

Original Research Article

Fetal Brain injury Associated with Maternal Caffeine Administration in Rats

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Abstract: The fetal brain injury associated with maternal caffeine administration in rats was studied using the Cajal reactive astrocytes method. Thirty (30) adult female rats were mated and pregnant female rats were divided into three major groups, namely A, B and C. Each group had ten pregnant rats. Group A served as the control and were served distilled water. Groups B and C received 50 mg and 90 mg/kg per body weight of caffeine (Sigma-Aldrich). On the 20th day of gestation, pregnant rats were sacrificed by chloroform inhalation method. Sixty fetuses were randomly selected and weighed. The brains of fetuses were harvested and fixed in 10 % neutral buffered formalin. Sections of the fetal brain were processed histologically using the Cajal reactive astrocytes staining method. Results revealed normal morphological appearance of astrocytes in the brain of fetal rats given distilled water while the sections of fetal brain of rats exposed to maternal administration of 50 mg and 90 mg/kg body weight of caffeine showed increased number of astrocytes that were hypertrophied and are termed reactive astrocytes. There was no significant difference in weights of animals in control group and test groups. Based on the presence of reactive astrocytes observed in the test group, the study suggests that maternal caffeine administration may induce fetal brain injury in rats.

Keywords: Caffeine, Fetus, Brain, Pregnancy, Astrocytes.

INTRODUCTION

Caffeine was first isolated from green coffee beans in 1820. It was later found in tea, mate and kolanuts. Somorin [1] reported that caffeine; theobromine and theophylline found in kolanuts are xanthine stimulants. Coffee, tea, carbonated beverages and cocoa are the main sources of caffeine [2]. An Ottawa study at Carleton University, Canada in 1983, analyzed the total caffeine intake from all sources in 286 pregnant women. In the first trimester of their pregnancy, coffee accounted for only 56 % of their total caffeine intake, tea accounted for 37 % of caffeine intake, while caffeinated soft drinks, chocolate bars, chocolate drinks and caffeinated medications accounted for approximately 7 % of caffeine intake. The content of caffeine in these various food items ranges from 40 to 180 mg/150 ml for coffee to 24 to 50 mg/150 ml for tea, 15 to 29 mg/180 ml for cola, 2 to 7 mg/150 ml for cocoa and 1 to 36 mg/28 g for chocolate [3, 4]. Caffeine absorption from the gastrointestinal tract is rapid and reaches 99 % in humans in about 45 minutes after ingestion [5, 9]. Absorption is, however, not complete when the substance is taken as coffee [10]. It is also known that when very large doses of caffeine are accidentally ingested, toxic effects appear, with an LD₅₀ of about 200 mg/kg body weight in rats [11]. Caffeine is

metabolized in the liver to form dimethyl and monomethylxanthines, dimethyl and monomethyl uric acids, trimethyl- and dimethylallantoin, and uracil derivatives [8, 9]. The hydrophobic properties of caffeine allows its passage through all biological membranes. There is no blood-brain barrier to caffeine in adult or the fetal animal [12, 13]. The equivalent of 80 – 100 mg/kg/day of caffeine is the dose usually required for development of malformations in rats [14]. In the monkey, spontaneous abortions and stillbirths have been recorded at 2 doses used, 10 – 15 and 25 – 35 mg/kg/day of caffeine (which is an equivalent of 2 - 3 cups and 5 – 8 cups of coffee, respectively) [15].

DEVELOPMENT OF THE BRAIN

During the third week of gestation the notochord sends signals to the overlying ectoderm, inducing it to become neuroectoderm [17]. This results in a strip of neuronal stem cells that runs along the back of the embryo [17]. This strip is called the neural plate, and is the origin of the entire nervous system [17]. The neural plate folds outwards to form the neural groove. Beginning in the future neck region, the neural folds of this groove close to create the neural tube by a process of neurulation [17].

The cephalic end of the neural tube shows three dilations and these are the primary brain vesicles. Primary brain vesicles are the prosencephalon, or forebrain, the mesencephalon, or midbrain and the rhombencephalon, or hindbrain [16].

Simultaneously two flexures are formed; these are the cervical flexure at the junction of the hindbrain and the spinal cord, and the cephalic flexure in the midbrain region [16].

When the embryo is 5 weeks old, the prosencephalon consists of two parts, namely, the telencephalon, formed by a midportion and two lateral outpocketings, the primitive cerebral hemispheres, and the diencephalon, characterized by outgrowth of the optic vesicles [16].

A deep furrow called the rhombencephalic isthmus, separates the mesencephalon from the Rhombencephalon [16].

The rhombencephalon also consists of two parts, namely, the metencephalon, which later forms the pons and cerebellum, and the myelencephalon [16].

