumulation in body tissues and posing risks to the developing fetus (Askari *et al.*, 2023). Health organizations, including the World Health Organization (WHO) and the Food and Drug Administration (FDA), have recommended restrictions on caffeine intake during pregnancy due to the uncertain effects of maternal caffeine consumption on the fetus. Given the increasing availability of

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caffeine-enhanced food products, there are growing concerns about the consumption of caffeine during pregnancy and its potential impact on maternal and fetal health (Pemathilaka *et al.*, 2019).

The use of caffeine during pregnancy has emerged as a prominent area of concern due to its potential implications for fetal development. Although studies have highlighted the impact of maternal caffeine consumption on fetal health, there remains a significant dearth of comprehensive investigations into the intrauterine effect of caffeine on placenta development. The placenta, acting as a vital intermediary between the mother and fetus, assumes a critical role in supporting fetal growth and development by facilitating the exchange of crucial nutrients and oxygen (Faudone *et al.*, 2021). Hence, the objective of this study was to evaluate the histological assessment of placental development following intrauterine exposure to caffeine in adult Wistar rat.

MATERIALS AND METHODS

Thirty-two (32) adult Wistar rats weighing between 170 g and 180 g were used for this study. The animals were kept in polypropylene cages at room temperature with 12-hour light and dark cycles. The animals were fed with pelleted feed and clean tap water. The animals were paired overnight at the estrous cycle with sexually active males in the ratio of 2:1. Estrous cycle was confirmed by vaginal lavage. The presence of a vaginal plug and/or sperm in the vaginal smear is GD0 as this indicates successful mating. The pregnant rats were divided into two groups (A and B) with sixteen (16) rats per group. Group A served as the Control that was administered with a single intraperitoneal injection of 1ml of normal saline on GD 11, in addition to free access to feed and water. Group B served as the treated group and was administered a single intraperitoneal injection of 150mg/kg/day from GD11 as previously described by Richard et al. (2012). On each gestational day (GD13, GD15, GD17, and GD19), four (4) animals were sampled from each group and sacrificed. The number of the fetus, maternal weight, fetal crown-rump length, placenta weight, placenta diameter major, placenta diameter minor, and fetal weight. The harvested placenta and fetus were fixed with 10% neutral buffered formalin before they were taken to the laboratory for histological assessment.

RESULTS AND DISCUSSION

There was a statistically significant difference (p<0.05) in the placental weight between the control and treated group on gestational days 17 and 19 (Fig. 1 [A]). There was a statistically significant difference (p<0.05) in the fetal weight between the control and treated group on gestational days 15 and 19 (Fig. 1 [B]). Also, there was a statistically significant difference (p<0.05) in the fetal:placental weight ratio between the control and treated group on gestational days 17 and 19 (Fig. 1 [C]). There was a statistically significant difference (p<0.05) in the crown-rump length between the control and treated group on gestational days 17 and 19 (Fig. 2 [A]). There was a statistically significant difference (p<0.05) in the major placental axis diameter between the control and treated group on gestational days 17 and 19 (Fig. 2 [B]). There was a statistically significant difference (p<0.05) in the Minor placental axis diameter between the control and treated group on gestational days 17 and 19 (Fig. 2 [C]). Histological findings showed normal developmental histoarchitecture in the junctional zone with mitotic trophoblast cells on GD13 (Plate 1 [A]), appearance of glycogen cell islands, spongiotrophoblasts and trophoblastic giants cells on GD15 (Plate 2 [A]) and regression of glycogen cell islands on GD17 (Plates 3 [A]) and GD19 (Plate 4 [A]). In contrast, caffeine-treated group showed apoptotic trophoblast cells with pyknotic nuclei on GD13 (Plate 1 [B]) as well as cystic degeneration of the glycogen cell islands on GD15 (Plate 2 [B]), GD17 (Plate 3 [B]) and GD19 (Plate 4 [B]). The Labyrinth zone of the control group showed normal developmental histoarchitecture with trophoblastic septa, maternal blood and fetal vessels (Plate 5 [A]) on GD13, GD15 (Plate 6 [A]), GD17 (Plate 7 [A]) and GD19 (Plate 8 [A]). In contrast, caffeine administration resulted in dilated fetal vessel and congested maternal sinusoids on GD13 (Plate 5 [B]), necrotic labyrinth tissues, dilated fetal vessels and cystically dilated maternal sinusoids on GD15 (Plate 6 [B]), necrotic labyrinth tissues, mildly congested maternal sinusoids on GD17 (Plate 7 [B]) and necrotic labyrinth tissues, dilated maternal sinusoids on GD19 (Plate 8 [B])

