

# Teratogenic Effects of Arabica Coffee (*Coffea arabica* L) on Rats During the Organogenesis Period

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**Abstract.** The purpose of the research was to evaluate the teratogenic effect caused by the provision of Arabica coffee solution with variations of 0.36 ml, 0.72 ml and 1.08 ml with positive control of caffeine at a dose of 300 mg / kg bw to rats during organogenesis. The measurement parameters in this study are the appearance of reproduction, external malformations and scalal malformations. The results in this study found that the administration of Arabica coffee solution with a volume of 1.08 ml and caffeine 300 mg / kg bb caused an abnormality in the reproductive appearance of weight loss and body length, whereas for extrenal malformation and scalal malforation no abnormalities were found in each administration of the solution Arabica coffee and caffeine.

**Keywords :** *Coffea arabica*, Caffeine, teratogenic effects, organogenesis.

**Abstrak.** Penelitian ini bertujuan untuk mengevaluasi efek teratogenik yang disebabkan pemberian larutan kopi arabika dengan variasi pemberian larutan 0,36 ml, 0,72 ml dan 1,08 ml dengan kontrol positif kafein dengan dosis 300 mg/kg bb terhadap tikus selama masa organogenesis. Parameter pengukuran dalam penelitian ini adalah tampilan reproduksi, malformasi eksternal dan malformasi skletal. Hasil dalam penelitian ini ditemukan bahwa pemberian larutan kopi arabika dengan volume 1,08 ml dan kafein 300 mg/kg bb menyebabkan kelainan pada tampilan reproduksi yaitu penurunan berat badan dan panjang badan, sedangkan untuk malformasi ekstrenal dan malforasi skletal tidak ditemukan kelainan pada setiap pemberian larutan kopi arabika dan kafein.

**Kata kunci :** Kopi arabika, kafein, efek teratogenik, organogenesis.

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## 1. Introduction

Coffee is one of the most important food commodities in the worldwide economy. The genus *coffea* presents more than 100 species, but commercial trade consists almost entirely of *Coffea arabica* (Arabica) and *Coffea canephora* (robusta)[1]. *Coffea arabica* is a plant that can grow in the tropics and subtropics in the highlands with an altitude of 1200 to 2200 mean sea level (MSL) and grows optimally at temperatures between 18-22 °C [2]. Morphologically, ripe

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arabica coffee fruit will be red with an oval shaped with a length of 12-18 mm and a diameter of 8-15 mm [3]. The genus Coffee belongs to the botanical family of Rubiaceae [4]. This beans are manually collected, washed, dried and finally roasted to produce the coffee [5]. The roasting process is carried out under temperatures usually above 200 °C, and the process is typically controlled by the time, weight loss, and color parameters. Roasting defines sensory characteristics and the quality of coffee products, affecting their chemical composition [6], [9]. Coffee contains several chemical components such as caffeine, chlorogenic acid, lignin, pectin, protein, trigonelline, nicotinic acid, diterpene, mineralism [10]. Arabica coffee has a superior flavor compared to other coffees, Arabica coffee caffeine content in 70 gr Arabica coffee beans contain caffeine as much as 63-112 mg / 100 ml [11]. Caffeine is a stimulant from the xanthine group, consumed worldwide from coffee intake. It is estimated that 89% of women aged 18-24 consume caffeinated beverages with an average intake of 166 mg of caffeine per day [12]. One study estimated that the average daily consumption of coffee in pregnant women in US and Europe varies from 1.5 to 4.6 cups [13]. The World Health Organization (WHO) and the US Centers for Disease Control and Prevention (CDC) encourage pregnant women not to consume caffeine. This is because caffeine consumption causes the risk of miscarriage and premature birth [14]. The effect of caffeine on pregnant women will interfere and cause the risk of Low Body Weigth (LBW), even to fetal malformation, this is likely caused by increased cyclic adenosine monophosphate (cAMP) which will interfere with cell development [15]. Teratogenicity test is a test to obtain information on fetal abnormalities that occur due to the provision of test preparations during the formation of fetal organs (during organogenesis). In this case teratogenic tests are carried out to assess the provision of Arabica coffee solution brewed with water solvents on the appearance of the reproduction display, external malformations and scletal malformations in the fetus compared to caffeine.

## **2. Materials and Methods**

### **2.1 Materials**

The materials used in this study are plant materials and chemicals. The plant material used is Coffee arabica (*Coffea arabica* L). The chemicals used are Caffeine (Merck), Nacl 0.9%, CMC Na 0.5% and distilled water.

