Original Research Article

MOTHER'S CAFFEINE INGESTION AFFECTS FECUNDITY AND OFFSPRING BIRTH WEIGHT IN MURINE MODELS

Abstract

Caffeine is the world most popularly consumed legal neurostimulant. It is naturally found in beverage drinks including coffee and tea. It is also artificially added to several soft and energy drinks, as well as medicinal drugs including analgesics. Caffeine itself can be employed for therapeutic purposes. The wide range of caffeine distribution in substances and its popularity in some cultures makes it almost impossible to regulate its consumption. Several people consume caffeine from one or more sources, daily and almost inadvertently. Yet, caffeine ingestion during pregnancy has been reported to have observable effects on female fertility as well as on embryo, foetal and child health. This investigation was conducted to analyse the effect of different doses of caffeine on pregnancy and foetus at birth with emphasis on the number of offspring and morphological parameters. Thirty two (n=32) adult female pregnant mice (*Mus musculus*) were divided into four groups- Group A as the Control, Group B was administered the low-dose caffeine (10mg/kg body weight), Group C was administered the medium-dose caffeine (50mg/kg body weight) and Group D was administered the high-dose caffeine (120 mg/kg body weight). Anhydrous caffeine was dissolved in distilled water to achieve the target dosage for each group and animals were administered caffeine daily throughout the period of pregnancy. At birth, the parameters of fecundity were examined especially with respect to the average litter number; total sum of litter weights as well as the average litters' weights across the experimental animal groups. Caffeine significantly affected birth weight of the offspring; treated groups had fewer offspring per birth and lower sum of offspring weights. Caffeine had observable effects on pregnancy and litters in manner that were negative especially at the higher doses.

Key words

Caffeine Pregnancy Fecundity Fertility Birth Weight

Introduction

Caffeine is produced commercially mainly as a by-product in making caffeine-free coffee. It can also be synthesized. When caffeine is administered orally, its Median Lethal Dose (LD_{50}) is 192 milligrams per kilogram in rats and 150 - 200 milligrams per kilogram of body mass in humans [1]; this amount of caffeine could be found in roughly 80 to 100 cups of coffee for an average human adult. The LD_{50} of caffeine in humans is also dependent on individual sensitivity [1]. It is not usual for a person to consume 80 to 100 cups of coffee at time, however this dosage can be achieved with overdose of caffeine pills or solutions of pure anhydrous caffeine powder.

Kuczkowski [2, 3] reported that caffeine ingestion during pregnancy was associated with an increased risk of foetal growth restriction and this association continued throughout pregnancy. It is also reportedly advisable to reduce caffeine intake throughout pregnancy. Furthermore, Fernandez *et al.* [4] found a small, but statistically significant increase in the risk of spontaneous abortion and Low Birth Weight infants in women consuming more than 150 mg of caffeine daily. Also, acute foetal arrhythmias secondary to excessive maternal intake of caffeine have been reported. Therefore, the physiologic effects and common use of caffeine during pregnancy calls for examination of maternal caffeine consumption and risk of birth defects. Epidemiologic studies have rather yielded mixed results [2, 3].

According to Weng *et al*., [5], an increasing dose of daily caffeine intake during pregnancy was associated with an increased risk of miscarriage, compared with no caffeine intake for caffeine intake of <200 mg/day. The same report concluded that high doses of caffeine intake during pregnancy increased the risk of miscarriage, independent of pregnancy-related symptoms. While Brent *et al.*, [6] remarked that some scientists have reported that caffeine consumption during pregnancy did not appear to increase the risk of congenital malformations, miscarriage or growth retardation even when consumed in moderate to high amounts. Kuczkowski [2] constructively noted that critically, the data supporting this conclusion was of poor quality. Other reports have simply suggested limiting caffeine consumption during pregnancy. Watkinson and Fried [7] reported that the most marked effects associated with heavy caffeine use (over 300 mg daily) in included reduced birth weight and smaller head circumference that was statistically significant.