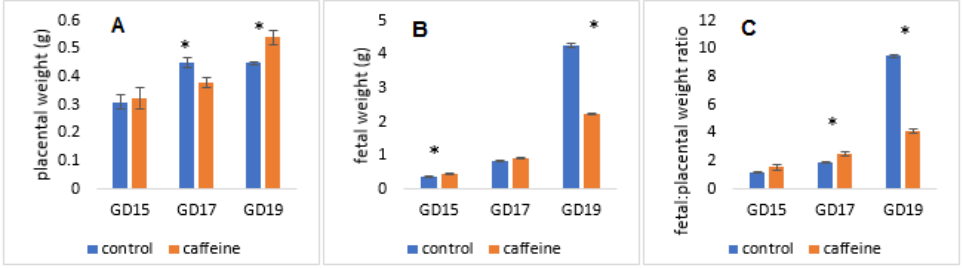

Fig. 1: Chart showing **[A]** Placental weight **[B]** Fetal weight **[C]** Fetal:placental weight ratio of control and treated groups on GD 15, GD 17, and GD 19.

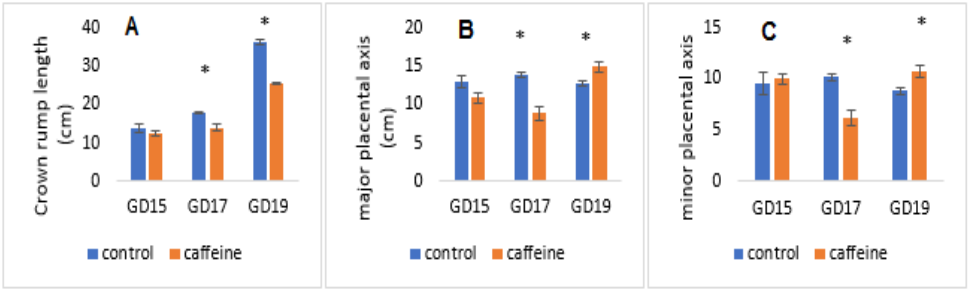

Fig. 2: Chart showing **[A]** Crown-rump length **[B]** Major placental axis diameter weight ratio **[C]** Minor placental axis diameter of control and treated groups on GD 15, GD 17, and GD 19.

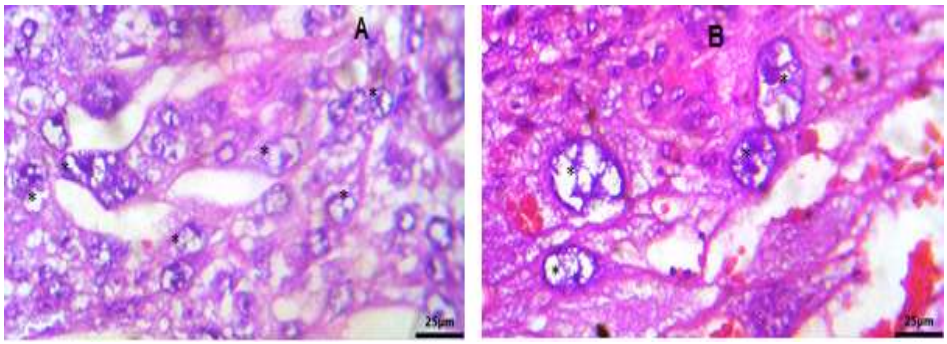

Plate 1: Photomicrograph of a section of the junctional zone of the placenta of **[A]** the control group showing mitotic trophoblast cells (*) and **[B]** the caffeine-treated group on gestational day 13 showing apoptotic trophoblast cells with pyknotic nuclei (*) (H&E; 10X; scale bar = 25 µm).

