

Histomorphological Study of the Developing Cerebral Cortex of Wistar Rats Exposed to Maternal Caffeine Administration

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Abstract: The histomorphological effects of caffeine on the developing cerebral cortex of rats were carefully studied. Thirty 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, five pregnant rats were randomly selected from each of the groups making a total of fifteen pregnant rats which were sacrificed by chloroform inhalation method. Thirty fetuses were selected and weighed. The brains of fetuses were harvested and fixed in 10 % neutral buffered formalin. The remaining fifteen pregnant rats were allowed to deliver normally on the twenty first day. Thirty pups were randomly selected and weighed. Four weeks after birth the pups were subjected to a series of neurobehavioural tests using the Morris water maze for spatial memory and learning. The day following the test, pups were weighed and sacrificed by using chloroform inhalation method. They were decapitated and the brains carefully removed and fixed in 10 % neutral buffered formalin. Sections of the cerebral cortex were processed histologically using the Haematoxylin and Eosin staining. Results revealed normal morphological appearance of cells in the cerebral cortex of fetal and young rats given distilled water while the sections of fetal and young rats exposed to maternal administration of 50 mg and 90 mg/kg body weight of caffeine showed the presence of pyknotic nuclei, hypertrophied cells and vacuolations. The findings indicated that there was no significant difference in weights and neurobehavioral assessment of animals in control group and test groups. Based on the cellular degenerative changes, and hypertrophy observed in the test group, the study suggests that caffeine may be harmful to the developing cerebral cortex.

Keywords: morphology, caffeine, cerebral cortex, wistar rats.

I. Introduction

Twenty-first (21st) century has brought with it the demand for energy drinks which come in canned form. Most of these energy drinks contain caffeine. Consumption of this drink may prove fatal for the developing brain due to the ability of caffeine to cross the placental and blood-brain barriers (Nehlig and Derby, 1994). Most of these energy drinks come in a range of flavoured shots developed especially for busy pubs and bars, and are very affordable. Unlike the conventional coffee which has a bitter taste, these energy drinks have a mild taste. These features coupled with its availability in the local market has made energy drinks to be highly patronized especially within the female folk. Hence, most women consume these products unmindful of its caffeine content. Katie Clemency (2013) in her findings revealed that busy moms consume more of this refined energy beverages than men, partly due to its portability.

Caffeine is the most widely consumed active substance in the world (Tanya, 2005) that affects the central nervous system (Tanya, 2005) by stimulating brain and nerve cells activities (United States Food and Drug Administration, 1980). Caffeine (1,3,7-trimethylxanthine) is a purine alkaloid present in high concentrations in tea, coffee and in a number of beverages such as Coca-cola product. An important source of caffeine for expectant mothers include coffee, chocolate, and cocoa drinks. Expectant mothers who consume these products pose a risk to the fetus. Unlike adults, the fetus cannot metabolize caffeine, and, this may encourage its accumulation within the tissues, and become harmful to the developing fetus (Nehlig and Derby, 1994). Caffeine may negatively impact fetal development, since it easily passes through the placental barrier, and may also reduce blood flow to the fetus. The effect of caffeine consumption during pregnancy has been implicated as a risk factor for delayed conception and also implicated as a risk factor for spontaneous abortions (Signorello and McLaughlin, 2004; Williams, Monson, Goldman, Mittendorf and Ryan, 1990; Hatch and Bracken 1993; Stanton and Gray, 1995; Bolumar, Olsen, Rebagliato and Bisanti, 1997; Jensen, et al., 1998; Srisuphan and Bracken, 1986).

Caffeine is a potent teratogen capable of causing serious harm to the fetus because it readily crosses the placental and the blood brain barriers. The teratogenic effect of caffeine has been clearly demonstrated in rodents (Nehlig and Derby, 1994). The quantity of caffeine needed to induce malformations in rodents reaches

doses that are toxic to man. The equivalent of 80 – 100 mg/kg/day of caffeine is the dose usually required for development of malformations in rats (Nehlig and Derby, 1994). 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) (Watkinson and Fried, 1985). The exact effect of caffeine on neurodevelopment have been studied on chick embryos, showing the inhibition of neural tube closure and neural crest cell migration differences when exposed to high amounts of caffeine (Ma et al., 2012).