The currently available literatures have largely indicated the possibilities of transient and persistent effects of mothers' caffeine ingestion on their offspring [2, 5, 7]. However, it is important to determine the influence of dose intake. It should also be noted that several safe-for-consumption agents and substances can become harmful to pregnancy and conceptus if they are abused or consumed at excessively high doses. This investigation also modelled the manners in which humans use caffeine in the experimental animals in order to produce data that can have relevance to human conditions and provide reliable basis for applications and further investigations, especially in humans.

Therefore, the specific aim of this investigation was to assess the effects of prenatal caffeine exposure resulting from maternal ingestion on fertility and offspring physical health parameters including litters number and offspring birth weight.

Materials and Methods

Thirty two(32) mated and pregnant female mice were used for the investigation after a monitored mating exercise, confirmed with the presence of a vaginal plug. Pure anhydrous caffeine powder was dissolved in distilled water to achieve the dose for each group. Effort was made to associate the various dose used with human situations of caffeine use. The lower dose of 10 mg/kg/day is roughly equivalent to taking about 2-3 normal cups of coffee/tea per day or 2-3 coffee tablets or chewing 2-3 bar of caffeine-containing chocolate or equivalent [8]. Thus, 10 mg/kg/day is equivalent to 2–3 cups of coffee/day in humans based on a metabolic body weight conversion [8]. This represented habitual mild and almost unconscious yet regular consumption of caffeine in coffee, tea or other sources such as in caffeinated drinks or in form of pills. This is a pre-caffeinism level of consumption which may not induce caffeinism or caffeine dependency. The medium caffeine dose represented caffeine excessive use and abuse while the highest dose represented a caffeine dependent condition that is abnormal, yet possible. Animals were treated throughout pregnancy that lasted 20-21 days. Each animal was given the daily dose of caffeine using oral gavages once between the hours of 7:00 and 9:00. At parturition, the offspring were collected and observed based on the parameters of interest.

Table 1: Table showing the Experimental Animal Grouping, Dosages and Rationale

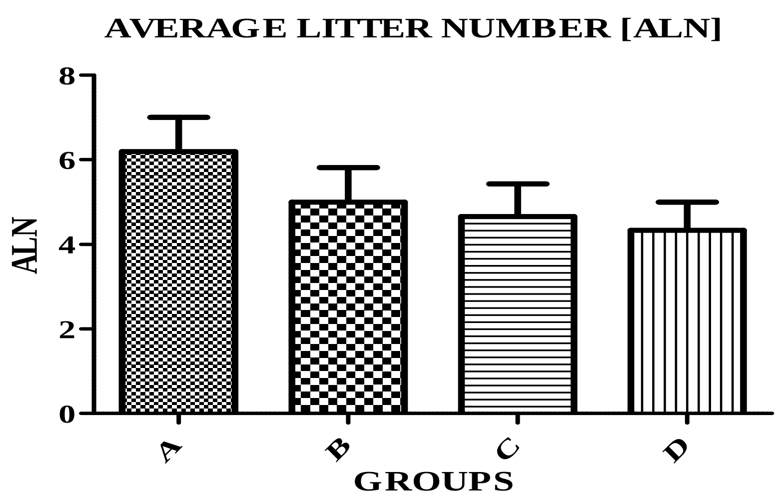
Grouping	**Animals**	**Dosage**	**Description**	**Rationale**
Group A	8	Control	No caffeine treatment;	

			animals receive a placebo of 5% sucrose solution	
Group B	8	10mg/kg body weight	Lower caffeine dosage is administered to pregnant animals	Lower dose treatment
Group C	8	50 mg/kg body weight	Medium caffeine dosage is administered to pregnant animals	Medium dose treatment
Group D	8	120 mg/kg body weight	High caffeine dosage is administered to pregnant animals	High dose treatment

Results

Caffeine negatively affected fecundity and birth weights of the offspring. The effects were observable in the number of offspring weight per mother as well as the total number of offspring per mother. Also, the sum of litters' weight per mother was also affected. Altogether, caffeine ingestion affected fertility and offspring weights; and the effects were dose dependent: birth weight reduced as caffeine dose increased, so also the number of offspring per mother. The figure below provide further details.