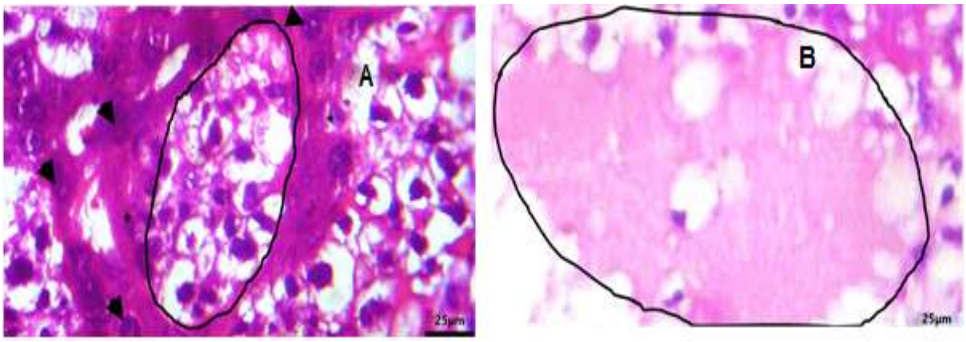

Plate 2: Photomicrograph of a section of the junctional zone of the placenta of **[A]** the control group showing glycogen cell island (encircled), spongiotrophoblasts (*), trophoblastic giant cells (arrowhead) and **[B]** the caffeine-treated group showing cystic degeneration of glycogen cell islands (encircled) on gestational day 15 (H&E; 10X; scale bar = 25 µm).

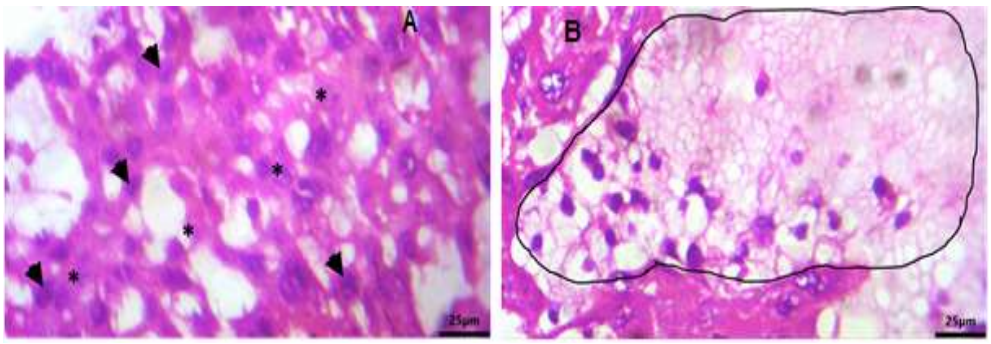

Plate 3: Photomicrograph of a section of the junctional zone of the placenta of **[A]** the control group showing showing spongiotrophoblasts (*), trophoblastic giant cells (arrow head) and **[B]** the caffeine-treated group showing cystic degeneration of glycogen cell islands (encircled) on gestational day 17 (H&E; 10X; scale bar = 25 µm).