Caffeine metabolites are naturally decreased in the pregnant mother by natural biological mechanisms, though it is not sure why this phenomenon occurs. The inability to metabolize caffeine results in caffeine buildup over time and this also relates to disruption of neurotransmitter signals which may affect development through organogenesis and growth in the third trimester of humans (Li et al., 2012).

The cerebral cortex is the outermost layered structure of neural tissue of the cerebrum (brain), in humans and other mammals. It covers the cerebrum and is divided into two cortices, along with the sagittal plane, covering the left and right cerebral hemispheres. The medial longitudinal fissure is a deep groove that separates these two hemispheres. The cerebral cortex plays a key role in memory, attention, perceptual awareness, thought, language, consciousness and also integrates higher mental functions, general movement, visceral functions, and behavioral reactions (Brodal, 1977; Cauller, 1995). The neocortex which is the major part of the cortex consists of up to six horizontal layers in humans, each with a different composition in terms of neurons and connectivity. Neurons in various layers connect vertically to form small microcircuits, called cortical columns. Different neocortical regions known as Brodmann areas are distinguished by variations in their cytoarchitectonics.

II. Methodology

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 45 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. The control group was served with distilled water. On the 20th day of gestation, five pregnant rats were randomly selected from each of the groups making a total of fifteen pregnant rats which were sacrificed under chloroform anesthesia. Thirty fetuses were harvested, selected and weighed. The brains of fetuses were harvested and fixed in 10 % neutral buffered formalin. The remaining fifteen pregnant rats were allowed to deliver normally on the twenty first day. Thirty pups were randomly selected and weighed. Four weeks after birth the pups were subjected to a series of neurobehavioural tests using the Morris water maze for spatial memory and learning. Thereafter, animals were decapitated and the brains samples were carefully removed and fixed in 10 % neutral buffered formalin. Sections of the cerebral cortex were processed histologically following the Haematoxylin and Eosin staining technique.

III. Results

The sections of the cerebral cortex of wistar rats given distilled water and stained using H & E staining showed normal morphological appearance of cells in the cerebral cortex, but the sections of wistar rats exposed to maternal caffeine administration of 50 mg and 90 mg/kg body weight of caffeine showed the presence of pyknotic nuclei, hypertrophied cells and vacuolation. No significant difference is observed in body weights changes and neurobehavioural assessment among the experimental groups.

Comparison of Body Weight Changes

Table 1: Body Weight Changes

Groups	Body weight of fetus	Body weight at birth	Body weight at four weeks
Group A	2.167 ± 0.389	6.917 ± 0.900	51.750 ± 6.062
Group B	2.750 ± 0.754 ^{NS}	5.675 ± 0.449 ^N	38.000 ± 4.090 ^{NS}
Group C	4.392 ± 0.610 ^{NS}	5.658 ± 0.645 ^{NS}	56.000 ± 5.394 ^{NS}

Neurobehavioral Assessment

Table 2: The Morris Water Maze Test

Groups	Trial day 1	Trial day 2	Trial day 3
Group A	44.376 ± 6.062	24.992 ± 23.109	17.301 ± 6.917
Group B	49.322 ± 4.090 ^{NS}	34.101 ± 24.031 ^{NS}	15.005 ± 5.961 ^{NS}
Group C	43.515 ± 5.394 ^{NS}	19.724 ± 15.489 ^{NS}	25.453 ± 4.107 ^{NS}