### **2.2 Sample Preparation and Extraction**

Arabica coffee beans (*Coffea arabica* L) obtained from coffee plantations Kelupak Mata Village, Kebayaan District, Central Aceh District, Nanggroe Aceh Darussalam Province. Plant identification was confirmed by Medan Herbarium (MEDA). Sample then picked stripping from the skin of the flesh of the fruit then carried out the drying process, after drying the roasting process carried out at a temperature of 149-213 °C, then smooth into powder using a grinder machine. The determination of the dose is based on the research of Meaning of Bhara (2009),

where the dose for humans weight 70 kilograms and converted to mice weighing 200 grams using the Laurance-Bacharach conversion table with a conversion factor of 0.018 [16,17]. in 1 cup of coffee 200 ml of water contains 10 grams of coffee powder (concentration 1 time), and the conversion dose to the volume of solution for rats which is equivalent to 200 ml of water that is  $0.018 \times 200$  ml is 3.6 ml. For 2 cups is equivalent to 7.2 ml and for 3 cups of coffee is equivalent to 10.8 ml. In this study Arabica coffee and Robusta coffee solutions were made for 100 grams of coffee powder (10 times concentration), brewed in 200 ml of water at 92 °C. The maximum volume of water given to rats is 5 ml.

### 2.3 Animal

All procedures were evaluated by the Animal Research Ethics Committee under number 0118 / KEPH-FMIPA / 2019 at the Faculty of Mathematics and Natural Sciences, Department of Biology, University of North Sumatra. The mice used were as many as 40 female mice weighing around 150-185 g with age of 2-3 months in nullipara. Before the experiment began the rats were acclimatized for 14 days. Rats were kept in cages that were given husks and regulated lighting 12 hours bright and 12 hours dark. Rats were fed standard pellets and provided access to water.

### 2.4 Treatment

Animals are divided into 5 groups, each consisting of 5 rat.

- I : CMC Na 0.5% suspension
- II : Coffea arabica solution 0.36 ml per day
- III : Coffea arabica solution 0.72 ml per day
- IV : Coffea arabica solution 1.08 ml per day
- V : Caffeine 300 mg / kg bw

Provision of the solution is carried out during the organogenesis period, on days 6 to 15 pregnancies in rats. On the 19<sup>th</sup> day of pregnancy the animal is anesthetized, dissected and taken by the fetus and then an observation is made of the living fetus, the fetus is resorbed, weighing and body length then the number of fetus is calculated. Then put into a bouin solution for 3 days to see fetal anatomy, including hydrocephalus, anencephaly, cleft palate, spina bifida, humpback body, limb defect, heart, liver and kidney. and at the same time for observation of scaletal malformations fixed with 95% alcohol for 7 days then the contents of the stomach and internal organs are removed. Then soaked in 1% KOH solution for 12 hours. Furthermore, the fetus was soaked in alizarin solution 24 hours and then soaked again in 2% KOH for 12 hours.

## 2.5 Statistic Analysis

Data were analyzed using the SPSS 25 program. Data were analyzed using the Oneway ANOVA test to determine differences in the mean parent body weight, fetus weight and length and the number of fetuses between groups. If there are differences, proceed with the Post Hoc LSD test and Tukey to see the real difference in treatment with significance ( $p > 0.05$ ) or more than 0,05

## 3. Results and Discussion

### 3.1 Display reproduction

Fetus biometrics is quantitative data that is used to see the effect of teratogens tested. One of the fetus biometrics data includes the number of deaths, number of live fetus, number of dead fetus. The results of observations of the number of live and dead fetus can be seen in Table 1.

**Table 1.** The number of live and dead fetus from each parent rat.

Test group	Number of parents	Number of Fetuses		
		Life	Die	Resorpsi
Control (CMC Na 0.5%)	5	31 (100%)	0 (0%)	0 (0%)
Coffea arabica solution 0.36 ml	5	30 (100%)	0 (0%)	0 (0%)
Coffea arabica solution 0.72 ml	5	37 (100%)	0 (0%)	0 (0%)
Coffea arabica solution 1.08 ml	5	32 (100%)	0 (0%)	0 (0%)
Caffeine 300 mg / kg bb	5	31 (100%)	0 (0%)	0 (0%)

Table 1 shows that administration of Coffea arabica solution and caffeine does not cause death and resorption in the fetus. Fetus that have been separated from the uterus and placenta are cleaned and dried with tissue then weighed in weight and length one by one and recorded in number. The observation of fetus weight and length can be seen in Table 2.