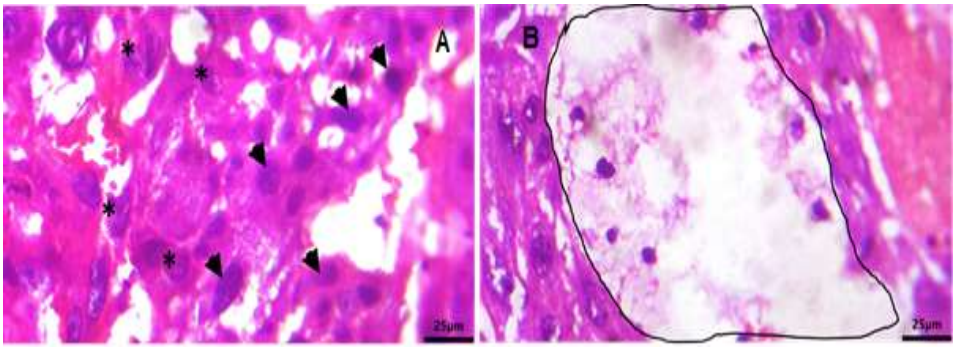

Plate 4: Photomicrograph of a section of the junctional zone of the placenta of **[A]** the control group showing completely regressed glycogen cell islands, spongiotrophoblasts (*), trophoblastic giant cells (arrow head). and **[B]** caffeine-treated group on gestational day 19 showing cystic degeneration of glycogen cell islands (encircled) on gestational day 19 (H&E; 10X; scale bar = 25 µm).

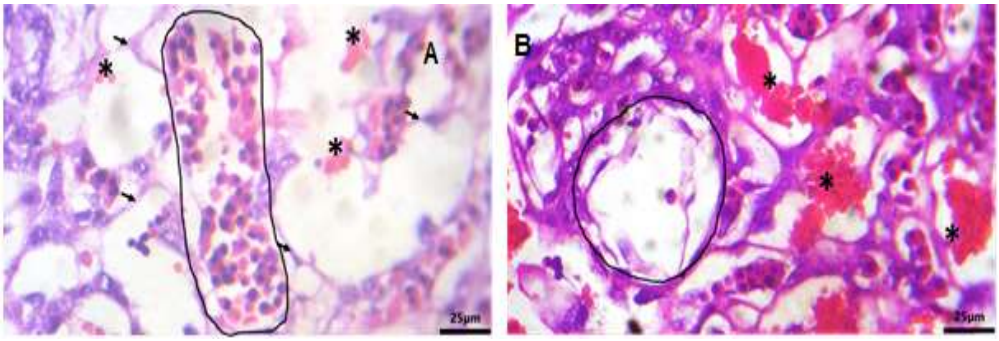

Plate 5: Photomicrograph of a section of the Lanbyrinth zone of the placenta of **[A]** the control group showing trophoblastic septa (arrow), maternal blood (*) and fetal vessels (encircled) and **[B]** the caffeine-treated group showing dilated fetal vessel (encircled) and congested maternal sinusoids (*) on gestational day 13 (H&E; 10X; scale bar = 25 µm).

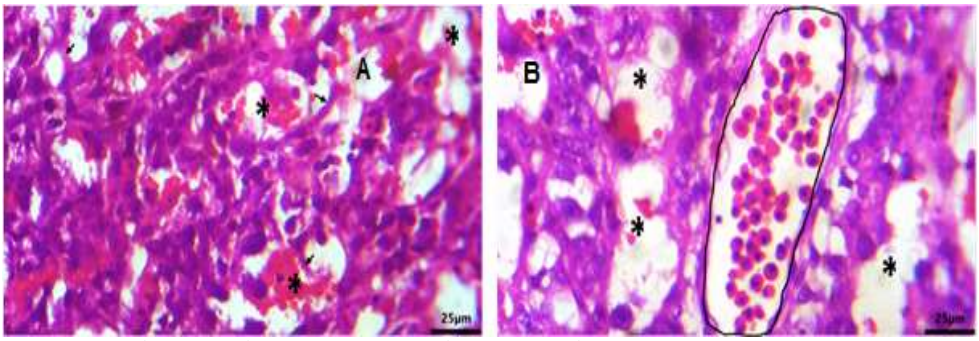

Plate 6: Photomicrograph of a section of the Lanbyrinth zone of the placenta of **[A]** the control group showing trophoblastic septa (arrow) and maternal sinusoids (*) and **[B]** the caffeine-treated group showing necrotic labyrinth tissues, dilated fetal vessels (encircled)and cystically dilated maternal sinusoids (*) on gestational day 15 (H&E; 10X; scale bar = 25 µm)