The boundary between these two portions is marked by the pontine flexure. The lumen of the spinal cord, the central canal, is continuous with that of the brain vesicles [16].

MATERIALS AND METHODS

Experimental Animals

Thirty adult female albino rats were obtained from the Animal House, College of Health Sciences, University of Uyo, Akwa Ibom State, Nigeria. The animals were housed in cages under standard laboratory conditions and fed with growers mash (Pfizer Nigeria Limited) and water ad libitum. All animals were housed in a cross-ventilated room.

Purchase and Preparation of Caffeine solution

The Caffeine used for this study was purchased from Rovet Nigeria Plc. The caffeine solution was prepared by dissolving 0.5 g of pure caffeine in 20 ml of water. This yielded a stock solution of 25 mg/ml of caffeine. Each reconstituted caffeine solution was stored in a cool dry place at room temperature and used within 2 days. The caffeine suspension was administered to the animals based on their body weight orally with the aid of orogastric tubes.

Experimental Design

The adult female rats were weighed, labeled and confined in cages. The rats were divided into three groups, n =10. Group A was the control group. Groups B and C served as experimental groups fed with the caffeine solution for a period of 20 days. Vaginal smear was carried out daily to study the oestrus cycle of the rats and to determine when they were in estrus phase for mating. Adult female albino rats were mated with males at the ratio of 2:1 (2 females to 1 male). The presence of tailed structures in the vaginal smear confirmed coitus and the sperm positive day was taken as day zero of pregnancy. The pregnant rats in groups B and C received 50 mg and 90 mg/kg per body weight of caffeine for a period of 20 days. The control group was served with distilled water. On the 20th day of gestation, pregnant rats were sacrificed under chloroform anesthesia. Sixty fetuses were randomly selected and weighed, the fetal brains were harvested. Sections of the fetal brain were processed histologically using Cajal reactive astrocyte staining method.

RESULTS

The sections of fetal brain of rats exposed to 50mg and 90mg/kg/body weight of caffeine showed evidence of brain injury characterized by reactive astrocytes compared to the control group which revealed normal morphology of the astrocytes. There was no significant difference in body weights between the test groups and the control group.

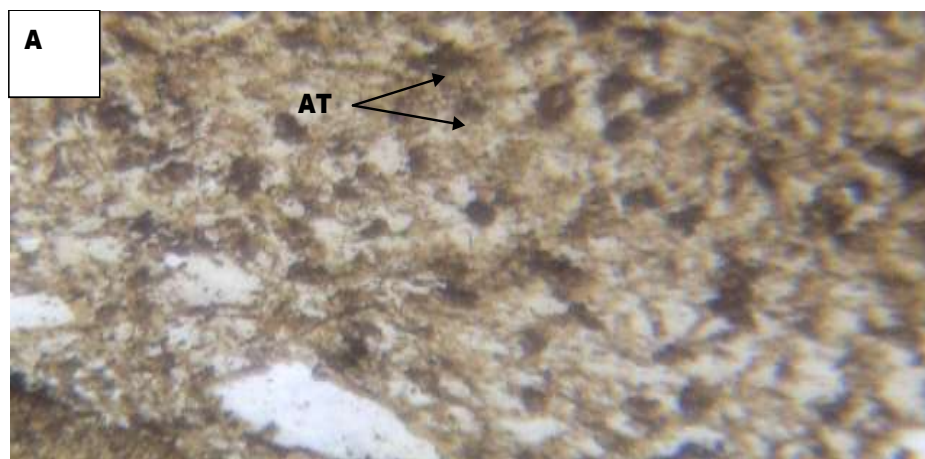


Fig-1: Section of fetal brain of rats given distilled water showing black stained astrocytes (AT) with normal cellular architecture