Figure 1: Bar Chart Showing Average Litters Number Of The Experimental Animal [Mothers] Groups A-D. The average numbers of litters per group in the treated groups were generally lower than the number for the Control Group. Litter numbers reduced as the dosage of caffeine administration increased.



* Indicates Statistical Significance [P ≤ 0.05]

A: Control Group Animals

B: Group B Animals Subject to the Low-dose [10mg/kg body weight] Prenatal Caffeine Administration

C: Group C Animals Subject to the Medium-dose [50mg/kg body weight] Prenatal Caffeine Administration

D: Group D Animals Subject to the High-dose [120mg/kg body weight] Prenatal Caffeine Administration

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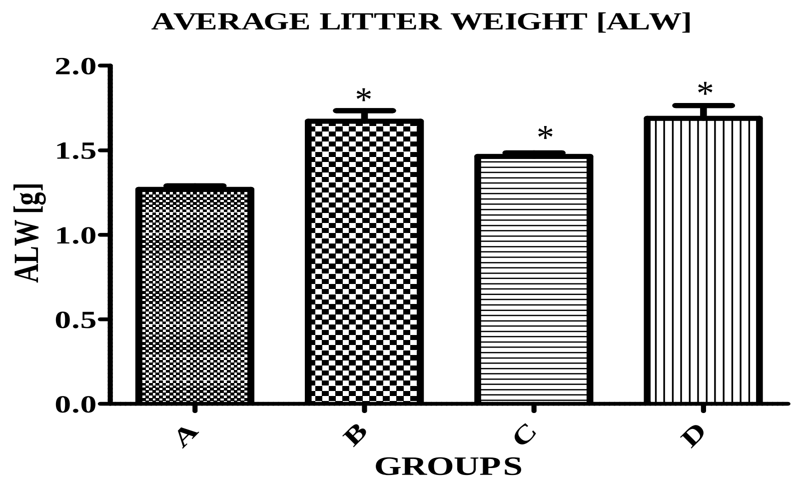
Figure 2: Bar Charts Showing the Average Litters Weight [ALW] of the Experimental Animals Groups A-D. The offspring of the treated animals had higher average weights at birth. These treated groups however had lower number of litters per animal and group.



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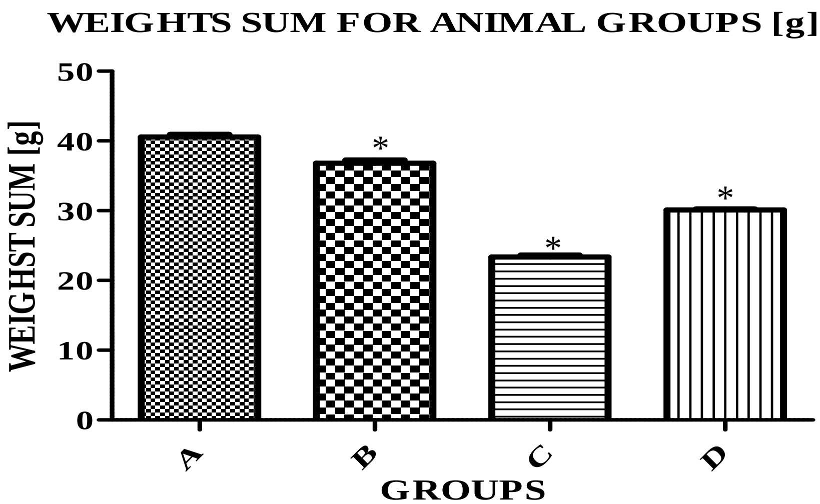
D: Group D Animals Subject to the High-dose [120mg/kg body weight] Prenatal Caffeine Administration

Figure 3: Bar Charts Showing the Sums of Litter Weights Per Group [SLWG]
The treated groups had lower number of litters per animal cum group; subsequently, the sum of litter birth-weights per group [SLWG] was higher in the Group A than the treated groups. Group C had the least value of the SLWG followed by Group D.