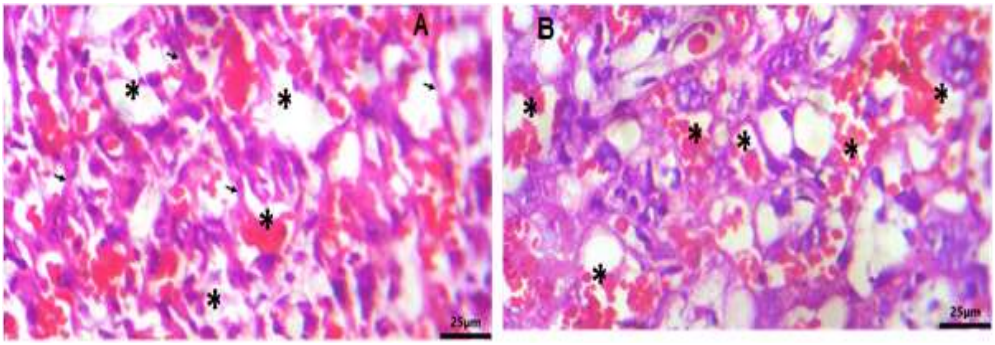

Plate 7: Photomicrograph of a section of the Lanbyrinth zone of the placenta of **[A]** the control group showing trophoblastic septa (arrow) and maternal sinusoids (*) and **[B]** the caffeine-treated group showing necrotic labyrinth tissues, mildly congested maternal sinusoids (*) on gestational day 17 (H&E; 10X; scale bar = 25 µm).

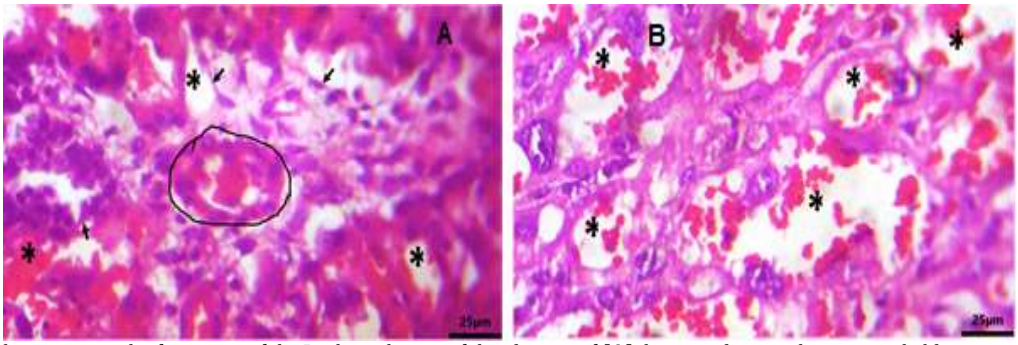

Plate 8: Photomicrograph of a section of the Lanbyrinth zone of the placenta of **[A]** the control group showing trophoblastic septa (arrow), maternal sinusoids (*) and fetal blood vessel (encircled) and **[B]** the caffeine-treated group showing necrotic labyrinth tissues, dilated maternal sinusoids (*) on gestational day 19 (H&E; 10X; scale bar = 25 µm).