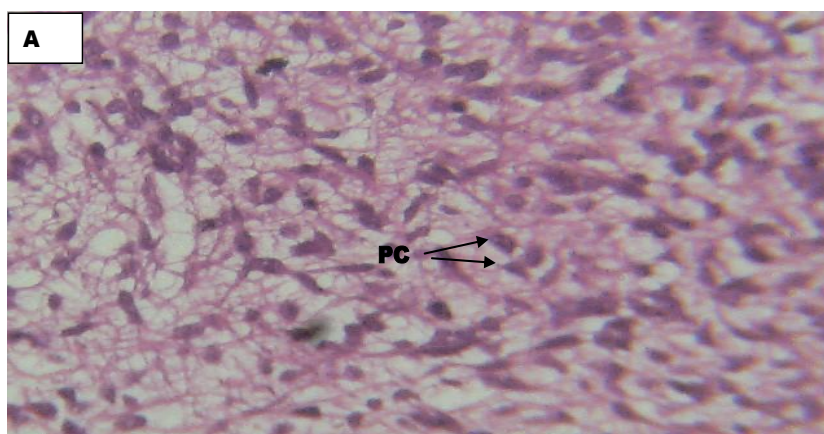


Plate 1: Control section of the fetal rat cerebral cortex showing: normal appearance of pyramidal cells (PC). (H & E x400)

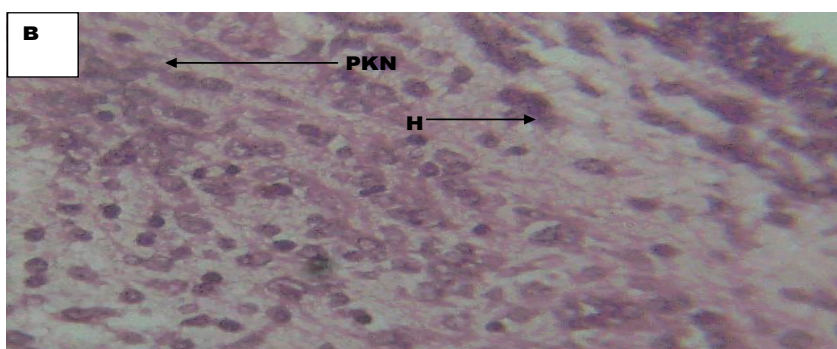
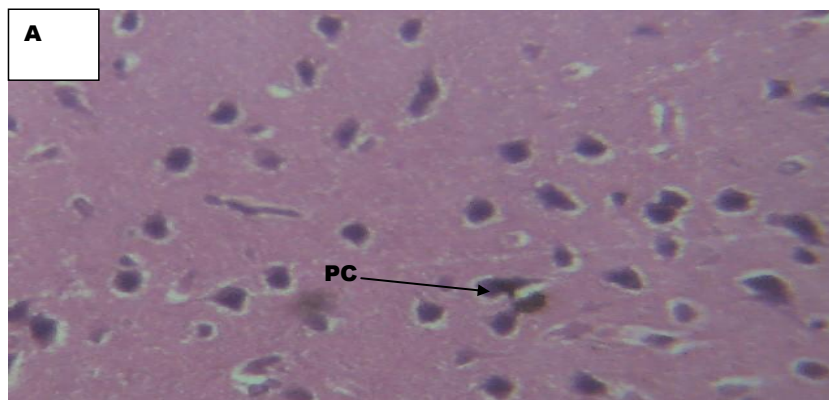


Plate 3: Section of fetal cerebral cortex of rat exposed to 50mg/kg caffeine showing: pyknotic nuclei (PKN) and hypertrophy (H). (H & E x400)