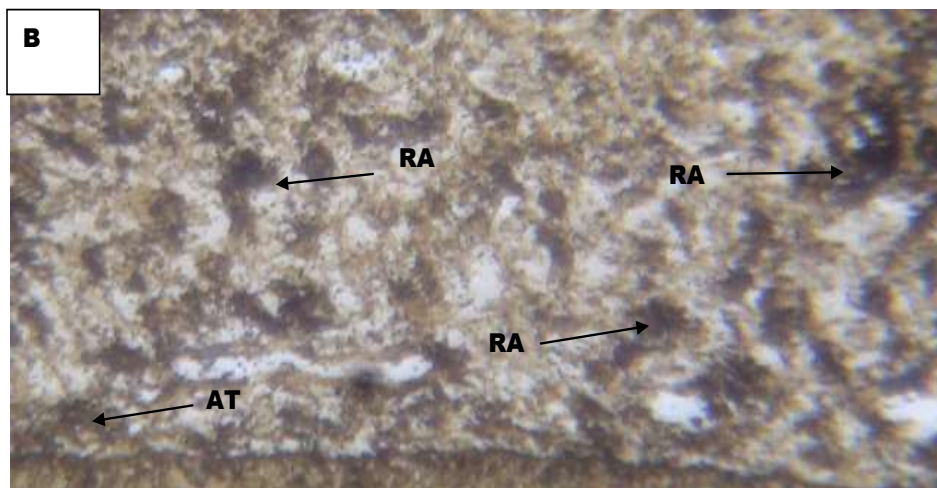


Fig-2: Section of fetal brain of rats exposed to 50 mg/kg per body weight of caffeine showing black stained reactive astrocytes (RA) and astrocytes (AT)

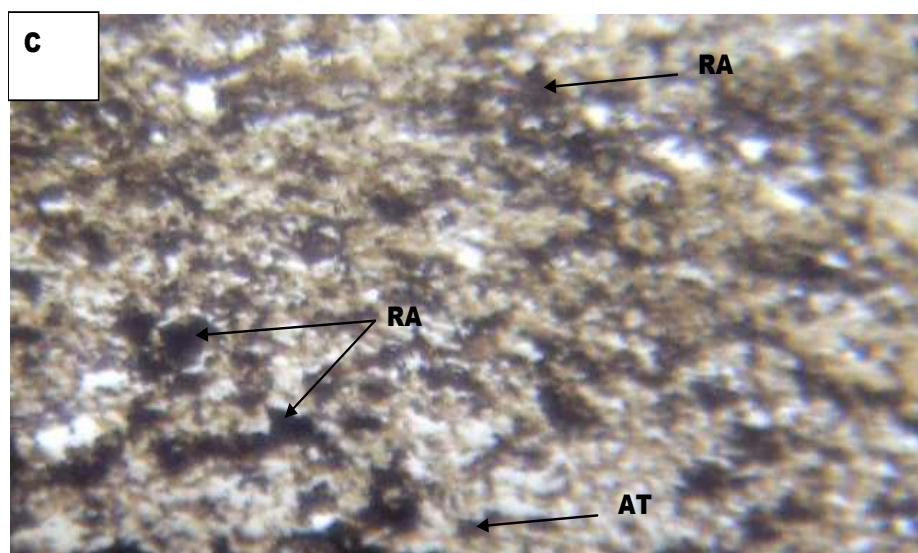


Fig-3: Section of fetal brain of rats exposed to 90 mg/kg per body weight of caffeine showing numerous black stained reactive astrocytes (RA), and astrocytes (AT)

DISCUSSION

The encyclopedia Britannica describes astrocytes as star shaped glial cells in the CNS which are usually of two forms in the mammalian brain namely; fibrous astrocytes related to the white matter which have small cell body with long processes. Protoplasmic astrocytes are another type of astrocytes with more frequent processes that are thicker and shorter than those seen in fibrous astrocytes. These astrocytes contain multiples of small, round and dark glycogen granules that make them appear dark stained [18]. Astrocytes are neuroprotective cells involved in healing and recovering of neurons in various nervous system pathology and are involved in the neuroinflammatory response [19].

The functions of astrocytes include the provision of metabolic support for neurons in the CNS

and synaptic means of communication within the brain [20]. They regulate ionic concentration for tight junctions, blood brain barrier and serve as intermediary stations for converging nutrients, gases and removal of waste products between neurons [21]. Astrocytes exert strong influence on neurons during synaptic transmission through modulation of their volume, concentration and composition of the neurotransmitters glutamate and ATPase receptor [20]. Astrocyte reactivity is characterized by morphological changes (hypertrophy, remodeling of processes) and the overexpression of the intermediate filament glial fibrillary acidic protein (GFAP) [19].

In this study, the sections of fetal brain of rats exposed to 50 mg/kg and 90 mg/kg of caffeine per body weight showed increased population of black stained reactive astrocytes.

Ekanem, Salami, Ekong, Eluwa, and Akpantah, [22] and Peter *et al.*, [20] documented that in the CNS injury, astrocytes acts as neuroprotective shealths, increasing in number and filling injury zone, heals and recovers neurones, hence they are called reactive astrocytes. Kumar, Abbas, and Fausto, [23] reported that the presence of reactive astrocytes is an indication of early signs of cell loss serving as an indicator of pathologic process. Thus, the activities of caffeine on astrocytes as seen in this present study might suggest a neurodegenerative potential, which could further explain the neurological effects of this product on the brain when consumed even by adults in high doses. This suggests that caffeine may induce fetal brain injury in rats. In conclusion, our finding from this study showed that caffeine consumption during gestation may induce fetal brain injury in rats.

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