The study findings reveal significant differences in the placental weight of caffeine-treated rats on GD 17 and 19. This suggests that exposure to caffeine can affect placental growth, potentially influencing nutrient exchange and fetal development. These results are consistent with prior studies that have linked caffeine intake to changes in placental development (Kaur *et al.*, 2013; Yu *et al.*, 2022). Regarding fetal weight, a significant decrease was observed in caffeine-treated rats on GD 15 and 19. The decrease in fetal weight suggests that caffeine exposure results in intrauterine growth restriction. Findings from this study align with previous studies highlighting the negative effects of caffeine consumption during pregnancy on fetal weight (Grosso *et al.*, 2001; Lunde *et al.*, 2016). The fetal-to-placental weight ratio is a metric used to assess placental efficiency, which is crucial for understanding fetal growth and development. Changes in this ratio can be indicative of disruptions in placental function, potentially leading to nutrient insufficiency for the developing fetus. Findings from this study showed significant differences in the fetal-to-placental weight ratio in caffeine-treated rats on GD 17 and 19. This ratio is a crucial indicator of placental efficiency, and the observed changes in this study suggest that caffeine interferes with placental function, potentially leading to nutrient insufficiency for the developing fetus. This is consistent with previous studies emphasizing the impact of caffeine on placental efficiency and fetal nutrition (Felicioni *et al.*, 2019; Qian *et al.*, 2020). Crown-rump length is a measurement commonly used to estimate fetal age and assess fetal growth and development during pregnancy. It provides important information about fetal health and development. Changes in crown-rump length can indicate growth abnormalities or developmental issues in the fetus. Findings from this study showed a significant difference in the crown-rump length of Caffeine-treated rats on GD 17 and 19. This measurement reflects fetal growth and development, and the observed decrease suggests that caffeine exposure may hinder fetal growth. This finding is consistent with previous studies linking caffeine intake to impaired fetal growth (Lunde *et al.*, 2016; Felicioni *et al.*, 2019). Changes in the major and minor axes can indicate abnormalities or disruptions in placental growth and morphology, which can impact fetal health and development. In this study, there was also a significant difference in the major and minor placental axis diameter in caffeine-treated rats on GD 17 and 19. These changes further highlight the disruptive effect of caffeine on placental morphology and function. Similar findings have been reported by Felicioni *et al.* (2019), emphasizing the negative consequences of caffeine exposure on placental development. Trophoblast cells are crucial for implantation and the formation of the placenta. Apoptosis, or programmed cell death, in trophoblast cells, can disrupt placental development and function, leading to potential complications such as intrauterine growth restriction (IUGR) and preeclampsia (Malhotra *et al.*, 2016). Pyknotic nuclei are condensed and fragmented nuclei typically seen in cells undergoing apoptosis or necrosis (Sharp *et al.*, 2010). Glycogen cells in the placenta store and release glycogen, which is an essential energy source for fetal development (Sharp *et al.*, 2010; Malhotra *et al.*, 2016). The degeneration of glycogen cell islands suggests impaired glycogen metabolism and availability, which can lead to inadequate nutrient supply to the fetus (Knöfler *et al.*, 2019). In this study, the caffeine-treated group showed signs of trophoblast cell apoptosis, pyknotic nuclei, and glycogen cell island degeneration on GD 13 and 15. The presence of trophoblast cell apoptosis suggests that caffeine exposure may interfere with the normal development of the placenta, compromising its ability to support fetal growth and development. The presence of pyknotic nuclei in the placental tissue of caffeine-treated rats further supports the notion of increased cell death and dysfunction within the placenta. This finding indicates that caffeine may induce cellular damage within the placenta, which could have long-term consequences for fetal health. The degeneration of glycogen cell islands suggests that caffeine exposure may disrupt the placental nutrient supply chain, potentially affecting fetal growth and development. The labyrinth zone is crucial for nutrient and gas exchange between the mother and fetus (Awad *et al.*, 2017). Necrotic tissues in this zone indicate cell death and tissue damage, which can impair the placenta's ability to transport nutrients and oxygen to the fetus (Kent *et al.*, 2023). This finding suggests that

caffeine exposure may lead to insufficient nutrient and oxygen supply to the fetus, potentially compromising fetal growth and development. Dilated fetal vessels in the placenta can be a sign of increased blood flow, which may indicate a compensatory response to the impaired placental function (Awad *et al.,* 2017; Kent *et al.,* 2023), in this case, caused by caffeine exposure. However, chronic dilation can lead to increased pressure and stress on the fetal circulatory system, which can have negative consequences for fetal cardiovascular development. Maternal sinusoids are blood spaces where maternal blood comes into close contact with the placental tissue for nutrient and waste exchange. Congestion in these sinusoids can impede blood flow and nutrient exchange, further compromising placental function. The findings from this study showed necrotic labyrinth tissues, dilated fetal vessels, and congested maternal sinusoids in the labyrinth zone of the placenta in the caffeine-treated group on gestational days 13, 15, 17, and 19. This finding suggests that caffeine exposure may disrupt the normal blood flow within the placenta, affecting nutrient delivery to the fetus.