A: Control Group Animals

B: Group B Animals Subject to the Low-dose [10mg/kg body weight] Prenatal Caffeine Administration

C: Group C Animals Subject to the Medium-dose [50mg/kg body weight] Prenatal Caffeine Administration

D: Group D Animals Subject to the High-dose [120mg/kg body weight] Prenatal Caffeine Administration

Discussion

The average litter number provides insight into fecundity of the experimental animals. All the animal groups administered caffeine had less number of litters compared to the control group. Also, the average litter in the treated groups reduced with increase in the dosage of caffeine. It therefore implies that the number of litter was inversely proportional to the dosage of caffeine administered to the animals. This simply suggests that caffeine affected fertility or fecundity and this relationship is dosage dependent. Caffeine in the current investigation reduced the average number of litter per mother. This shares similarities with some previous investigations that have suggested that caffeine has negative effects on conception and pregnancy in female humans [9, 10]. Caffeine effects also reportedly included spontaneous abortions and still births in female humans [11, 12, 13]; and such negative effects have been reported in mice or rodents and mammals generally [14, 15, 16].

Noting that the animals were administered caffeine beginning from the day of copulation (D0); caffeine supposedly had effects that could possibly influence the rate of viability of the embryos through the process of pregnancy. Though the mechanism(s) involved in the reduction of litter per birth cannot be specifically established; it is logical to examine the possibilities from the known processes- especially the critical stages. Caffeine could not have influenced ovulation and spermatogenesis in this context, but implantation and embryo implantations and survival till parturition.

Variations in the Average [Mean] Litter Weight [ALW] show that the offspring of the treated animals generally had higher average weights at birth. Values varied between groups and the pattern was not specifically consistent with trends in dosage variations. Interestingly, most reports from human reproductive health investigations have suggested that caffeine consumption by the mother during pregnancy could cause reduction in birth weight of the offspring [17, 18, 19]. These have been complemented by animal-model investigations as well [20]. It is however important to relate these values with the average number of litter per mother as previously presented. The Control Group A had the highest number of average litter or offspring per birth. Obviously, it is important to note that more offspring would have resulted in high total sum of litter weight per birth as indicated on the second chart.

If both results [average litter weight and total sum of litter weight per birth] are considered altogether; caffeine did not necessarily have to influence growth and stimulate either cellular proliferation or tissue hypertrophy to have caused the relative higher average litter weights in the treated groups. It is logical to observe the variations in the number of litter per birth in the caffeine-treated groups relative to the Control Group A. Thus, when summed up, on the average, the caffeine-treated animal Groups B, C and D did not necessarily have higher total-offspring birth weight. Actually, they had less sums of litter weights per group. It is therefore important to consider the average litter in relation to the total number of litter per group and mother to be able to have a useful comparison to the human situation in which single-birth is prevalent contrary to the predominant multiple births in the rodents. When taken from both perspectives, caffeine actually reduced birth weight sums in the treated groups and Group C had the least sum of birth weight. Group D might have higher sum and average weight per litter than C but the number of litter per mother was quite relatively low in Group D. Generally, these results are consistent with many previous findings about caffeine’s potential to reduce birth weight [21, 22, 23]. Even the lowest dosage employed affected litter’s weight per animal and the effect increased with dosage.

Conclusion

The current study investigated the nature of caffeine effects on pregnancy after copulation and fertilisation. It is an attempt to model how caffeine might affect foetal health, birth weight and potentials of multiple births when the pregnant mother ingests caffeine- at various doses. Caffeine had observable negative effect on the birth weights of litters. It also caused reduction in the number of litters. Mother that ingested caffeine also had lower sums of weight litters per birth; this can be likened to low birth weight in human or other mammals with typical single birth. Therefore, it can be concluded from this study that caffeine use, especially at relatively high doses had negative effects of pregnancy, manifested in the weights of the offspring.

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