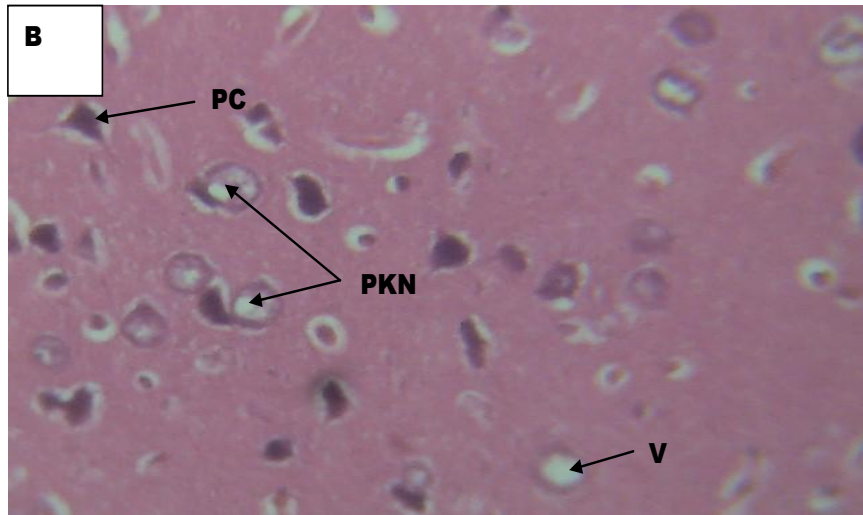


Plate 4: Section of young rat cerebral cortex of exposed to 50mg/kg caffeine showing: pyknotic nuclei (PKN) and Vacuolation (V). (H & E x400)

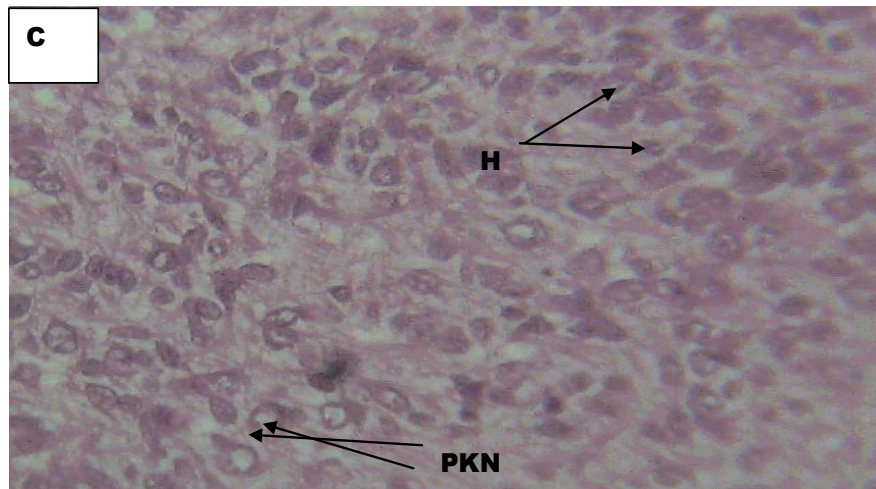


Plate 5: Section of fetal rat cerebral cortex of exposed to 90mg/kg caffeine showing: pyknotic nuclei (PKN) and hypertrophy (H). (H & E x400).

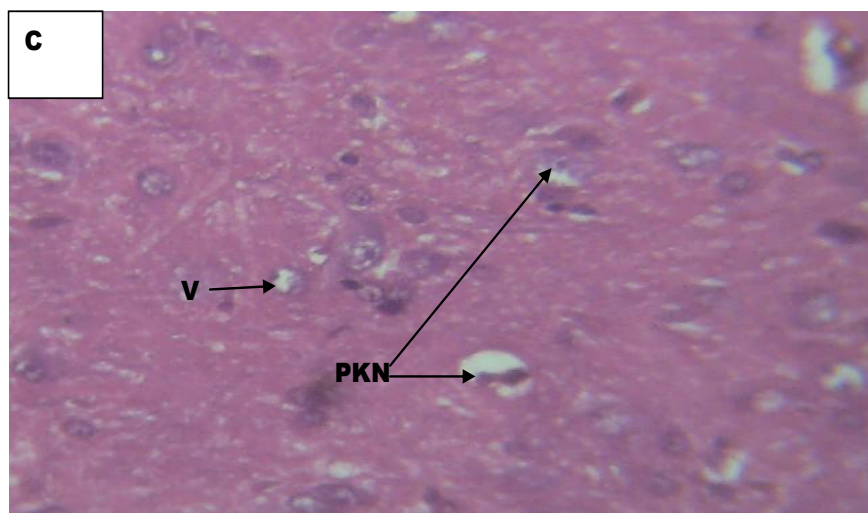


Plate 6: Section of young rat cerebral cortex of exposed to 90mg/kg caffeine showing: pyknotic nuclei (PKN) and Vacuolation (V). (H & E x400).