Conclusion: Findings from the study revealed evidence of placental toxicity from caffeine exposure in Wistar dams, highlighting the need to monitor caffeine intake during pregnancy. Expectant mothers should be informed of the risks to ensure the best outcomes for maternal and fetal health. Further research is needed to understand the mechanisms and develop preventive measures for caffeine-related risks during pregnancy.

REFERENCES

Askari, M; Bazshahi, E; Payande, N; Mobaderi, T; Fahimfar, N; Azadbakht, L (2023). Relationship between caffeine intake and small for gestational age and preterm birth: a dose-response meta-analysis. *Crit. Rev. Food Sci. Nutr.* 1-11.

Awad, O; Malek, A; Ogeng'o, J (2017). Differential effects of chronic iron deficiency anaemia on junctional and labyrinthine zones of placenta in Sprague dawely rat. *Anat. J. Africa.* 6(1): 840-846.

Charnock-Jones, DS; Kaufmann, P; Mayhew, TM (2004). Aspects of human fetoplacental vasculogenesis and angiogenesis. I. Molecular regulation. *Placenta*. 25(2-3): 103-113.

Faudone, G; Arifi, S; Merk, D (2021). The medicinal chemistry of caffeine. *J. Med. Chem*. 64(11): 7156-7178.

Felicioni, F; Santos, TG; Paula, TDMD; Chiarini-Garcia, H; de Almeida, FRCL (2019). Intrauterine growth restriction: screening and diagnosis using animal models. *Anim. Reprod.* 16(1): 66.

Grosso, LM; Rosenberg, KD; Belanger, K; Saftlas, AF; Leaderer, B; Bracken, MB (2001). Maternal caffeine intake and intrauterine growth retardation. *Epidemiology*, 447-455.

Kaur, P; Shorey, LE; Ho, E; Dashwood, RH; Williams, DE (2013). The epigenome as a potential mediator of cancer and disease prevention in prenatal development. *Nutrition reviews*. 71(7): 441-457.

Kent, NL; Atluri, SC; Moritz, KM; Cuffe, JS (2023). Maternal hypothyroidism in rats impairs placental nutrient transporter expression, increases labyrinth zone size, and impairs fetal growth. *Placenta*. 31(3): 218-227.

Knöfler, M; Haider, S; Saleh, L; Pollheimer, J; Gamage, TK; James, J (2019). Human placenta and trophoblast development: key molecular mechanisms and model systems. *Cell. Mol. Life Sci.* 76: 3479-3496.

Lunde, ER; Washburn, SE; Golding, MC; Bake, S; Miranda, RC; Ramadoss, J (2016). Alcohol-induced developmental origins of adult-onset diseases. Alcoholism*: Clin. Exp. Res.* 40(7): 1403-1414.

Malhotra, SS; Banerjee, P; Gupta, SK (2016). Regulation of trophoblast differentiation during embryo implantation and placentation: implications in pregnancy complications. *J. Reprod. Health Med.* 2: S26-S36.

Pemathilaka, RL; Caplin, JD; Aykar, SS; Montazami, R; Hashemi, NN (2019). Placenta-on-a-Chip: in vitro study of caffeine transport across placental barrier using liquid chromatography mass spectrometry. *Global Challenges.* 3(3): 1800112.

Qian, J; Chen, Q; Ward, SM; Duan, E; Zhang, Y (2020). Impacts of caffeine during pregnancy. *Trends Endocrinol. Metab*. 31(3): 218-227.

Sharp, AN; Heazell, AE; Crocker, IP; Mor, G (2010). Placental apoptosis in health and disease. *Am. J. Reprod. Immunol.* 64(3): 159-169.Weinberg, BA; Bealer, BK (2004). *The world of caffeine: the science and culture of the world's most popular drug*. Routledge.

Yu, P., Zhou, J., Ge, C., Fang, M., Zhang, Y., & Wang, H. (2022). Differential expression of placental 11β-HSD2 induced by high maternal glucocorticoid exposure mediates sex differences in placental and fetal development. *Sci. Total Environ.* 827: 154396.