**Table 2.** Average fetus weight and length in each treatment group.

Test group	Fetus Weight (grams)	Fetus Length (cm)
	X ± SD	X ± SD
CMC Na 0.5%	4.31 ± 0.43	3.77 ± 0.37
Coffea arabica solution 0.36 ml	3.95 ± 0.41	3.67 ± 0.33
Coffea arabica solution 0.72 ml	4.03 ± 0.37	3.61 ± 0.33
Coffea arabica solution 1.08 ml	3.86 ± 0.58 *	3.40 ± 0.37 *
Caffeine 300 mg / kg bw	3.65 ± 0.59 *	3.37 ± 0.53 *

Note: X = Average value, SD = Standard Deviation

\* = Significantly different from the normal control group ( $p < 0.05$ )

Based on table 2, fetus weight and length showed significant differences ( $p < 0.05$ ) in the *Coffea arabica* solution group 1.08 ml and caffeine dose 300 mg / kg bw with 0.5% CMC Na control. It was concluded that the *Coffea arabica* solution group of 1.08 ml and Caffeine 300 mg / kg bw had an effect on the weight and length of the fetus compared to the control group. Increase in weight and length of the fetus is influenced by hormones. Growth hormone is very important because it will affect the metabolism of protein, electrolytes, carbohydrates and fats [18]. The caffeine in coffee inhibits mRNA synthesis and can affect the decrease in Bcl-2 which causes blood flow in the placenta, causing fetus weight loss [19]. Metabolism that occurs in pregnant women can trigger an increase in cAMP which makes the flow less than the intervillous space [20]. An increase in cAMP due to caffeine can cause a decrease in mitosis which ultimately inhibits growth acceleration [21]. Fetus that has been examined the number, length and weight will be examined sex ratio produced based on the total fetus value of each parent in each test group. The value of sex ratio comparison is presented in table 3.

**Table 3.** Comparison of fetus sex between treatment groups.

Parents of Rat	Gender					Total fetus
	I	II	III	IV	V	
Control ( CMC Na 0.5%)	M: 2	M: 4	M: 4	M: 4	M: 5	M: 19
	F: 3	F: 2	F: 1	F: 3	F: 3	F: 12
Coffea arabica solution 0.36 ml	M: 3	M: 2	M: 5	M: 2	M: 4	M: 16
	F: 3	F: 4	F: 2	F: 4	F: 1	F: 14
Coffea arabica solution 0.72 ml	M: 4	M: 5	M: 4	M: 2	M: 4	M: 19
	F: 2	F: 3	F: 4	F: 6	F: 3	F: 18
Coffea arabica solution 1.08 ml	M: 5	M: 5	M: 3	M: 2	M: 4	M: 19
	F: 2	F: 3	F: 3	F: 3	F: 2	F: 13
Caffeine 300 mg / bw	M: 4	M: 4	M: 2	M: 5	M: 4	M: 19
	F: 1	F: 3	F: 4	F: 2	F: 2	F: 12

Note : M = Male. F = Female

Based on Table 3. it can be seen that *Coffea arabica* solution and caffeine does not affect the determination of the dominant sex of the resulting fetus. All data indicate that the sex of male and female have a comparable value of each parent in each treatment.

### 3.2 External malformations

The fetus that has been assessed for its reproductive appearance is further divided into 3 parts. 2/3 fetus from each parent were immersed in bouin solution for 3 days for observation of external malformations, the remaining 1/3 were immersed in 95% alcohol fixation solution for 2 weeks to prepare for observing fetal skeletal before being soaked in alizarin red s. no abnormalities were found in external malformations from the administration of *coffea arabica* solution and caffeine.

### 3.3 Malformation scletal

Fetus that have been immersed in a 95% alcohol solution for a week aim to maintain tissue morphology so that skeletal preparations can be seen that are not much different from the initial conditions. Fixated with 95% alcohol, the fetus is then soaked with 1% KOH solution as a cleansing solution for  $\pm$  12 hours so that the bone structure is clearly visible. The fetus is soaked in alizarin red s solution for no more than 24 hours which is useful for giving the skeletal color. After soaking with alizarin red s solution, the fetus is again soaked in a 2% KOH solution for  $\pm$  12 hours to clean the dyes that remain in the muscle tissue. The fetus is put into a purifying solution A, B and C containing glycerin, each for 1 day to make the scletal appearance transparent and easily observable.

Based on observations of scletal malformation, no abnormalities were found in the parent given coffea arabica solution 0.36, 0.72, 1.08 and Caffeine 300 mg / kg bw.

## 4. Conclusion

Provision of Arabica coffee solution in 1.08 administration and caffeine 300 mg / kg bw causes weight loss and body length. Where as the observation of external malformations and scletal malformations were not found abnormalities.

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