IV. Discussion

The cerebral cortex plays a key role in memory, attention, perceptual awareness, thought, language, and consciousness. Damages to some parts of the cerebral cortex such as damages in form of the presence of pyknotic nuclei, hypertrophied cells and vacuolations in the cerebral cortex upon maternal administration of caffeine as seen in this study may impair some functions of the cerebral cortex such as memory, attention, perceptual awareness, thought and consciousness which may affect proper learning. Memory is an organism's ability to retain and subsequently retrieve information (Osim, 2008).

Spatial memory, in neuroscience and cognitive psychology, is the part of memory responsible for recording information about one's environment and its spatial orientation (Johnson and Adamo-Villani, 2010). It is believed that a person's spatial memory is required in order to navigate around a familiar city. Similarly, it is believed that the rat spatial memory is needed to learn the location of food at the end of a maze. Spatial memory is seen as a cognitive process that enables a person to remember different locations as well as the spatial relationship between an object (Johnson and Adamo-Villani, 2010).

Specific areas of the brain have been associated with spatial memory function. These include the hippocampus which provides animals with a spatial map of their environment (O'keefe and Dostrovsky, 1971). The medial prefrontal cortex is said to process egocentric spatial information. It participates in the processing of short-term spatial memory used to guide planned search behaviour and is believed to join spatial information with its motivational significance (Pratt et al., 2003). The medial prefrontal cortex is also implicated in the temporal organization of information (Kesner and Holbrook, 1987). Prefrontal Cortex is shown to exhibit hemisphere specialization in its spatial memory function (Slotnick and Moo, 2006). Damage to this region is associated with spatial memory deficit in the rat, though performance can gradually improve due to previous experience (Becker et al., 1980; Aggleton et al., 1995; Lecroix et al., 2002).

Pyramidal neurons are the types of neurons found in the pyramidal cell layer of the cerebral cortex. A pyramidal neuron has a triangular shaped soma or cell body, a single axon and a large apical dendrite and multiple basal dendrites with dendritic spines. The functions of the pyramidal neurons include involvement in circuitry responsible for the vision and guided motor function. Pyknosis from Greek word pyknono meaning to thicken up, close or to condense, is the irreversible condensation of chromatin in the nucleus of a cell undergoing necrosis or apoptosis (Kumar et al., 2005). Vacuolation is a feature of autophagy. Autophagy which is a highly regulated process involving the bulk degradation of cytoplasmic macromolecules and organelles in mammalian cells via lysosomal system is induced under starvation, differentiation, and normal growth control to mammalian cellular homeostasis and survival (Komatsu et al., 2005; Kuma et al., 2004; Shintani and Klionsky, 2004). Simultaneously, it has also been evident that autophagy participates in various neurodegenerative disorders (Zhu et al., 2007; Nixon, 2006; Koike et al., 2005; Chu, 2006) and further that it can trigger a form of cell death distinct from apoptosis in neurons (Canu et al., 2005; Clarke, 1990; Ohsawa et al., 1998; Uchinoyama, 2001).

The study revealed that cerebral cortexes of wistar rats exposed to maternal administration of 50 and 90 mg/kg per body weight of caffeine, showed hypertrophied cells, pyknotic nuclei and vacuolation, which are all neurodegenerative features. Pagnussat, Faccioni_Heuser, Netto, and Achaval (2007) reported the presence of pyknotic nucleus in the pyramidal cells of the hippocampus as well as vacuolation of the surrounding glia and oligodendrocytes are features of type II neuron death. Similar type II neuron death features were observed in the pyramidal cells of the cerebral cortexes of wistar rats exposed to maternal caffeine administration. This suggests that caffeine poses a risk to the developing cerebral cortex.

V. Conclusion

The study revealed that maternal consumption of caffeine resulted in morphological changes in the cells of the cerebral cortex of wistar rats. These changes may affect the functions of the cerebral cortex which plays a key role in memory, attention, perceptual awareness, thought, language and consciousness.

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